Genetic Analysis of a Hybrid Cross between *Cryptococcus neoformans* and *Cryptococcus deneoformans*

GENETIC ANALYSIS OF A HYBRID CROSS BETWEEN *CRYPTOCOCCUS NEOFORMANS* AND *CRYPTOCOCCUS DENEOFORMANS*

BY

AARON A. VOGAN,

A THESIS

SUBMITTED TO THE DEPARTMENT OF BIOLOGY

AND THE SCHOOL OF GRADUATE STUDIES

OF MCMASTER UNIVERSITY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Doctor of Philosophy (2015) McMaster University

(Biology) Hamilton, Ontario, Canada

TITLE:	Genetic Analysis of a Hybrid Cross between *Cryptococcus neoformans* and *Cryptococcus deneoformans*
AUTHOR:	Aaron A. Vogan B.Sc., (Biochemistry) McMaster University, Hamilton, Canada
SUPERVISOR:	Dr. Jianping Xu
NUMBER OF PAGES:	xv, 200

Abstract

Cryptococcus neoformans is a deadly human fungal pathogen that primarily affects immunocompromised individuals. Matings with the closely related species *Cryptococcus deneoformans* produce viable, yet atypical offspring. Previous research has demonstrated that the hybrids are aneuploid or diploid, and have low germination rates and suppressed recombination. In addition, numerous large-scale genomic rearrangements have been identified between *C. deneoformans* and *C. neoformans*. We conducted a cross between CDC15, a clinical drug-resistant isolate of *C. neoformans* and JEC20, a laboratory derived strain of *C. deneoformans*. We collected 230 hybrid progeny and determined their genotypes using codominant PCR-RFLP markers. Three separate analyses were conducted in order to better understand the genetics of these hybrids. These revealed that there is mitotic recombination during the production of the hybrids, that there are genetic incompatibilities between *C. neoformans* and *C. deneoformans*, and that differences between the two species in virulence-related traits are controlled by multiple quantitative genetic loci. The results not only improve our understanding of the hybrid progeny themselves, but also of *Cryptococcus* in general.

Acknowledgements

I want to thank my advisor Dr. Jianping Xu, for the support and freedoms that he has given me throughout the completion of this thesis. He has allowed me to pursue my own ideas and projects, and as a result I feel as though I have learned far more than I may have otherwise. Additionally, I thank my committee, Dr. Paul Higgs and Dr. Ben Evans, for aiding me in statistical analyses and for always being available to sanity check my logic on many of these projects.

There have been many students to come and go in the lab since I began, I have valued working with all of them, but there are a few who stand out above the rest. Amanda Wilson was the first person in the lab to work with me and she taught me a lot of what I know about wet lab work. Greg Armstrong, Jake Alter, and Jasmine Dautovich welcomed me with open arms and made me feel a part of the lab almost instantly. Without them the ups and downs of beginning a masters project would have driven me to wits' end. Judy (Zixuan) Wang livened up the lab with her great personality and never ending gifts. Jordan Khankhet was an exceptional student as an undergrad and helped me extensively with my project. It was great to have him join the lab as a graduate student and continue to work together. Himeshi Samarasinghe did great work for me as an undergrad and, along with Wenjing Hua, I know she will

continue doing great work with her masters project. I was very excited when Eta Ashu joined the lab, as I would finally have another PhD student in the lab to discuss research with. It has been a pleasure having him around. Adiran Forsythe has been an excellent addition to the lab. He is following closely in my footsteps, and I've been doing my best to mold him into an appropriate replacement for me. It's a big lab coat to fill, but I know he will be able to.

Outside of the lab, I owe a lot to my close friend Ben Furman. Constant caffeine-fuelled discussions about science and evolution have kept me sharp and up to date with the current literature. While the alcohol-fuelled discussions have relaxed me and kept me sane. Chris Dejong has aided in many of those discussion and always provided a venue and atmosphere to unwind.

Lastly, I need to thank my lovely partner Ranya (Saranya) Amirthamanoharan. Without her love and support I know I never could have completed any of this. From cooking me meals when I was in lab late, to cheering me up with surprise presents after a hard week (or month). She has been a motivation for me to do well in all that I try and a comfort for when I fail. I eagerly look forward to stumbling through the darkness that is research with her holding my hand.

Abbreviations

Acronyms

BDM Bateson – Dobzhansky – Muller

CIM composite interval mapping

FDR false discovery rate

IL incompatibility loci

IP incompatibile pair

IT incompatibile trio

MIC minimal inhibitory concentration

MIM multiple interval mapping

MQM multiple QTL mapping

NSERC the Natural Sciences and Engineering Research Council

QTL quantitative trait loci

SD Sabouraud dextrose

YEPD yeast extract peptone dextrose

Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 Cryptococcus

The genus *Cryptococcus* was defined by Kützing in 1883 to include yeasts which did not form endospores (Casadevall and Perfect, 1998), but it was not until 1956 that the genus was described in detail by Benham (Benham, 1956). As a result of this long period during which the definition of the genus was in flux, *Cryptococcus* is grossly polyphyletic with member species distributed across multiple orders (Fell *et al.*, 2000). *Cryptococcus neoformans* was first described in 1894 by Busse, who isolated this species from a human patient. It was originally classified under *Saccharomyces* and although Vuillemin placed it in the genus *Cryptococcus*, it underwent multiple name changes between then and 1956 (Casadevall and Perfect, 1998). By passing polysaccharide of *C. neoformans* through rabbits, Evans (1949) was able to classify three separate serotypes of *C. neoformans*, simply lettered A, B, and C (Evans, 1949). In 1968, a broader survey of *Cryptococcus* strains conducted by Wilson *et al.* identified a fourth serotype, D, as well as strains which typed as both

A and D, designated AD (Wilson *et al.*, 1968). In 1975 Kwon-Chung identified a sexual state within the A and D serotypes, creating the new genus *Filobasidiella* for this telomorph (Kwon-Chung, 1975). The following year, she was able to identify the sexual state of serotypes B and C, and this telomorph was given the species name *Filobasidiella bacillisporus* (Kwon-Chung, 1976b). In another two years, Kwon-Chung would further reclassify the anamorph state, *C. neoformans* serotypes B and C as the species *Cryptococcus bacillisporus* (Kwon-Chung *et al.*, 1978). However, the discovery that mating was possible between *C. neoformans* and *C. bacillisporus* led to a reversal on the decision to split *C. neoformans* into two groups. Instead, serotypes A, D, and AD were classified as *C. neoformans* var. *neoformans* and serotypes B, and C were classified as *C. neoformans* var. *gattii* (Kwon-Chung *et al.*, 1982).

For the following two decades the taxonomy of *C. neoformans* would enjoy an era of stability, but the rise of molecular techniques would soon end this. In 1999, results from DNA fingerprinting and sequence data from the URA5 gene led to the elevation of serotype A to varietal status, *C. neoformans* var. *grubii* (Franzot *et al.*, 1999). Soon after, four separate molecular groups would be defined for *C. neoformans*: var. *neoformans*(VNIV), var. *grubii* (VNI, and VNII), and serotype AD (VNIII) (Meyer *et al.*, 1999) and an additional four for var.*gattii* (VGI, VGII, VGIII, and VGIV) (Ellis *et al.*, 2000). Then in 2002, the decision to reverse the splitting of *C. neoformans* into two species was itself reversed, and *C. neoformans* var. *gattii* became *C. gattii* (Kwon-Chung *et al.*, 2002). Evidence slowly amounted over the following years that hinted towards true differences between the molecular types (Bovers *et al.*, 2008). Most recently, this has precipitated the reclassification of *C. neoformans* and *C. gattii* once again, now into seven separate species: *C. neoformans* (formally *C. neoformans*

var. *grubii*, serotype A), *C. deneoformans* (formally *C. neoformans* var. *neoformans, serotype D*), *C. gattii* (formally *C. gattii*, VNI), *C. deuterogattii* (formally *C. gattii*, VNII), *C. bacillisporus* (formally *C. gattii*, VNIII), *C. tetragattii* (formally *C. gattii*, VNIV), and *C. decagattii* (formally *C. gattii*, VNIV/VNIIIc) (Hagen *et al.*, 2015). As this body of work began in 2010, chapters 2 and 3 refer to serotype A as *C. neoformans* var. *grubii* and serotype D as *C. neoformans* var. *neoformans* while chapter 4 refers to them as *C. neoformans* and *C. deneoformans*, respectively.

Mating in *Cryptococcus* generally follows that of other basidomycete fungi, with one major difference, which is briefly described below. In the environment *Cryptococcus* generally grows as a yeast. When conditions are correct and another strain of *Cryptococcus* of the opposite mating type is present, a morphological change will occur that results in the production of dikaryotic hyphae. These hyphae are binucleate, with one nucleus coming from the MATa parent and one from the MATα parent. The terminal ends of the hyphae will form a basdium in which the two haploid parental nuclei will fuse to form one diploid nucleus. This nucleus will undergo a single round of meiosis to produce four recombinant daughter nuclei. For most basidiomycetes, these daughter nuclei are packaged into four individual basidiospores. However, in *Cryptococcus*, the daughter nuclei undergo continued rounds of mitosis, which results in long chains of basidiospores produced from each basidium (Kwon-Chung, 1975; Idnurm *et al.*, 2005; Idnurm, 2010) (Figure 1.1).

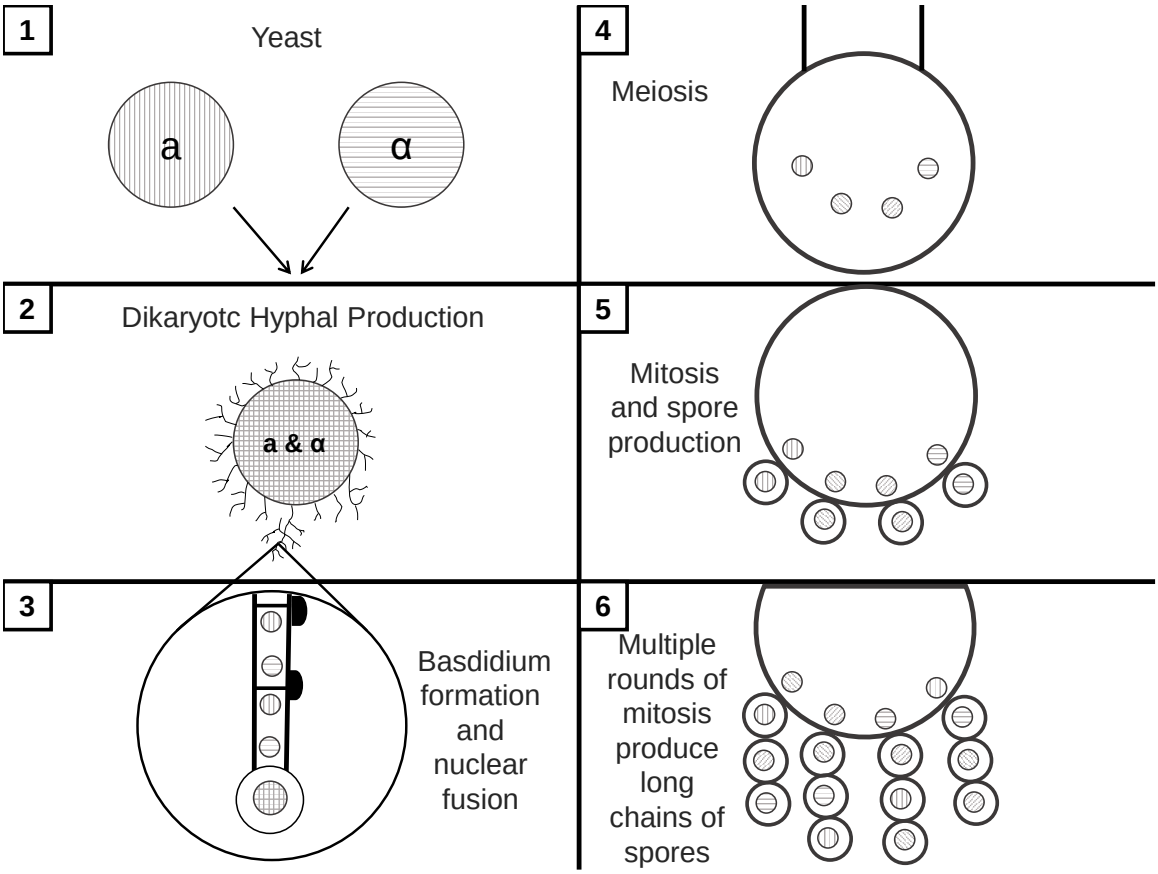

Figure 1.1: The life cycle of *Cryptococcus neoformans*. **1.** Growth as free living yeast. Strains can be either MATa or MATα **2.** Production of dikaryotic hyphae. **3.** Basidium formation and fusion of haploid nuclei into a diploid nucleus **4.** A single round of meiosis produces four recombinant daughter nuclei. **5.** Daughter nuclei undergo mitosis and are packaged into basidiospores **6.** Multiple rounds of mitosis produce long chains of basidiospores.

1.2 Hybridization

Throughout the history of *C. neoformans* research, the serotype AD, received little attention beyond the occasional footnote in serotype surveys. It had long been suspected that they represented a hybrid lineage between serotype A and D, but this was not conclusively demonstrated until Lengeler *et al.* successfully performed an experimental laboratory cross. They were not only able to demonstrate that serotype AD strains could be obtained from a hybridization event, but also determined that the strains are aneuploid/diploid and have low germination rates (Lengeler *et al.*, 2001). Further studies revealed that hybridization in *Cryptococcus* is relatively recent and ongoing, rather than the result of rare events in the past (Xu *et al.*, 2002; Litvintseva *et al.*, 2007).

Hybridization is an oft debated topic in the evolutionary biology literature. The primary reason for this is the effect hybridization has on"the tree of life". Classically, the phylogenetic tree that contains all life is a continuously bifurcating lineage, where a split is made at every "speciation event". In actuality, this tree is much more complex, exhibiting the properties of a network where horizontal gene transfer is frequent enough to skew the boundaries between species (such as in bacteria) and containing reticulations where past hybridization events have led to the emergence of new lineages. In theory, hybridization has the ability to either breakdown speciation barriers or reinforce them (Barton, 2001). Breakdown is thought to occur through relatively straightforward mechanisms. As long as hybrid individuals can mate with parental populations, genes can be introgressed into either population, eroding the differences between the parental populations (Wu, 2001). In the simplest sense, reinforcement

occurs when hybrids have lower fitness cannot mate with parental populations. In this case, it is expected that two separate species will be maintained with a zone of hybridization between them. A third outcome exists in the case that hybrids are fertile and can mate with each other, then it is possible that they may diverge and eventually establish a new population or species (Abbott *et al.*, 2013).

The impact of hybridization on speciation in fungi can be considerably more complicated. A primary reason for this is the sexually facultative nature of many fungal species. In animals, if hybrid offspring are sterile then it is essentially the same as if the parental populations were completely incompatible. For organisms with both sexual and asexual life cycles, sterile hybrids can persist for indeterminate generations through clonal reproduction. This circumvents one of the mechanisms of reinforcement. Another unique feature of fungi as compared to animals is the relative low cost of reproduction. An animal may need to spend a significant amount of time and energy to bear offspring, which can promote the evolution of prezygotic barriers in the cases where those offspring are unfit. Sexual spores and structures in fungi are generally less costly, so there are weaker pressures to evolve these barriers, again removing a potential mechanism of reinforcement (Giraud *et al.*, 2008). These confounds would suggest that prezygotic barriers may occur less frequently in fungi than in animals, and in fact *Cryptococcus* exemplifies this. It is estimated that *C. neoformans* and *C. deneoformans* have diverged by 18 million years (Xu *et al.*, 2000a), yet they are still able to form viable hybrids. Compare this to the model animal genus *Drosophila*, in which certain members show approximately equal levels of incompatibility to each other as in *Cryptococcus*, but have only diverged for roughly 250,000 years (Fang *et al.*, 2012).

Natural and artificial hybridization has been observed across multiple lineages of yeasts. Strains of *Saccharomyces* are used for a large number of different commercial fermentation processes and hybrid strains have proven useful for everything from the production of lagers to that of vinegar (Morales and Dujon, 2012). This diverse utilization highlights the ability of hybridization to provide a selective advantage to strains in novel environments. Among pathogens, novel environments can translate into the infection of new host species or increased species ranges and there are numerous examples of this in plant pathogenic fungi (Schardl and Craven, 2003; Olson and Stenlid, 2002). Additionally, hybrids may display hypervirulence (Farrer *et al.*, 2011). While theory does support these observations, the genetic mechanisms that lead to these virulence trait are unknown.

In *Cryptococcus* there are three main virulence traits: growth at high temperature, the production of melanin, and the ability to produce an extracellular polysaccharide capsule (Kwon-Chung and Rhodes, 1986; Idnurm *et al.*, 2005). Although most strains possess all of these traits, most environmental isolates are in fact unable to cause disease (Litvintseva and Mitchell, 2009). Studies have suggested that as *cryptococcus* is not an obligate pathogen, these virulence traits likely evolved and are maintained for the purpose of defending against natural predators in its native environment (Steenbergen *et al.*, 2001; Casadevall *et al.*, 2003). It is unclear what genetic differences exist between disease causing and non-disease causing strains, but multiple lines of work suggest that they may be relatively few yet diverse changes (Beale *et al.*, 2015; Campbell *et al.*, 2015; Franzot *et al.*, 1998; Gupta and Fries, 2010; Hu *et al.*, 2011; Lin *et al.*, 2008; Morrow *et al.*, 2012; Movahed *et al.*, 2015).

Fungal infections appear to be on the rise (Fisher *et al.*, 2012). It is likely that increased globalization is introducing fungi to new environments around the world. This can have catastrophic outcomes, especially when naïve hosts in a new environment have close relatives in the fungi's native range as occurred with the etiological agent of white nose syndrome, *Pseudogymnoascus destructans* (Warnecke *et al.*, 2012). Such introductions can also result in secondary contact of previously allopatric populations and followed by hybridization. This has been demonstrated in the causal agent of dutch elm disease (Brasier, 2001), and may be behind the origin of AD hybrids in *Cryptococcus* (Litvintseva *et al.*, 2007).

The main goal of this thesis is to improve our understanding of hybridization in *Cryptococcus*. From previous work, we know that hybridization is ongoing, but the mechanisms remain poorly understood. Chapters 2 - 4 address the following three questions: Why are the progeny often aneuploid? Do any genetic incompatibilites exist between *C. neoformans* and *C. deneoformans*? And what are the genetic bases for the differential virulence traits noted between *C. neoformans* and *C. deneoformans*?

To answer the first question a tetrad dissection was performed to determine the number of genotypes produced from a single sexual structure. In the intraspecies cross, it has been demonstrated that one round of meiosis occurs per basidium, which in turn produces four separate haploid genotypes. Multiple mechanisms can cause aneuploidy after meiosis. These have their own unique signatures in the genotypes that they produce. By observing these, it is then possible to work backwards and make informed hypotheses about which specific processes might be occurring during

meiosis to lead to the hybrid individuals.

For the second question we scanned the genome to look for regions of incompatibility. The parental alleles should be present in equal proportions for any given locus if no incompatibilities exist. Therefor any pairwise combinations of alleles which do not show a 50:50 distribution of alleles is evidence for the existence of incompatible loci. These loci are predicted by evolutionary theory, but are often difficult to observe in practice.

The final question is centred around the fact that *C. neoformans* and *C. deneoformans* differ in clinical abundance. The difference is likely due to specific alleles, which can be uncovered through the use of quantitative genetics. Identification of virulence related QTLs should greatly expand our understanding of why *Cryptococcus* is able to cause disease. It is unclear what impact hybridization is having on the evolution and virulence of *Cryptococcus*, but this study should help us understand if hybridization could lead to increased virulence and burden of disease.

Chapter 2

Evidence for Mitotic Recombination within the Basidia of a Hybrid Cross of *Cryptococcus neoformans*

2.1 Preface

It has been known for sometime that hybridization between *C. neoformans* and *C. deneoformans* results in diploid/aneuploid offspring. Multiple authors have assumed that this is a result of non-disjunction during meiosis, but no detailed studies had been conducted. we conducted a hybrid cross between two well studied strains of *Cryptococcus*. Full tetrads were collected using a micro-manipulator. With this methodology, it is possible to determine how many genotypes are produced from a single mating structure. The results then allow us to infer the mechanism which is responsible for the abnormal ploidy of the hybrid progeny.

This work has been published as (Vogan *et al.*, 2013)

I am the primary contributor of this work. The majority of the experiments were conducted by me as well as the analyses and writing of the manuscript.

Abstract

In the majority of diploid eukaryotes, each meiotic process generates four haploid gametes with each containing a single recombinant nucleus. In some species and/or some meiotic processes, aneuploid or diploid gametes can also be generated due to chromosomal non-disjunction and/or the co-packaging of two of the four haploid nuclei into the same gamete. Here we show that another process is involved in generating genotypes of sexual progeny from a hybrid cross between two divergent lineages of the human fungal pathogen *Cryptococcus neoformans*. Through micro-dissection of 1358 basidiospores from 194 basidia and genotyping using 33 co-dominant genetic markers, the genotypes of all 230 germinated basidiospores from 94 basidia were obtained. The minimum haploid genotypes required to constitute the observed genotypes from each basidium were then inferred. Our results demonstrated that more than four haploid nuclear genotypes are required to explain the observed genotypes of basidiospores in seven of the 94 basidia. Our results suggest that mitotic recombination within basidia must be involved to produce the observed genotypes in these seven basidia. The mitotic recombination likely includes both chromosomal loss and crossing over. This novel recombination process could play an important role in generating the genotypic and phenotypic diversities of this important human pathogen.

2.2 Introduction

Since the first discovery of meiosis in the late 1800's in sea urchin eggs (Hertwig, 1876), the fundamental genetic features of meiosis have proven to be virtually universal for sexual reproduction throughout the Eukarya Domain (Hamoir, 1992). Specifically,

a typical meiosis involves one round of genome duplication, followed by pairing and crossing-over between homologous chromosomes and two rounds of reductive division to generate four genetically different nuclei with each having half of the nuclear genetic material as the original cell. Some of the cytological processes in meiosis vary among organisms. For example, in the fungal subkingdom Dikarya, which includes mushrooms, yeasts, and molds, sexual reproduction includes the dikaryotic phase – a binucleate hyphal structure formed from the fusion of two haploid monokaryotic individuals with compatible mating types. In most cases, the haploid nuclei remain separate in the dikaryon and only fuse to form a single diploid nucleus right before meiosis. The diploid nucleus then undergoes one replication and two reductive divisions to produce four haploid recombinant daughter nuclei, which are subsequently packaged into four sexual spores (Herskowitz, 1988; Raper and Raper, 1966).

Cryptococcus neoformans is a dimorphic basidiomyceteous fungus, consisting of a haploid, asexual yeast form and a dikaryotic, sexual filamentous form (Kwon-Chung, 1976a). It is an opportunistic human pathogen, infecting up to one million people a year (Park *et al.*, 2009). Its medical significance and ease of genetic manipulation in the laboratory have made *C. neoformans* a model organism for fungal pathogen research (Bauer *et al.*, 2012; Heitman, 2011). *C. neoformans* is composed of two varieties var. *grubii* and var. *neoformans*, which have been traditionally identified as serotype A and serotype D respectively based on their cell surface antigenic properties. Different from sexual reproduction in the majority of basidiomycetes, the production of four haploid daughter nuclei in each *C. neoformans* basidium is typically followed by multiple rounds of mitosis, with each haploid nucleus entering into one spore and each basidium bearing four chains of basidiospores. Interestingly, basidiospores from

each of the four chains are genetically heterogeneous, suggesting that haploid nuclei in each basidium are randomly distributed into the spores (Idnurm, 2010; Kwon-Chung, 1980). Analyses of micro-dissected chains of basidiospores from intra-variety crosses (i.e. between serotype A strains and between serotype D strains) have revealed that only four haploid genotypes are found for spores isolated from each basidium, consistent with the hypothesis that only one round of meiosis occurs in each basidium (Idnurm, 2010; Kwon-Chung, 1980).

Epidemiological surveys have identified that strains of serotype AD are commonly found in both environmental and clinical populations of *C. neoformans* (Ikeda *et al.*, 1982), (Brandt *et al.*, 1996b). In certain geographic regions, serotype AD strains can account for a significant percentage of clinical isolates (Brandt *et al.*, 1996b). Gene genealogical analyses have indicated that serotype AD strains are the results of recent hybridizations between strains of serotypes A and D (Xu *et al.*, 2002; Xu and Mitchell, 2003). Interestingly, in contrast to the haploid status for strains of serotypes A and D, most serotype AD strains are diploid or aneuploid and are heterozygous for at least some loci. These results suggested that sexual reproduction (i.e. meiosis and sporulation) in AD hybrids might be different from that in intra-varietal crosses between serotype A strains or between serotype D strains.

In the genetic analyses of a hybrid cross between strains JEC20 (serotype D, mating type a or MATa) and CDC15 (serotype A, MATα), 157 of the 163 analyzed progeny were found to contain at least one heterozygous locus out of the 114 screened co-dominant loci (Sun and Xu, 2007). The mean heterozygosity per locus was estimated at 74.81 % among the 163 progeny. The high level heterozygosity was attributed

to non-disjunction during meiosis (Sun and Xu, 2007). However, due to the random nature of the examined progeny population, other potential processes that might have contributed to the observed genotype diversity in the progeny could not be excluded.

The objective of this study is to analyze the processes that might be involved in generating progeny genotypes in a hybrid cross of serotypes A and D. To accomplish this, we dissected 1358 basidiospores from 194 basidia using a micromanipulator. The genotypes of the germinated spores were analyzed using 33 co-dominant genetic markers. Based on the observed genotypes from the germinated spores of each basidium, we infer the minimum number of haploid genotypes and the events that would be required in order to reconstitute the observed genotypes. Our analyses provided evidence for mitotic recombination within the basidia in the inter-variety hybrid cross. Furthermore, the results indicated that the mitotic recombination within the basidia likely included both chromosomal loss and crossing over.

2.3 Materials and Methods

2.3.1 Hybrid Progeny

Parental strains JEC20 (Serotype D, MATa) and CDC15 (Serotype A, MATα) were mated on V8 agar, following the same protocol as that described in Sun and Xu (2007) (Sun and Xu, 2007). After 1–4 weeks of incubation at 23 °C, basidiospores were collected through microdissection from individual basidia. Specifically, each entire mating spot containing hyphae and basidiospores was first cut from the V8-mating medium and transferred to a slightly bigger hole in a new plate containing the

yeast extract peptone dextrose (YEPD) medium. Chains of basidiospores from each individual basidium that were well - separated from other chains of basidiospores on other basidia were transferred to separately marked fresh spots on the YEPD medium using a micromanipulator (MSM System 300, Singer Instruments). Individual basidiospores were then picked and transferred to pre-determined spots on the agar to allow easy tracking of the relationships among basidiospores with respect to the dissected basidia. Basidiospores were incubated at 23 °C for up to 3 weeks to ensure that slow-germinating and/or slow-growing basidiospores could form colonies for genotyping. DNA was extracted from these colonies using the method described in Xu *et al.* (2000) (Xu *et al.*, 2000a).

2.3.2 Genotyping

A total of thirty-three co-dominant markers were used to genotype all progeny. These include 32 PCR-RFLP markers distributed on 4 chromosomes with 23 markers on Chromosome 1, 4 on Chromosome 3, 2 on Chromosome 4, and 3 on Chromosome 7. The reasons for including a large number of markers for Chromosome 1 were to: (i) help identify potentially multiple recombination breakpoints within individual basidia on one chromosome; (ii) reveal reciprocity of recombinant products at a fine scale; and (iii) investigate potential discordance between the physical map and the linkage map for the largest chromosome in the hybrid cross. Protocols for obtaining the PCR-RFLP genotypes followed that described in Sun and Xu (2007) (Sun and Xu, 2007). 15 of the 32 markers were the same as those used by Sun and Xu (2007) (Sun and Xu, 2007) while the remaining 17 markers were designed using `Prifi` (Fredslund *et al.*, 2005) based on the whole genome sequences of JEC21 and H99 (Table 2.S1).

In addition to the 32 PCR-RFLP markers, we also screened the mating types of these progeny using both the MATa and MATα –specific primer pairs located on Chromosome 4, following the protocol described in Yan *et al.* (2003) (Yan and Xu, 2003). The physical relationships among these markers in the JEC21 genome are shown in Figure 2.1. `MapMaker 3.0` was used to construct a linkage map for Chromosome 1. Individuals that showed an identical genotype to another basidiospore from the same basidium were excluded from the mapping population.

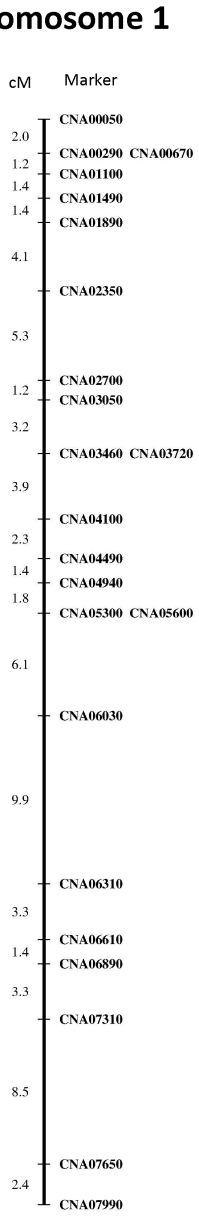

Figure 2.1: Linkage relationships among the examined PCR-RFLP markers on Chromosome 1.

Distances are in centi-Morgan, markers are evenly spaced with ∼1/100 kb. Markers on the same line had less than 1 cM distance between them. No recombination was observed between markers CNA05300 and CNA05600.

doi:10.1371/journal.pone.0062790.g001

2.3.3 Haplotype Inference

To infer the number of unique haploid genotypes from the observed genotypes for each basidium, we employed two different approaches. In the first, we assumed that any of the progeny that had at least one heterozygous locus were diploid and all loci containing an allele from only one parent were treated as homozygous at these loci (i.e. each with two identical alleles). The observed genotypes from each basidium were then analyzed with the `PHASE 2.1` program (Stephens and Scheet, 2005; Stephens *et al.*, 2001) to infer the minimum number of haploid genotypes that were required to explain the observed genotypes of each basidium. In the second approach, we assumed that only heterozygous loci had two alleles and that the loci with alleles from only one parent were hemizygous, containing only one allele at the specified locus. These genotypes were then used to infer the minimum set of haploid genotypes for each basidium. While the first approach would result in inferred haploid genotypes each with a whole complement of all chromosomes, the second approach would result in certain haploid genotypes with only a subset of the chromosomes.

2.3.4 Microscopy

To investigate whether individual basidiospores are uninucleated (monokaryotic) or binucleated (dikaryotic), basidiospores were stained and examined using fluorescence microscopy. Briefly, hyphae produced from mating spots (described above) were scraped with a loop and fixed to a slide. The slide was placed in a solution with DAPI and Calcofluor as described by Wickes *et al.* (1996) (Wickes *et al.*, 1996) for 30 min to stain the nuclei and cell walls respectively of the basidia and basidiospores for visualization.

2.4 Results and Discussion

A total of1358 basidiospores were individually picked using a micromanipulator and incubated on YEPD agar for up to three weeks. Each progeny that germinated and formed a colony on the agar medium was genotyped using the 33 co-dominant molecular markers. Below we describe the germination rates and genotypes of the progeny, followed by comparisons of the genotype data with those derived from two models that are known to generate atypical meiotic products.

2.4.1 Basidiospore Germination

Of the 1358 basidiospores that were micro-dissected from 194 basidia, 230 basidiospores (17 %) from 94 basidia germinated and grew into full colonies that could be genotyped and analyzed. Our overall germination rate was higher than the 5.5 % observed by Lengeler *et al.* (2001) (Lengeler *et al.*, 2001) in their meiotic progeny of a serotype AD strain. One factor that could have contributed to the difference in germination rates observed between our study and that by Lengeler *et al.* (2001) (Lengeler *et al.*, 2001) was the different parental strains used. Specifically, the parental strain that Lengeler *et al.* (2001) (Lengeler *et al.*, 2001) used was a self-fertile natural AD hybrid while ours were two self-sterile strains with different mating types. Their natural hybrid parent strain might be hemizygous for certain loci, lacking certain genetic complements from either the serotype A or D parental strains that could have contributed to a higher percentage of inviable basidiospores. In contrast, our zygotes would contain the whole complement of genetic materials from both the serotypes A (strain CDC15) and D (strain JEC20) parents.

Although a single basidium can potentially produce over 100 basidiospores (Idnurm, 2010), only 7 basidiospores were dissected for the majority of the basidia examined (159) (table 2.S2 shows the full distribution of the number of spores plated per basidium). This was done in an effort to obtain a genotypically diverse set of progeny from many basidia. Of the 94 basidia that contained germinated basidiospores, the number of basidiospores germinated ranged from 1 to 7 per basidium, with the mean and median of 2.4 and 2 respectively. Large differences in basidiospore germination rates were found among the dissected basidia. However, due to the relatively low number of basidiospores dissected from each basidium, germination rate comparisons among these basidia were not conducted.

2.4.2 Distributions of Observed Genotypes and Inferred Haplotypes

All 230 germinated basidiospores were genotyped at the 33 loci (Table 2.S3). The combined multilocus genotype data were used to determine the number of unique genotypes for each basidium. Our analyses identified that the largest number of unique genotypes from one basidium was 6 (Table 2.1), present in basidium 51 (Table 2.S3). For the remaining 93 basidia, two contained 4 genotypes each for their basidiospores, 17 contained 3 genotypes each, 27 contained 2 genotypes each, and 47 contained only 1 genotype each (Table 2.1). Genetic linkage analyses of the 23 markers on Chromosome 1 indicated that recombination was observed between all but one adjacent pair of loci (Figure 2.1) and that the order of the markers on this linkage group matched their relative physical positions on this chromosome. Our markers were placed with approximately 1 marker every 100 kb, yielding an average

of 2.9 cM/100kb.

			Number of basidia with the inferred number of unique haploid genotypes per basidium	
Observed number of unique genotypes per basidium	**Number of basidia with the observed number of genotypes**	**Inferred number of unique haploid genotypes per basidium**	**Assuming all progeny are diploid**	**Assuming hemizygous progeny are aneuploid**
1	47	1	9	9
2	26	2	48	48
3	18	3	20	20
4	2	4	8	10
6	1	5	5	5
		6	3	1
		8	1	1

doi:10.1371/journal.pone.0062790.t001

Table 2.1: Distribution of unique genotypes and inferred haplotypes across the 94 basidia that contained at least one successfully germinated basidiospore.
doi:10.1371/journal.pone.0062790.t001

The observed genotypes were then used to infer the minimum number of haploid genotypes that are needed to explain the observed genotypes for each basidium. Though there are differences in the specific allelic combinations of the inferred genotypes, the two different approaches showed very little difference in the minimum numbers of inferred haploid genotypes for each basidium (Table 2.1). Of the 94 basidia, three showed differences in the number of haplotypes needed to explain the observed genotypes: basidia 17, 92 and 137. For both basidia 17 and 92, the `PHASE` program based on diploid data inferred 6 haplotypes. In contrast, assuming the genotype data as hemizygous, we found that a minimum of 4 and 5 haplotypes respectively could explain basidia 17 and 92 (Table 2.S5). For basidium 137, the `PHASE` analysis returned a minimum of five haplotypes while the other analysis resulted in a minimum of four haplotypes. Overall, the hemizygous approach was more conservative (Table 2.1). Using the conservative hemizygous approach, 87 of the 94 basidia could be explained by having four or fewer haploid genotypes within each basidium. Of the remaining seven basidia, five (basidia 28, 74, 90, 92, and 111) each required a minimum of 5 haploid genotypes to explain their respectively observed genotypes; one (basidium 24) required 6 haploid genotypes to explain the three observed genotypes; and one (basidium 51) required 8 haploid genotypes to explain the six observed genotypes (Table 2.S5). The observed genotypes and inferred haplotypes for five basidia (basidia 24, 51, 74, 92 and 137) are shown in 2.2. Based on the information in Figure 2.2 and Table 2.S5, each observed genotype could be reconstituted by a maximum of two inferred haploid genotypes. For example, the genotype of JK45 in basidium 24 can be reconstructed from the haploid genotypes of H1 and H2.

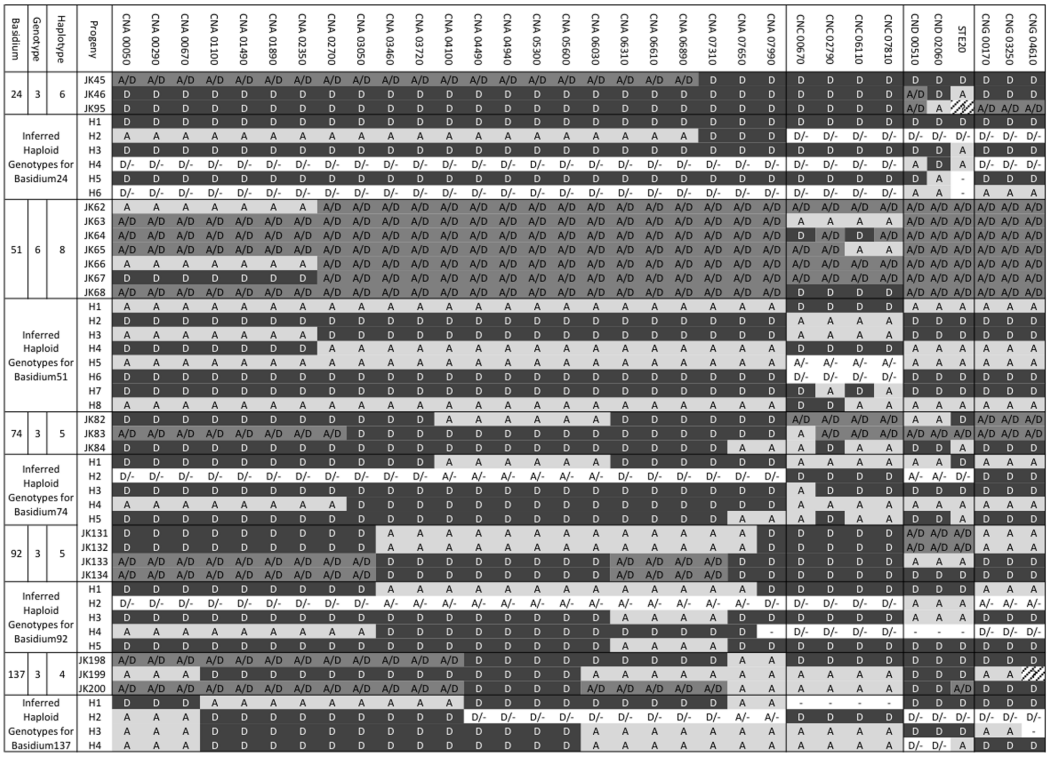

Figure 2.2: Examples of basidia with progeny genotypes that cannot be explained by the non-disjunction models and the nuclear co-packaging model. Nuclear locus information is presented in the first row and are arranged sequentially in the same order as that in Table 2.S1 and Figure 2.1 (for chromosome 1 markers). Allele "A" refers to alleles from the serotype A parent; "D" refers to alleles from the serotype D parent; "H" refers to a heterozygous locus containing both A and D alleles; "?" Refers to markers where no DNA fragment could be amplified in PCR after three attempts. H1 to H8 are the inferred haploid genotypes for the observed genotypes in the given basidium. When conflict exists between the two approaches for estimating the number of haplotypes (for basidia 17, 92, 137), only the conservative estimate (i.e. one based on the assumption of hemizygous state) is presented. "D/-" and "A/-" in the inferred haploid genotypes refer to two possibilities based on the assumptions used in our inferences: (i) the inferred haplotype has allele A or D if we assume all progeny are diploid; and (ii) the inferred haplotype doesn't have the locus if we assume that those with only one allele are hemizygous, "-" denotes chromosomes/markers which are not present, i.e. a full or partial chromosome loss has occurred. Evidence for mitotic chromosome crossing over is found for chromosome 1 of basidium 137, chromosome 3 of basidia 51 and 74, and chromosome 4 of basidium 24.
doi:10.1371/journal.pone.0062790.g002

Two processes have been previously found to generate atypical meiotic products in several eukaryotes: Non-disjunction (e.g. (Poss *et al.*, 2004; Chiavarino *et al.*, 2000; Sora *et al.*, 1982)) and meiotic nuclei co-packaging (e.g. (Kerrigan *et al.*, 1994; Summerbell *et al.*, 1989; Raper *et al.*, 1972)). Below we compare our observed results with the expectations of these two models.

2.4.3 Comparison of the Genotype Data to the Non-disjunction Models

Non-disjunction is an aberrant meiotic process known to cause abnormal ploidy. It occurs when one (or more) pair(s) of chromosomes fails to segregate during either meiosis I or meiosis II. Non-disjunction has been found in the generation of sexual progeny in a variety of eukaryotes, including many model organisms such as the Zebra fish (*Danio rerio*) (Poss *et al.*, 2004), maize (*Zea mays*) (Chiavarino *et al.*, 2000), and the Baker's yeast (*Saccharomyces cerevisiae*) (Sora *et al.*, 1982). While evidence for non-disjunction has not been reported for intra-variety crosses in *C. neoformans*, it has been suggested as the mechanism behind the diploidy/aneuploidy in the meiotic progeny of hybrid crosses between strains of serotypes A and D (Sun and Xu, 2007; Lengeler *et al.*, 2001).

Among the 94 basidia containing successfully germinated basidiospores, 47 had only one genotype each and these were not analyzed further for model comparisons because the limited information was insufficient to refute any of the models. The remaining 47 basidia with two or more observed basidiospore genotypes were individually compared to the expectations of the non-disjunction models to determine

whether these models could be the sole mechanism(s) to account for the observed genotypes for each basidium. We considered non-disjunction at both meiosis I and meiosis II. Specifically, if non-disjunction occurred during meiosis I, then recombinant non-sister chromatids should be found in the same basidiospore. In addition, each basidium should contain two reciprocal diploid genotypes or reciprocal aneuploidy genotypes for each of the four analyzed chromosomes. Furthermore, the presence of individual basidiospores revealing the same recombination events, but with different degrees of heterozygosity for a given chromosome would be inconsistent with the model. Of the 47 basidia, 17 could be explained by nuclear non-disjunction during meiosis I while 30 could not (Table 2.S3). If non-disjunction occurred during meiosis II, then only sister chromatids would be found together. Our analyses of the observed genotypes for individual basidia showed that 36 of the 47 basidia couldn't be explained by non-disjunction during meiosis II (Table 2.S3). In total, the genotypes for 30 of the 47 basidia could not be explained by either non-disjunction model (Table 2.S3).

2.4.4 Comparison of the Genotype Data to the Nuclear Co-Packaging Model

The nuclear co-packing model was proposed to describe sexual reproduction in the commercial button mushroom, *Agaricus bisporus* (Kerrigan *et al.*, 1994; Summerbell *et al.*, 1989; Raper *et al.*, 1972). This mechanism for generating atypical meiotic progeny refers to the process where the four haploid meiotic daughter nuclei are packaged as pairs into basidiospores, with a bias towards the pairing of non-sister nuclei into the same spore. Sister nuclei refer to the two daughter nuclei formed

during the second round of meiosis with both derived from the same nucleus generated after meiosis I. Thus the expectations of this model are that: (i) most basidiospores should each have two nuclei; (ii) the minimum number of inferred haplotypes from each basidium should not exceed four; and (iii) each basidium should have two main highly heterozygous genotypes. However, a recent study found that a high percentage of chromosomes/chromosomal segments in the hybrid progeny of the serotype A and D cross were homozygous (Sun and Xu, 2007). Thus, an alternative nuclear co-packaging scenario is also possible where the daughter nuclei are co-packaged at random. If the co-packaging is random, the four haploid daughter nuclei could be packaged to produce 6 potential diploid genotypes for the basidiospores from a single basidium. Furthermore, each of the four individual haploid nuclei could form a basidiospore and potentially generate a total of 10 different genotypes on a single basidium.

We compared the 47 basidia that contained two or more observed genotypes each to the expectations of nuclear co-packaging after meiosis. Among the 47 basidia, the co-packaging model (either random or non-random) could explain the genotypes of 40 basidia (Table 2.S3). However, the co-packaging model could not explain the observed genotypes of the remaining seven basidia, due to the greater than four haploid genotypes that were needed to reconstitute the observed genotypes from each of the seven basidia. Furthermore, over 1000 basidiospores were examined under the microscope and none were observed to have two nuclei (Figure 2.3). Of the 230 germinated and analyzed basidiospores, 64 were completely homozygous at all the 33 examined loci (Table 2.S3) while the remaining 166 were heterozygous for at least one of these loci. Assuming that the 64 basidiospore progeny were haploid (and thus uninucleated) and were representative of the overall spore population, we should

expect 72 % (166/230) of all examined basidiospores to be binucleated based on the co-packaging hypothesis but without nuclear fusion in the basidia). However, none were observed to be binucleated. Therefore, for this model to be correct, we would have to modify it to indicate that the co-packaged nuclei fused immediately after they were co-packaged into basidiospores and before their germination.

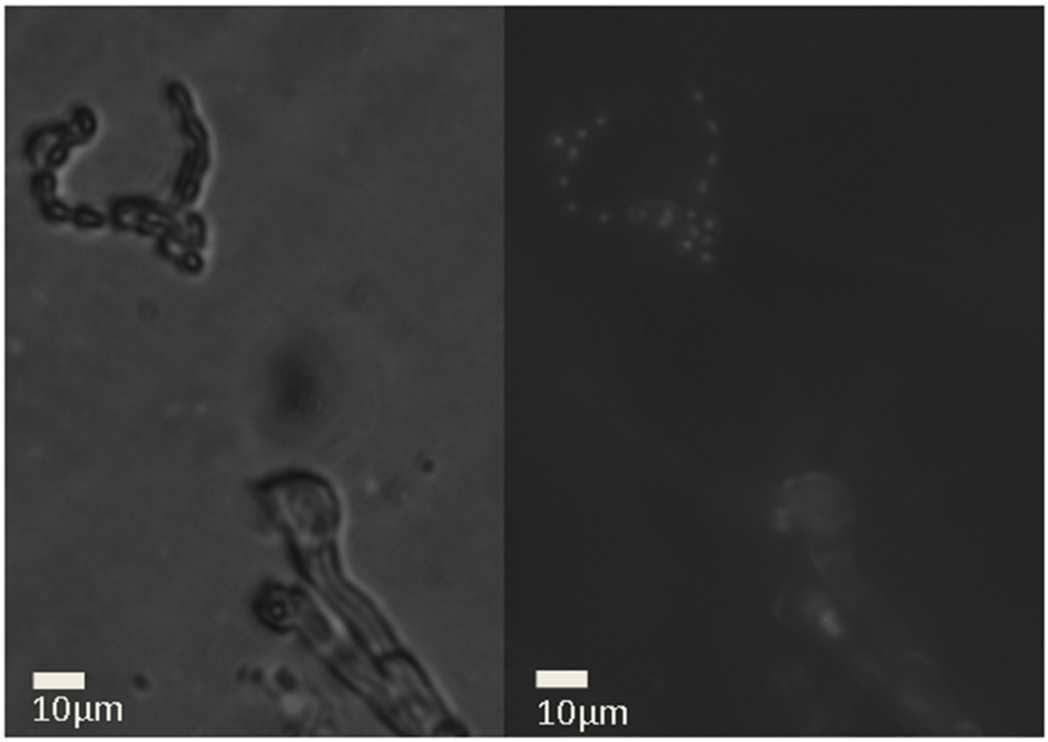

Figure 2.3: Lack of evidence for binucleated basidiospores in a hybrid cross between strains of serotypes A and D in *C. neoformans*.
Left: Standard light image. Right: DAPI and Calcofluor White fluorescence. Scale bar: 10 µm.
doi:10.1371/journal.pone.0062790.g003

In summary, based on the conservative approach, seven basidia each required more than four haploid genotypes to explain the observed genotypes of their basidiospores. Unless we assumed that complete or partial chromosome loss had occurred immediately after germination and that all cells with the ancestral genotype had died out prior to the DNA extraction, none of these seven basidia could be explained through either the non-disjunction or the co-packaging models proposed above. As the cells were only subcultured once after germination, we believe this scenario is extremely unlikely. Instead, we believe other mechanisms were likely involved to cause the observed genotypes and these mechanisms are discussed below.

2.4.5 Potential Mechanisms

In the first potential model, there might be more than one round of meiosis in each basidium in our hybrid cross. However, a previous study by Idnurm *et al.* (2010) (Idnurm, 2010) has shown that in intra-variety crosses between strains of serotype A in *C. neoformans*, a maximum of four haploid genotypes were found for basidiospores from each basidium and thus only one round of meiosis was needed in each basidium to generate their genotypes. Indeed, as far as we know, there has been no report of more than one round of meiosis for any taxon in the generation of an individual gamete. While plausible, this hypothesis would require an additional new regulatory pathway to initiate a second round of meiosis soon after the first one ended in the basidia. In addition, without other processes, the additional meiosis by itself could not generate the prevalent heterozygous genotypes observed here. Thus, we believe this model is not a good fit.

The second possibility involves both non-disjunction during meiosis I and mitotic recombination within nuclei after meiosis II but before the nuclei enter into basidiospores. In this model, there is non-disjunction during meiosis I in the basidia that would generate two diploid heterozygous nuclei at the end of meiosis II. During subsequent mitotic divisions that generate progeny nuclei for basidiospores, recombination could occur between non-sister homologous chromosomes which could produce a diversity of diploid/aneuploid genotypes for basidiospores (Figure 2.4).

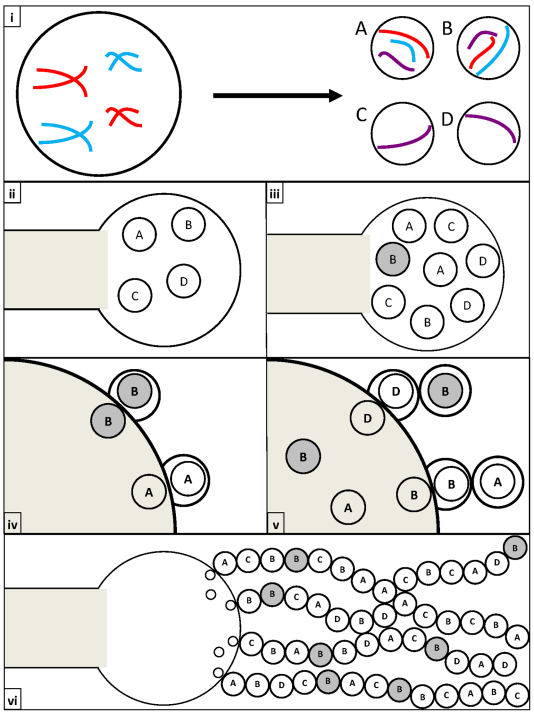

Figure 2.4: Non-disjunction followed by mitotic recombination within the basidium.
(i) Mis-segregation occurs during meiosis to produce some nuclei with additional chromosome copies (n+x) and some without the corresponding chromosomes (n-x). [Purple chromosomes represent copies which have undergone recombination]. (ii) & (iii) The four diploid/aneuploid daughter nuclei produce additional copies of themselves through mitosis. At this point some nuclei go through mitotic recombination (Grey circles) to form novel genotypes. (iv) & (v) The daughter nuclei migrate to the edge of the basidium where they go through an additional round of mitosis to produce a nucleus which is packaged into a basidiospore. (vi) Nuclei divide and stochastically migrate towards the basidiospores forming long heterogeneous chains of diploid/aneuploid basidiospores.
doi:10.1371/journal.pone.0062790.g004

The third possibility involves non-disjunction during meiosis II, and followed by nuclei fusion and mitotic recombination within the basidia. In this model, the non-disjunction during meiosis II would generate haploid nuclei with additional or missing chromosomes. These nuclei would then fuse within the basidia and during subsequent mitotic divisions to generate progeny nuclei for basidiospores. Recombination could occur between non-sister homologous chromosomes which could produce a diversity of diploid/aneuploid genotypes for basidiospores.

The fourth possibility involves a regular meiosis to generate four haploid nuclei within each basidium but followed by preferential fusion of non-sister haploid nuclei into two diploid nuclei, and then through mitosis to generate multiple diploid nuclei to enter into basidiospores. During replication of the diploid nuclei within the basidium, mitotic recombination could be involved to produce a diversity of genotypes, consequently leading to greater than four inferred haploid nuclear genotypes (Figure 2.5).

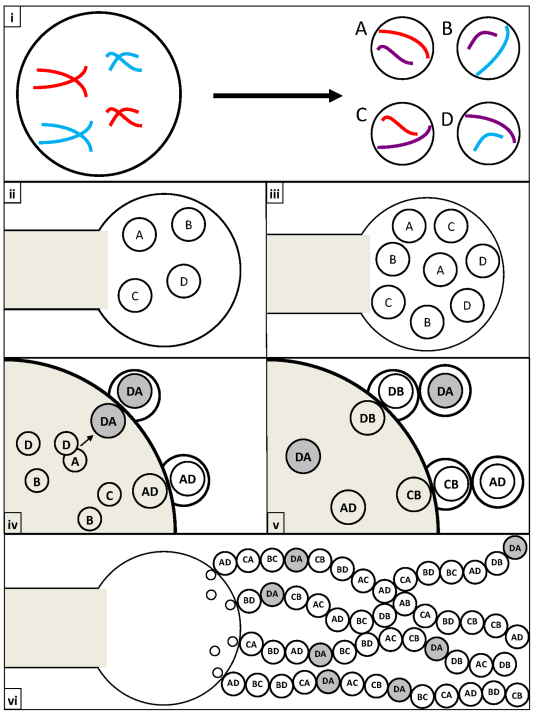

Figure 2.5: Non-random co-packaging and fusion of daughter nuclei followed by mitotic recombination within the basidium.
(i) Meiosis occurs properly to form four haploid daughter nuclei. [Purple chromosomes represent copies which have undergone recombination]. (ii) & (iii) The four haploid daughter nuclei produce additional copies of themselves through mitosis. (iv) & (v) The daughter nuclei fuse to form diploid nuclei. Subsequent rounds of mitosis produce multiple copies of the diploid nuclei. Through these mitotic divisions, recombinant genotypes (Grey circles) may be produced and packaged into basidiospores. (vi) Nuclei divide and stochastically migrate towards the basidiospores forming long heterogeneous chains of diploid/aneuploid basidiospores.
doi:10.1371/journal.pone.0062790.g005

Our current genotype data are unable to distinguish the proposed models 2, 3 and 4. Instead, we believe detailed cytological observations of nuclear and chromosomal dynamics within basidia are needed in order to understand the underlying processes involved in generating the diversity of genotypes. However, potential models 2, 3, and 4 all require mitotic recombination within the basidia, before the nuclei enter into basidiospores. Indeed, the proposed mitotic recombination could generate a large number of unique genotypes within a given basidium, regardless of their original ploidy and/or genotypic state after meiosis. If true, the result would suggest that the rate of meiotic recombination would likely be lower than what was determined by Sun *et al.* (2007) (Sun and Xu, 2007), which already showed a decreased in recombination as compared to the intra-variety cross.

2.4.6 Mitotic Recombination within Basidia Likely Includes both Chromosomal Loss and Crossing Over

Based on genotype comparisons, the inferred mitotic recombination within basidia likely included both chromosomal loss and crossing over. Differences in the patterns of heterozygosity among the chromosomes within many of the individual basidiospores are consistent with chromosomal losses (Tables 2.S3 and 2.S5). However, to identify mitotic chromosome crossing over within individual basidia, we looked for basidium that contained more than four reciprocal haploid genotypes for an individual chromosome. Four reciprocal haploid genotypes per chromosome are the maximum of what we should expect from a single round of meiosis and deviations from this expectation would be consistent with mitotic chromosomal crossing over within the basidium.

Our screening revealed that four basidia (24, 51, 74, and 137, Figure 2.2) each had one chromosome that contained more than four reciprocal haploid nuclear genotypes (inferred haploid genotype plus the reciprocals of the inferred haploid genotypes if the reciprocals are not present in the inferred genotype group already). Specifically, Chromosome 4 (represented by three CND markers) in basidium 24 contained five inferred haploid genotypes plus five reciprocals of the inferred five; Chromosome 3 (represented by four CNC markers) in basidium 51 contained four inferred plus 2 additional reciprocals; Chromosome 3 in basidium 74 contained four inferred plus 2 additional reciprocals; and Chromosome 1 in basidium 137 contained three inferred and three additional reciprocals) (Figure 2.2 and Figure 2.6).

Basidium	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990
84	JK99 - 1	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	JK100	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
	JK100 - 1	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
	JK100 - 2	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
137	JK198	H	H	H	H	H	H	H	H	H	H	H	H	D	D	D	D	D	D	D	D	D	A	A
	JK199	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A
	JK200	H	H	H	H	H	H	H	H	H	H	H	H	D	D	D	D	H	H	H	H	H	A	A
	JK200 - 1	H	H	H	H	H	H	H	H	H	H	H	H	D	D	D	D	H	H	H	H	H	A	A
	JK200 - 2	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A
	137-H1	D	D	D	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	A	A
	137-H2	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A
	137-H3	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A
	137-H4-cH1	A	A	A	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	D	D
	137-H5-cH2	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D
	137-H6-cH3	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D

Figure 2.6: Evidence for reciprocity of recombinant haploid genotypes (basidium 84) and for mitotic chromosomal crossing-over within Chromosome 1 (basidium 137).

JK100-1 and JK100-2 were two subcultures from a single basidiospore (JK100) of basidium 84. Basidiospore JK100 was completely heterozygous for all the marker loci on chromosome 1. However, analyses of the two subcultures identified that the two subcultures showed reciprocal genotypes for chromosome 1 with JK100-2 showing an identical genotype to the genotype of a different spore JK99. JK200-1 and JK200-2 were two subcultures from a single basidiospore JK200. Four recombination breakpoints were identified for chromosome 1 within this basidium and a minimum of three Chromosome 1 haploid genotypes (137-H1 to 137-H3) was inferred for this basidium to explain the observed genotypes. However, meiosis generates reciprocal recombinant genotypes. Thus, three additional Chromosome 1 genotypes are further inferred. Haploid genotypes 137-H4-cH1, 137-H5-cH2, and 137-H6-cH3 are the reciprocal genotypes for 137-H1, 137-H2, and 137-H3 genotypes respectively. Since each round of meiosis generates a maximum of four haploid nuclear genotypes, mitotic chromosomal crossing-over must be involved to produce the six haploid genotypes chromosome 1.

doi:10.1371/journal.pone.0062790.g006

An example of reciprocal chromosome genotype inference is shown in Figure 2.6 for basidium 137. In this basidium, basidiospore JK200 was subcultured and two segregant colonies were genotyped for Chromosome 1. Subculture JK200-1 was found to have an identical genotype to JK200 while JK200-2 showed the loss of heterozygosity on chromosome 1 (Figure 2.6; Table 2.S5). This loss of heterozygosity allowed us to infer the exact allelic composition of the two copies of Chromosome 1 within the original basidiospore JK200 as well as the minimum number of haploid genotypes that are required to reconstitute the observed genotypes for basidium 137. Our analyses identified a total of four recombination breakpoints and a minimum of three haploid genotypes for chromosome 1 within basidium 137 (Figure 2.6). However, none of these three haploid genotypes represented reciprocal genotypes of each other (as would be expected from meiosis) and three other reciprocal haploid genotypes were thus required to fully reconstitute the meiotic process. These observations are thus consistent with mitotic chromosome crossing over within this basidium that contributed to generating the six haploid genotypes for chromosome 1.

Direct evidence for reciprocity of recombinant genotypes was found in a progeny from basidium 84 that originally typed ambiguously, i.e. multiple markers on chromosome 1 showed heterozygosity but with one of the two alleles (DNA fragments on the gel) being weaker than the other allele at all the marker loci on this chromosome. The progeny (JK100) was subcultured and the resulting pure subcultures were re-typed for markers on chromosome 1, showing the presence of two genotypes (Figure 2.6). Specifically, JK100-1 and JK100-2 were purified from the same colony formed by a single basidiospore and they were found to possess reciprocal genotypes at chromosome 1. Interestingly, subculture JK100-2 had the same genotype at chromosome 1

as an independent progeny JK99-1 from the same basidium, which strongly suggests that the reciprocity was generated during meiosis and that chromosome 1 of JK100-1 and JK100-2 were likely co-packaged together into the same basidiospore JK100. Alternatively, mitotic crossing over after germination could also give rise to the reciprocal genotypes. However, this scenario is very unlikely and would require that the meiotic recombination (as shown in basidiospore JK99) within the basidium and the mitotic recombination after germination (for basidiospore JK100) to happen within the same chromosomal region between adjacent markers CNA01890 and CNA02350.

We would like to note that for several reasons, the observed and inferred numbers of genotypes shown here were likely underestimates of the true genotype diversities and recombination processes. First, only four of the 14 chromosomes were genotyped and with the majority of the markers on one chromosome. If more markers were genotyped on these four chromosomes as well as with markers on the remaining 10 chromosomes, more genotypes would likely be found for spores from each basidium. Second, basidiospores that did not germinate likely contained other genotypes that we had missed from all basidia. Third, at present, the inferred haploid genotypes for each basidium were the minimum set required to re-constitute the observed genotypes. A greater number of haplotypes could have existed for these and other spores from each basidium. More detailed analyses using more markers will likely increase the number of observed and inferred genotypes substantially and lead to greater evidence for mitotic recombination within these basidia.

Since early in their discovery, it has been suspected that the genomes of the AD hybrids could undergo mitotic recombination to generate novel genotypes, as serotyping

of isolates after subsequent sub-culturing could lead to different results (Brandt *et al.*, 1996a). If chromosome loss occurs through mitotic recombination within the basidium, it is possible that the same mechanisms could revert AD hybrids to serotype A or D during sporulation. This would imply that the genomes of AD hybrids are inherently flexible as opposed to varying solely in response to stress and selective pressure. In addition, mitotic recombination within basidia could potentially subvert any mechanisms which may have evolved to suppress recombination during meiosis between the two varieties, preventing the varieties from undergoing full speciation within sympatric populations and increasing the viability and genetic diversity of recombinant hybrids.

2.5 Supporting Information

Table 2.S1: Information for the 33 genetic loci analyzed in this study. Column A: marker code (1–33); column B: locus code/name as annotated in the *C. neoformans* var. *neoformans* (serotype D) strain JEC21 genome; column C: primer sequences; columns D: locus location on the annotated *C. neoformans* var. *grubii* (serotype A) strain H99 genome; column E: size of amplified fragment for the parent strain CDC15; columns F: locus location on the annotated *C. neoformans* var. *neoformans* (serotype D) strain JEC21 genome; column G: size of amplified fragment for the parent strain JEC20; column H: restriction enzyme used to distinguish the parental alleles.
doi:10.1371/journal.pone.0062790.s001

ID	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Size D (bp)	Enzyme
CNA00050F	AGCAATTCCAAACCGACCCC	31347	1440	17738	1436	HaeIII
CNA00050R	TTACGCCCACACCAAGGCAT	29908		19173		
CNA00290F	AAACTCGCGGTTGCCAATCC	120407	1170	95921	1205	SacI
CNA00290R	TTGGAGGATGCAGGCGGTTT	121576		97125		
CNA00670F	TTGGGCCAGGAGTGAGTGAT	213956	1316	192305	1309	HaeIII
CNA00670R	AAGGCAGAAAGAGCCGCGTT	215271		193613		
CNA01100_2F	GCAAAGACTGACACCAGCGACC	322838	1060	298164	1061	HaeIII
CNA01100_2R	CTGCCTCTTTTCCAAGCACAGC	323897		299224		
CNA01490F	AGGCAGGCGCGTCAGCTTTTTG	438028	1303	401640	1320	HaeIII
CNA01490R	AAACAGGCGACCATTGCGGGAG	439330		402959		
CNA01890F	ATCACGCAACCCGTTGCCAT	543126	1227	508335	1230	SacI
CNA01890R	TGCCTTGGCGTTGGCTCTTT	544352		509564		
CNA02350F	CGAGGCTTTGAGAGGATGGGG	643257	1156	613955	1167	HindIII
CNA02350R	CCGCTCGAACATGTACTCACCC	644412		615121		
CNA02700F	TTGGATGTTGTCCATCCCGC	729033	1368	703078	1368	EcoRI
CNA02700R	TCAAGACCGGCATTGTCGCA	730400		704445		
CNA03050F	CCCCCATTGTCGCCAAACAT	819061	1183	795229	1178	HaeIII
CNA03050R	AAGCAATTCCCCACGGCGAT	820243		796406		
CNA03460F	CCACGGTTGGGCATGAACA	911508	1667	900629	1667	HaeIII
CNA03460R	TTGCCTGGCTATCCATTC	909842		902295		
CNA03720F	GCTGAGAGGTGCCCAGTTGAG	1008429	1177	999753	1170	HinfI
CNA03720R	ACAGCTTGATCGTGCCATCAGG	1009605		1000922		
CNA04100F	CCACCAGCGCAAAAACGAC	1109343	1726	1100766	1748	HindIII
CNA04100R	TGATGCGCCGCCTTTCTCTT	1111068		1102513		
CNA04490F	TGCCCGACCTCCAAAAGCAGAC	1211780	1225	1203345	1222	HindIII
CNA04490R	CCAGATGCCATCACGATCCACG	1213004		1204566		
CNA04940F	GCTCAAAAAGACCAGCCGCTTC	1305670	1023	1307790	1026	PvuII
CNA04940R	GGACCTGCTAGCGTTGTGAGAG	1306692		1308815		
CNA05300F	TCGGACAGCAAGTGAGTCCAGG	1399036	1097	1406625	1096	HaeIII
CNA05300R	TAAGCCTGTTGAGCGAATCGGG	1400132		1407720		
CNA05600F	TGGGTTTGGCTTCAGGCAG	1484100	1068	1500087	1068	HaeIII
CNA05600R	CCGCTCTTCACATGCAGCAA	1485167		1501154		
CNA06030F	GGGTTACCAGCGGTGGCGATC	1596113	1099	1612358	1096	PstI
CNA06030R	GTGACGGCCAACCCTGAAGGAG	1597211		1613453		
CNA06310F	CGTATCCAGCGGCTGACCAC	1696056	1100	1715148	1105	PvuII
CNA06310R	AATGGTCGCTAGACTGGACGGG	1697155		1716252		
CNA06610F	GCCTCAATTCACGCCCAGACCC	1776337	1126	1803026	1123	SacI

ID	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Size D (bp)	Enzyme
CNA06610R	CTCCTTCGCTCCCTTCTTCGTC	1777462		1804148		
CNA06890-F	CGTGCCCTTCCCCAAACAAT	1853447	1453	1879077	1455	BamHI
CNA06890-R	TCATTGTGCGAGATGCCTGC	1851995		1880531		
CNA07310F	ACACCCCAAATTCCCCAACC	1973202	975	2000368	975	PstI
CNA07310R	ACATCCCAAACGAACCCAC	1974176		2001342		
CNA07650F	AGAAGAGCATGGCAGCGGAA	2073146	1344	2098757	1352	HinfI
CNA07650R	ATGTCGTCGTCGTTTCGCCC	2074489		2100108		
CNA07990F	TCCAATGGACGAGGACGATG	2177600	1430	2200900	1424	HinfI
CNA07990R	TGACCGGTGTGGGTTGCAAT	2179029		2202323		
CNC00670F	TGTGCGGCTTTGGGATTGGT	195225	1110	191360	1108	HaeIII
CNC00670R	TTGCAGGTATGGCCGAATGG	196334		192467		
CNC02790F	GATGAAATGGCGAGGACGCA	866572	1469	799711	1470	EcoRI
CNC02790R	TTCCGCGTTGCAACACAACC	868040		801180		
CNC06110F	AAGGGCGTTGATCCGGCAAT	235559	1404	1795499	1403	HaeIII
CNC06110R	TTCCCCAGGTCGTTTGGGAT	236962		1796901		
CNC07180F	TGGAGGCGTTGGGCGAAATAGAG	15726	1316	2100876	1309	HaeIII
CNC07180R	TTCAGCCGTCGCCTTTACCACAA	17041		2102184		
CND00510F	CGGTGCCGCTTTATTTGTGGC	1650153	1325	150300	1327	HinfI
CND00510R	TCTAGCGCCAAAGCGTGCAAG	1651477		151626		
CND02060F	TCAGGTTCAAACCGCCAGCA	1261617	1223	553800	1222	HaeIII
CND02060R	AGTTTTGCCCCGCTTCGCTTGC	1262839		555021		
STE20DaF	GATCTGTCTCAGCAGCCAC	-	-	-	440	-
STE20DaR	AATATCAGCTGCCCAGGTGA	-		-		
STE20AaF	CCAAAAGCTGATGCTGTGGA	-	588	-		-
STE20AaR	AGGACATCTATAGCAGAT	-		-		
CNG00170F	TTTCTTCCGCCGCTTCTCAC	1348500	1245	42764	1232	HaeIII
CNG00170R	ACAGCGCGTTGAGTTTCGGT	1349744		43995		
CNG03250F	TTGCCAATAACGTGGCACGG	444807	1424	916601	1423	HindIII
CNG03250R	AAAGGGAGGCGGCTGATGATA	446230		918023		
CNG04610F	TGTTTCCCACAGGCCAAGGACT	59766	1466	1308769	1514	HinfI
CNG04610R	CGTGCGCGAATGCATCGATAT	61231		1310282		

Table 2.S2: Summary information for the numbers of basidiospores dissected and germinated from the 194 basidia.
doi:10.1371/journal.pone.0062790.s002

Number of basidiospores dissected per basidium	Total number of basidia dissected in each spore number category	Number of basidia in each catagory without any germinated basidiospores	Number of basidia in each category with germinated basidiospores	Range of germination rate for basidia with germinated spores in each category
1	2	2	0	-
2	3	3	0	-
3	1	1	0	-
4	2	2	0	-
5	2	1	1	3
6	13	6	7	1-5
7	159	78	81	1-7
8	4	2	2	1-3
10	3	2	1	4
11	2	1	1	1
12	1	0	1	4
17	1	1	0	-
24	1	1	0	-

Table 2.S3: Genotypes of all 230 germinated basidiospores. The genotype data are organized based on their basidium affiliation. Also included in the table are the number of genotypes observed for spores from each basidium (column B), the number of inferred haplotypes based on the observed genotypes of basidiospores (column C), and whether the known meiotic mechanisms could explain the observed and inferred genotypes (Columns D, E, and F). Y: the known mechanism can explain the observed results; N: the specific mechanism cannot explain the observed results. The details of the three mechanisms are discussed in the Main Text.

doi:10.1371/journal.pone.0062790.s003

Basidium	Number of Genotypes	Genotypes Number of Haploid	Meiosis I Model Non-Disjunction	Meiosis II Model Non-Disjunction	Co-Packaging Model	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490
2	3	3	N	N	Y	JK1	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK2	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK71	D	D	D	D	D	D	D	D	D	D	D	D	D
3	3	4	N	N	Y	JK3	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK4	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK5	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK13	D	D	D	D	D	D	D	D	D	D	D	D	D
6	1	1	Y	Y	Y	JK71	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK8	D	D	D	D	D	D	D	D	D	D	D	D	D
7	1	2	Y	Y	Y	JK6	D	D	D	D	D	D	D	D	D	D	D	D	D
10	2	3	Y	N	Y	JK14	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK80	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
13	1	2	Y	Y	Y	JK9	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK10	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK11	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK12	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
14	1	2	Y	Y	Y	JK20	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D
17	4	4	N	N	N	JK15	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK19	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK21	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK22	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
18	1	1	Y	Y	Y	JK81	A	A	A	A	A	A	A	A	A	A	A	A	A
19	1	2	Y	Y	Y	JK37	A	A	A	A	A	A	A	A	A	A	A	A	A
22	1	2	Y	Y	Y	JK38	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK39	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK40	A	A	A	A	A	A	A	A	A	A	A	A	A
23	3	3	N	N	Y	JK41	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK42	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK43	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK44	A	A	A	A	A	A	A	A	A	A	A	A	A
24	3	6	N	N	N	JK45	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK46	D	D	D	D	D	D	D	D	D	D	D	D	D

Basidium	Number of Genotypes	Genotypes Number of Haploid	Meiosis I Model	Non-Disjunction Meiosis II Model	Non-Disjunction Model	Co-Packaging	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490
							JK95	D	D	D	D	D	D	D	D	D	D	D	D	D
27	2	2	Y	Y	Y		JK47	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK48	D	D	D	D	D	D	D	D	D	D	D	D	D
28	4	5	N	N	N		JK16	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK17	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK18	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK24	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK25	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK26	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK148	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
29	1	1	Y	Y	Y		JK149	D	D	D	D	D	D	D	D	D	D	D	A	A
30	3	3	N	N	Y		JK27	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK28	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK29	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK30	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK31	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK32	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK98	D	D	D	D	D	D	D	D	D	D	D	D	D
34	2	3	Y	Y	Y		JK33	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK34	A	A	A	A	D	D	D	D	D	D	D	D	D
35	1	2	Y	Y	Y		JK35	D	A/D	A/D	A/D	A/D	A/D	D	D	D	A/D	A/D	A/D	D
37	2	2	Y	Y	Y		JK72	A	A	A	A	A	A	A	A	A	A	A	D	D
							JK73	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK74	D	D	D	D	D	D	D	D	D	D	D	D	D
40	1	2	Y	Y	Y		JK75	D	D	D	D	D	D	D	D	D	D	D	D	D
42	1	2	Y	Y	Y		JK76	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D
44	1	1	Y	Y	Y		JK77	A	A	A	A	A	A	A	A	A	A	A	A	A
45	1	2	Y	Y	Y		JK78	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D		A/D	A/D	?	A/D
							JK79	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
46	3	4	Y	N	Y		JK59	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK60	A	A	A	A	A	A	A	A	A	A	A	A	A
							JK118	A	A	A	A	A	A	A	A	A	A	A	A	A
48	1	2	Y	Y	Y		JK61	D	D	D	D	D	D	D	D	D	D	D	D	D
51	6	8	N	N	N		JK62	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D
							JK63	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK64	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK65	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
							JK66	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D
							JK67	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D
							JK68	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
53	2	3	Y	Y	Y		JK69	A	A	A	A	A	A	A	A	A	A	A	A	A
							JK70	D	D	D	D	D	D	D	D	D	D	D	D	D
56	2	3	Y	Y	Y		JK120	D	D	D	D	D	D	D	D	D	D	D	D	D

Basidium	Number of Genotypes	Genotypes Number of Haploid Meiosis I Model	Non-Disjunction Meiosis II Model	Non-Disjunction Model	Co-Packaging	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490
						JK121	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK122	D	D	D	D	D	D	D	D	D	D	D	D	D
57	1	1	Y	Y	Y	JK123	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK23	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK49	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK50	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
58	3	3	N	N	Y	JK51	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK52	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK53	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK54	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK55	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK56	D	D	D	D	D	D	D	D	D	D	D	D	D
59	3	4	N	N	Y	JK57	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK58	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK124	A	A	A	A	A	A	A	D	D	D	D	D	D
60	1	1	Y	Y	Y	JK125	D	D	D	D	D	D	D	D	D	D	D	D	D
62	1	2	Y	Y	Y	JK126	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK127	D	D	D	D	A	A	A	A	A	A	A	A	D
63	2	3	Y	Y	Y	JK128	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK129	D	D	D	D	A	A	A	A	A	A	A	A	D
64	1	2	Y	Y	Y	JK147	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK150	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK151	D	D	D	D	D	D	D	D	D	D	D	D	D
65	2	2	Y	Y	Y	JK152	D	D	D	D	D	D	D	D	D	D	D	?	D
						JK153	D	D	D	D	D	D	D	D	D	D	D	?	D
67	1	2	Y	Y	Y	JK154	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK155	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
68	2	3	N	N	Y	JK156	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK157	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK158	D	D	D	D	D	D	D	D	D	D	D	D	D
69	2	4	N	N	Y	JK159	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK160	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK82	D	D	D	D	D	D	D	D	D	D	D	A	A
74	3	5	N	N	N	JK83	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D
						JK84	D	D	D	D	D	D	D	D	D	D	D	D	D
75	2	3	Y	N	Y	JK85	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK116	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK86	D	D	D	D	D	D	D	D	D	D	D	D	D
76	2	2	N	N	Y	JK87	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	?	A/D
						JK88	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
77	1	2	Y	Y	Y	JK119	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK36	D	D	D	D	D	D	D	D	D	D	D	D	D
79	2	3	Y	Y	Y	JK89	D	D	D	D	D	D	D	D	D	D	D	?	D

Basidium	Number of Genotypes	Genotypes Number of Haploid	Meiosis I Model	Non-Disjunction Meiosis II Model	Non-Disjunction Model	Co-Packaging Model	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490
							JK90	D	D	D	D	D	D	D	D	D	D	D	D	D
							JK117	D	D	D	D	D	D	D	D	D	D	D	D	D
81	1	2	Y	Y	Y	JK91	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
83	1	2	Y	Y	Y	JK96	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK97	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
84	2	3	Y	N	Y	JK99	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK100	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
85	3	4	N	N	Y	JK101	D	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK102	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK103	D	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK104	D	D	D	D	D	D	D	D	D	D	D	D	D	D
86	2	4	N	N	Y	JK105	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A
						JK106	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
87	1	2	Y	Y	Y	JK107	D	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK108	D	D	D	D	D	D	D	D	D	D	D	D	D	D
90	3	5	N	N	Y	JK92	D	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK93	D	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK94	D	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK109	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
91	1	2	Y	Y	Y	JK130	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
92	3	5	N	N	N	JK131	D	D	D	D	D	D	D	D	D	A	A	A	A	A
						JK132	D	D	D	D	D	D	D	D	D	A	A	A	A	A
						JK133	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D
						JK134	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D
93	3	4	N	N	Y	JK135	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK136	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK137	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK138	A	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK139	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK140	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
94	1	2	Y	Y	Y	JK141	A	A	A	A	A	A	D	D	D	D	D	D	D	D
95	2	3	Y	Y	Y	JK142	A	A	A	A	A	A	A	A	A	D	D	D	D	D
						JK143	D	D	D	D	D	D	D	D	D	D	D	D	D	D
96	2	2	Y	Y	Y	JK144	D	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK145	A	A	A	A	A	A	A	A	A	A	A	A	A	A
99	1	2	Y	Y	Y	JK146	A	A	A	A	A	A	A	A	A	A	A	A	A	A
100	1	2	Y	Y	Y	JK110	D	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK111	D	D	D	D	D	D	D	D	D	D	D	D	D	D
101	1	1	Y	Y	Y	JK112	A	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK113	A	A	A	A	A	A	A	?	A	A	A	A	A	A
						JK114	A	A	A	A	A	A	A	A	A	?	A	A	A	A
104	1	2	Y	Y	Y	JK115	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK170	D	D	D	D	D	D	D	D	D	D	D	D	D	D
111	3	5	N	N	N															

Basidium	Number of Genotypes	Genotypes Number of Haploid Meiosis I Model	Non-Disjunction Meiosis II Model	Non-Disjunction Model	Co-Packaging	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490
						JK171	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK172	D	D	A	A	A	A	A	A	A	A	A	A	A
113	1	2	Y	Y	Y	JK173	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D
						JK174	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D
114	3	3	N	N	Y	JK175	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK176	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK177	D	D	D	D	?	D	D	D	D	D	D	D	D
						JK178	D	D	D	D	D	D	D	D	D	D	D	D	D
118	2	4	Y	N	Y	JK161	A	A	A	A	A	A	A	D	D	D	D	D	D
						JK162	A	?	A	A	?	A	?	D	D	D	D	?	D
						JK163	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D
121	1	2	Y	Y	Y	JK164	D	D	D	D	D	D	D	D	D	D	D	D	D
122	1	2	Y	Y	Y	JK165	A	A	A	A	A	D	D	D	D	D	D	D	D
123	1	2	Y	Y	Y	JK166	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
126	3	3	N	N	Y	JK167	A	A	A	A	A	A	A	A	D	D	D	D	D
						JK168	A	A	A	A	A	A	A	A	D	D	D	D	D
						JK169	A	A	A	A	A	A	A	A	D	D	D	D	D
132	1	1	Y	Y	Y	JK179	A	A	A	A	A	A	A	A	A	A	A	A	A
134	2	2	Y	Y	Y	JK180	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK181	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK182	D	D	D	D	?	D	D	D	D	D	D	D	D
135	2	2	N	N	Y	JK183	A	D	D	D	D	D	A	A	A	D	D	D	D
						JK184	A	D	D	D	D	D	A	A	A	D	D	D	D
136	1	1	Y	Y	Y	JK197	D	D	D	D	D	D	D	D	D	D	D	D	D
137	3	4	N	N	Y	JK198	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D
						JK199	A	A	A	D	D	D	D	D	D	D	D	D	D
						JK200	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D
141	1	2	Y	Y	Y	JK201	D	D	D	D	D	D	?	D	D	D	D	D	D
146	2	3	N	N	Y	JK190	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A
						JK191	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A
						JK202	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A	A	A	?	A
						JK203	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A
149	2	2	Y	N	Y	JK192	A	A	A	A	A	A	A	A	A	A	A	A	A
						JK193	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK194	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
151	1	2	Y	Y	Y	JK195	A	A	A	A	A	A	A	A	A	A	A	A	A
152	1	2	Y	Y	Y	JK196	A/D	A/D	D	D	D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D
157	1	2	Y	Y	Y	JK185	D	D	D	D	D	D	D	D	D	D	D	D	D
						JK186	D	D	D	D	D	D	?	D	D	D	D	D	D
159	2	3	N	N	Y	JK187	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
						JK188	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
162	1	2	Y	Y	Y	JK189	D	D	D	D	D	D	D	D	D	D	D	D	D

Basidium	Number of Genotypes	Number of Haploid Genotypes	Meiosis I Model	Non-Disjunction Meiosis II Model	Non-Disjunction Model	Co-Packaging Model	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490
167	1	2	Y	Y	Y		AV1	D	D	D	D	D	D	D	D	D	D	D	D	D
176	1	2	Y	Y	Y		AV2	D	D	D	D	D	D	D	D	D	D	D	?	A/D
							AV3	D	D	?	D	D	D	D	D	D	D	D	D	A/D
							AV4	D	D	D	D	?	D	D	D	?	D	D	D	A/D
							AV5	D	D	D	D	D	D	D	D	D	D	D	D	A/D
177	2	2	N	N	Y		AV6	A	A	A	A	A	A	A	A	A	A	?	D	D
							AV7	A	A	A	A	A	A	A	A	A	A	A	D	D
							AV8	A	A	A	A	A	A	A	A	A	A	A	D	D
							AV9	A	A	A	A	A	A	A	A	A	A	A	D	D
179	1	2	Y	Y	Y		AV10	A	A	A	A	A	A	?	A	A	A	A	?	A
180	1	2	Y	Y	Y		AV11	D	D	D	D	D	D	D	D	?	D	D	D	A
187	1	2	Y	Y	Y		AV12	D	D	D	D	D	D	D	A	A	A	A	A	A
							AV13	D	D	D	D	D	D	D	A	A	A	A	A	A
							AV14	D	D	D	D	D	D	D	A	A	A	?	?	A
189	2	3	N	N	Y		AV15	D	D	D	D	D	D	D	D	?	D	D	D	D
							AV16	D	D	D	D	D	D	D	D	D	D	D	D	D
190	1	2	Y	Y	Y		AV17	A	A	A	A	A	A	A	A	?	A	A	A	A
194	3	3	N	N	Y		AV18	A	A	?	A	A	A	A	A	?	A	A	A	A
							AV19	A	A	A	?	A	A	A	A	?	A	A	A	A
							AV20	A	A	A	A	A	A	A	A	?	A	A	?	A
							AV21	A	A	A	A	?	A	A	A	?	A	A	?	A
196	1	2	Y	Y	Y		AV22	?	A	A	A	A	A	A	?	?	D	?	D	D
							AV23	D	A	A	A	A	A	A	?	?	D	?	D	D
197	2	2	N	N	Y		AV24	D	A	A	A	A	A	A	A	?	A	?	A	A
							AV25	D	A	A	A	A	A	A	A	?	?	A	A	A
							AV26	D	A	A	A	A	A	A	A	?	A	A	A	A
							AV27	D	A	?	A	A	A	A	A	?	A	A	A	A

Progeny	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
JK1	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	A	D	A/D	D	D	D
JK2	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	D	D	D	D	D	A/D	?	A/D	D	D	D
JK71	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A/D	A/D	A/D	A/D
JK4	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A/D	A/D	A/D	A/D
JK5	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D
JK13	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	D	D	D	D
JK71	D	D	D	D	D	A	A	A	A	A	D	D	D	D	D	D	D	D	D	A
JK8	D	D	D	D	D	A	A	A	A	A	D	D	D	D	D	D	D	D	D	A
JK6	D	D	D	D	D	D	D	D	D	D	A	D	A/D	A/D	A	D	D	A	A	A

Progeny	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
JK14	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A/D	A/D	A/D	A	A	A
JK80	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A/D	A/D	A/D
JK9	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK10	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK11	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK12	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK20	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	D	A	D	A/D	A/D
JK15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
JK19	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	?
JK21	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A
JK22	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	D	D	D
JK81	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D
JK37	A	A	A	D	D	D	D	D	A	A	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D
JK38	A	A	A	A	D	D	D	D	D	D	A/D	A	A/D	A/D	A	A	A	A	A	A
JK39	A	A	A	A	D	D	D	D	D	D	A/D	A	A/D	A/D	A	A	A	A	A	A
JK40	A	A	A	A	D	D	D	D	D	D	A/D	A	A/D	A/D	A	A	A	A	A	A
JK41	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
JK42	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A	D	D	D
JK43	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A	A	A
JK44	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
JK45	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK46	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	A	D	D	D
JK95	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A	A	A/D	A/D	A/D
JK47	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK48	D	D	D	A	A	A	A	A	A	A	D	D	D	D	D	A	A	D	D	D
JK16	A/D	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK17	A/D	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK18	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK24	A/D	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK25	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK26	A/D	A/D	A/D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK148	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	D	D	D
JK149	A	A	A	D	D	D	D	D	D	D	D	A	D	D	D	D	D	D	D	D
JK27	D	D	D	D	D	A	A	A	A	A	A/D	A/D	A/D	D	A	A	A	D	D	D
JK28	D	D	D	D	D	A	A	A	A	A	A/D	A/D	A/D	D	A	A	A	D	D	D
JK29	D	D	D	D	D	A	A	A	A	A	A/D	A/D	A/D	D	A	A	A	D	D	D
JK30	D	D	D	D	D	A	A	A	?	A	A/D	A/D	A/D	D	A	A	A	D	D	D
JK31	D	D	D	D	D	A	A	A	A	A	A	A	A	D	A	A	A	D	D	D
JK32	D	D	D	D	D	A	A	A	A	A	A	A	A	D	A	A	A	D	D	D
JK98	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	D	D	D	D
JK33	D	D	D	D	D	D	D	D	D	D	A	A	A	A/D	D	D	A	D	A	A
JK34	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK35	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D
JK72	D	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	D	D	D	D
JK73	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A
JK74	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A

Progeny	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
JK75	D	D	D	D	D	D	D	D	A	A	A	A	A	D	A/D	A/D	A/D	D	D	D
JK76	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A	?
JK77	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A	D	D	D	D	D
JK78	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	A	A	D	D	D	D
JK79	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	A	A	D	D	D	D
JK59	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A
JK60	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A	A	A	A
JK118	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A	A	A
JK61	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A/D	?	D	D
JK62	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK63	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D
JK64	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK65	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A/D	A/D
JK66	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK67	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK68	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D
JK69	A	A	A	A	A	A	A	A	A	A	A/D	A	A/D	A	A/D	A/D	A	D	D	D
JK70	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK120	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D
JK121	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	A/D	A/D	A/D
JK122	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D
JK123	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	D
JK23	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK49	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A/D	A/D	A/D
JK50	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	A/D	A/D	A/D
JK51	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A	A	A	A/D	A/D	A/D
JK52	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A	A	A	A/D	A/D	A/D
JK53	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A/D	A/D	A/D
JK54	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	A/D	A/D	A/D
JK55	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK56	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK57	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	D
JK58	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A/D	?	A/D	?
JK124	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	D	D	A/D	D	A/D	A/D	A/D
JK125	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D	D
JK126	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	A	D	A/D	D	D	D
JK127	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	D	D	D
JK128	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D
JK129	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	D	D	D
JK147	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D
JK150	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D
JK151	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A	D	D	D
JK152	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A	D	D	D
JK153	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK154	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	D	D	D	A	A	A
JK155	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	D	D	D	A	A	?

Progeny	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
JK156	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	A/D	D	D	D	D	D	D
JK157	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	D	D	D	D	D
JK158	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A	?	D	D
JK159	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A	D	D	D
JK160	A	A	A	A	A	A	A	A	A	A	D	A/D	A/D	D	D	D	D	D	D	D
JK82	A	A	A	A	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	D	A/D	A/D	A/D
JK83	D	D	D	D	D	D	D	D	D	D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK84	D	D	D	D	D	D	D	D	A	A	A	D	A	A	D	D	A	D	D	D
JK85	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
JK116	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	D	D	D
JK86	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK87	A/D	A/D	A/D	A/D	D	D	D	D	?	D	D	D	D	D	A	A	A	?	D	D
JK88	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK119	D	D	D	D	D	D	D	D	A	A	A	A/D	A	A/D	D	D	D	A	A/D	A/D
JK36	D	D	D	D	D	D	D	D	A	A	D	D	D	D	D	D	D	D	D	D
JK89	D	D	D	D	D	D	D	D	A	A	D	D	D	D	D	D	D	D	D	D
JK90	D	D	D	D	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D
JK117	D	D	D	D	?	D	D	D	A	A	D	D	D	D	D	D	D	D	D	D
JK91	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	A/D	A	A	A	D	D	D	D	D	D
JK96	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	D	D
JK97	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D
JK99	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	D	D	A	A	D	D
JK100	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D
JK101	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D
JK102	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	?	D	D
JK103	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK104	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D
JK105	A/D	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	?	A	?
JK106	A	A	A	A	A	A	A	A	D	D	D	D	D	D	A/D	A/D	A/D	D	D	A/D
JK107	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	D	D	D	D	D
JK108	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	D	D	D	D	D
JK92	D	D	D	D	D	D	D	A	A	A	D	D	D	D	A	A	?	D	D	D
JK93	D	D	D	D	D	D	D	A	A	A	D	D	D	D	A	A	?	D	D	D
JK94	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A	A	D	D
JK109	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	A/D	A/D	A/D
JK130	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D
JK131	A	A	A	A	A	A	A	A	A	D	D	D	D	D	A/D	A/D	A/D	A	A	A
JK132	A	A	A	A	A	A	A	A	A	D	D	D	D	D	A/D	A/D	A/D	A	A	A
JK133	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	A	A	A	D	D	D
JK134	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D
JK135	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	A	A	D	D	D
JK136	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A
JK137	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK138	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
JK139	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK140	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A

Progeny	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
JK141	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	D	D	D	D	D
JK142	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK143	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	A	A	A
JK144	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D
JK145	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D
JK146	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A	A	A	A	A	A
JK110	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D
JK111	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D
JK112	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	?	A	A	A
JK113	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
JK114	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
JK115	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK170	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK171	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	?
JK172	A	A	A	A	A	A	A	A	D	D	D	D	D	D	A	A	D	D	D	D
JK173	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	?	D	?
JK174	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK175	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	?	D	D
JK176	D	D	D	D	D	A	A	A	A	A	D	D	D	A	D	D	?	?	D	?
JK177	D	D	D	D	D	A	A	A	A	A	D	D	D	A	D	D	D	?	D	?
JK178	D	D	D	D	D	A	A	A	A	A	D	D	D	A	D	D	D	D	D	A
JK161	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK162	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	?	D	?
JK163	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	?	D	?
JK164	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	?	D	?
JK165	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK166	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D
JK167	D	D	D	D	D	D	D	A	A	A	A	A	A	A	D	D	D	D	D	D
JK168	D	D	D	D	D	D	D	A	A	A	A	A	A	A	D	D	D	D	D	D
JK169	D	D	D	D	D	D	D	A	A	A	D	D	D	D	D	D	D	D	D	D
JK179	A	A	A	D	D	D	A	A	A	A	A	A	A	A	D	D	D	D	D	D
JK180	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK181	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK182	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
JK183	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK184	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
JK197	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D
JK198	D	D	D	D	D	D	D	D	A	A	D	D	D	D	D	D	D	D	D	D
JK199	D	D	D	A	A	A	A	A	A	A	A	A	A	A	D	D	D	A	A	?
JK200	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D	D	D
JK201	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	?	D	?	D	?
JK190	A	A	A	A	D	D	D	A	A	D	D	D	D	D	D	D	D	A	A	A
JK191	A	A	A	A	D	D	D	A	A	D	D	D	D	D	D	D	A	A	A	A
JK202	A	A	A	A	D	D	D	A	A	D	D	D	D	D	D	D	D	A	A	A
JK203	A	A	A	A	D	D	D	A	A	D	D	D	D	D	D	D	A	A	A	?
JK192	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A	A	A	?	D	?

Progeny	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
JK193	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	?	D	?
JK194	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	?	D	D	D
JK195	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	A	D	D	D	A
JK196	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	?	D	D
JK185	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D
JK186	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D
JK187	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	A	A	A
JK188	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	D
JK189	D	D	D	D	D	A	A	A	A	A	D	D	A	D	D	D	A	?	D	D
AV1	D	D	D	D	A	A	A	A	D	D	A	A	A	A	A	A	D	A	A	A
AV2	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D
AV3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D
AV4	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D
AV5	D	D	D	D	?	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D
AV6	D	D	D	D	?	D	D	D	D	?	D	D	D	D	A	A	D	D	A	?
AV7	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	A	?
AV8	D	D	D	D	D	D	D	D	D	D	D	D	?	D	A	A	D	D	A	A
AV9	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	A	A
AV10	A	A	A	A	D	D	D	D	D	D	A	A	A	A	D	A	D	D	A	A
AV11	A	A	A	A	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D
AV12	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D
AV13	A	A	A	A	?	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D
AV14	A	A	A	A	?	A	A	A	D	D	D	D	D	D	D	D	D	D	?	D
AV15	D	D	D	A	A	A	A	A	A	A	D	D	D	D	A	A	D	D	D	?
AV16	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D
AV17	A	A	A	A	D	D	D	D	D	D	A	D	A	A	D	D	A	D	D	D
AV18	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	?	D	D	D
AV19	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D
AV20	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D
AV21	A	A	A	A	?	A	A	A	A	A	D	D	D	D	A	A	A	D	?	D
AV22	D	A	A	A	?	A	A	A	A	A	D	D	D	D	A	A	D	?	D	?
AV23	D	A	A	A	?	A	A	A	A	A	D	D	D	D	A	A	A/D	A/D	A/D	?
AV24	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AV25	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AV26	A	A	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
AV27	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A	A	A	A	A	A

Table 2.S5: The observed genotypes and inferred haplotypes for spores from nine basidia that suggested evidence for mitotic recombination within basidia.

doi:10.1371/journal.pone.0062790.s004

Basidium	Genotype	Haplotype	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
17	4	4	JK15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
			JK19	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	?
			JK21	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A
			JK22	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	D	D	D
Inferred			H1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Haploid			H2	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A
Genotypes for			H3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/-	A/-	A/-	A/-	A/-	A/-	A/-	-	-	-
Basidium 17			H4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D
24	3	6	JK45	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	D
			JK46	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	A	D	D	D
			JK95	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A	?	A/D	A/D	A/D
Inferred			H1	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
Haploid			H2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-
Genotypes for			H3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D
Basidium 24			H4	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	A	D	A	D/-	D/-	D/-
			H5	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	-	D	D	D
			H6	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	A	A	-	A	A	A
28	4	5	JK16	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK17	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK18	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK24	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK25	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK26	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK148	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	D	D	D
Inferred			H1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	A	A	A
Haploid			H2	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
Genotypes for			H3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	D	D	D	D	A	A	A	D	D	D
Basidium 28			H4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	A	A	A	A	D	D	D	A	A	A
			H5	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/-	A/-	A/-	D/-	D/-	D/-

Basidium	Genotype	Haplotype	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
51	6	8	JK62	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK63	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D
			JK64	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK65	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A/D	A/D
			JK66	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK67	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK68	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D
Inferred Haploid Genotypes for Basidium 51			H1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A	A	A	A	A	A
			H2	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	D
			H3	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	D
			H4	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A	A	A	A	A	A
			H5	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/-	A/-	A/-	A/-	A	A	A	A	A	A
			H6	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D/-	D/-	D/-	D/-	D	D	D	D	D	D
			H7	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	A	D	D	D	D	D	D
			H8	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A	A	A	A	A	A	A	A
74	3	5	JK82	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	D	A/D	A/D	A/D
			JK83	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
			JK84	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	A	A	D	D	A	D	D	D
Inferred Haploid Genotypes for Basidium 74			H1	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D	D	D	A	A	A	A	A	A	D	A	A	A
			H2	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	A/-	A/-	A/-	A/-	A/-	A/-	D/-	D/-	D/-	D/-	D/-	D/-	D	D	D	D	A/-	A/-	D/-	D	D	D
			H3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D	D	D	D	D
			H4	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A
			H5	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	A	A	D	D	A	D	D	D
90	3	5	JK92	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D	D	A	A	?	D	D	D
			JK93	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D	D	A	A	?	D	D	D
			JK94	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A	A	D	D
			JK109	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	A/D	A/D	A/D
Inferred Haploid Genotypes for Basidium 90			H1	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D	D	A	A	-	D	D	D
			H2	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D
			H3	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	A	A	A	A	A/-	A/-	A/-	A/-	D/-	D/-
			H4	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
			H5	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D/-	D/-	D/-	A	A	A
92	3	5	JK131	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	A/D	A/D	A/D	A	A	A

Basidium	Genotype	Haplotype	Progeny	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06310	CNA06610	CNA06890	CNA07310	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	STE20	CNG00170	CNG03250	CNG04610
			JK132	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A/D	A/D	A/D	A	A	A
			JK133	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	D	A	A	A	D	D	D
			JK134	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D
Inferred			H1	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	A	A	A
Haploid			H2	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	D/-	D/-	D/-	D/-	D/-	A	A	A	A/-	A/-	A/-
Genotypes for			H3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	D	A	A	A	D	D	D
Basidium 92			H4	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	-	D/-	D/-	D/-	D/-	-	-	-	D/-	D/-	D/-
			H5	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D
			JK170	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
111	3	5	JK171	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	A/D	A/D	D	A	A	?
			JK172	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	D	A/D	A/D	A	A	D	D	D	D
Inferred			H1	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D
Haploid			H2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	A	-
Genotypes for			H3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/-	A/-	-
Basidium 111			H4	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	A	A	D	D	D	D
			H5	D/-	D/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	A/-	D	D	D	D	D	D	A/-	A/-	D/-	D/-	D/-	D/-
			JK198	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D	D	D	D	D	D	D
137	3	4	JK199	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	D	D	D	A	A	?
			JK200	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	D	D	A/D	D	D	D
Inferred			H1	D	D	D	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	A	A	-	-	-	-	D	D	D	D	D	D
Haploid			H2	A	A	A	D	D	D	D	D	D	D	D	D	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	D/-	A/-	A/-	D	D	D	D	D/-	D/-	D/-	D/-	D/-	D/-
Genotypes for			H3	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	D	D	D	A	A	-
Basidium 137			H4	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	D/-	D/-	A	D	D	D

2.6 Author Contributions

Conceived and designed the experiments: JX AV. Performed the experiments: AV JK. Analyzed the data: AV JX. Contributed reagents/materials/analysis tools: AV JK JX. Wrote the paper: AV JX JK.

Chapter 3

Evidence for genetic incompatibilities associated with post-zygotic reproductive isolation in the human fungal pathogen *Cryptococcus neoformans*

3.1 Preface

From previous observations of laboratory hybrid crosses between *C. neoformans* and *C. deneoformans*, it was determined that germination rates were only 5.5 %. One prediction of evolutionary theory states that low germination rates can be a result of genetic incompatibilities. To determine if there is evidence for these incompatibilities in *Cryptococcus*, two strains were mated under laboratory conditions. The genotypes of the progeny from this cross were determined and the subsequent analysis was used to locate incompatible regions.

This work has been published as (Vogan and Xu, 2014)

I am the primary contributor of this work. The majority of the experiments were conducted by me as well as the analyses and writing of the manuscript.

Abstract

Hybridization is a potent mechanism for generating unique strains with broad host ranges and increased virulence in fungal pathogens. In the opportunistic basidiomycete pathogen *Cryptococcus neoformans*, intervarietal hybrids are commonly found infecting patients. The two parental varieties *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* mate readily under laboratory conditions, but the hybrid basidiospores have germination rates about four times lower than those from intravarietal crosses. Here, we used microdissection to collect basidiospores from a hybrid cross and analysed the genotypes of germinated basidiospores to identify potentially antagonistic allelic combinations between loci that impact basidiospore germination. Our analyses showed clear evidence for Bateson – Dobzhansky – Muller (BDM) incompatibility affecting basidiospore viability. Antagonistic combinations of alleles from both two loci and three loci were found. Interestingly, most of the hybrid progeny showed segregation distortion in favour of the alleles from var. *neoformans*, consistent with large-scale epistatic interactions among loci affecting basidiospore viability. Our study presents the first evidence of BDM incompatibility between nuclear genes affecting post-zygotic reproductive isolation in this model basidiomycete yeast.

Keywords: hybridization, heterozygosity, homozygosity, basidiospore viability, genetic incompatibility, *Cryptococcus neoformans*

3.2 Introduction

Cryptococcus neoformans is an encapsulated, basidiomycetous yeast that can cause meningoencephalitis in individuals with compromised immunity (Mitchell and Perfect, 1995). Besides its unicellular growth, the fungus also has a filamentous sexual morph with a simple bipolar (a/α) mating system (Kwon-Chung, 1976a). *Cryptococcus neoformans* is composed of two evolutionarily diverged lineages, *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii*, traditionally recognized by their respective serotypes, D and A (Kwon-Chung and Varma, 2006). The two varieties differ in several aspects, including clinical manifestation, virulence, and geographic distribution. For example, var. *neoformans* is mostly found in temperate regions and is less virulent in animal models than var. *grubii* (Dromer *et al.*, 1996), whereas var. *grubii* has a global distribution and is the most common agent of systemic cryptococcosis (Lin, 2006). Apart from the varietal status, mating type is also an important contributor to virulence, whereby strains of MATα display higher virulence than MATa strains in both varieties (Lin *et al.*, 2008). The importance of *C. neoformans* as a pathogen, combined with the fact that representative genomes of both varieties have recently been published, has led it to become a prominent model basidiomycete.

Recent epidemiological and population genetic evidence suggests that the two varieties of *C. neoformans* diverged allopatrically, with var. *grubii* originating in Africa (Litvintseva and Mitchell, 2012) and var. *neoformans* in Europe (Boekhout *et al.*, 2001). Estimates placed the time of their divergence at about 18 million years ago (Xu *et al.*, 2000a,b), but human migrations and other associated anthropogenic activities have facilitated the pathogen's dispersal over the last few centuries. As a

result, the current geographic distributions of both varieties are broad and overlapping. This overlapping distribution has allowed hybridization to occur, and indeed, multiple recent hybridization events have been detected between the two lineages in nature (Xu *et al.*, 2002). The findings of natural hybrids and the ease of generating laboratory hybrids suggest that, despite their significant evolutionary divergence (Xu *et al.*, 2000b), there is little to no pre-zygotic reproductive isolation between the two lineages. However, two pieces of evidence suggest that significant post-zygotic isolation exists. First, most natural and laboratory hybrids are aneuploid or diploid and are heterozygous at multiple loci, as opposed to the haploid parents, consistent with frequent nuclear nondisjunction during meiosis (Sun and Xu, 2009). Second, the sexual products (the basidiospores) of the hybrid crosses have low germination rates (<20 %) (Lengeler *et al.*, 2001; Vogan *et al.*, 2013).

Reproductive isolation is the key indicator of speciation. While some significant progress has been made in understanding the underlying genetics of speciation, most research in the field has focused on plant and animal models, and relatively little has been explored in fungi (see a recent review by (Giraud *et al.*, 2008)). Traditionally, the mechanisms for reproductive isolation have been broadly grouped into pre-zygotic or post-zygotic reproductive isolation. The genetic and molecular bases of pre-zygotic reproductive isolation are intrinsically difficult to study, so most research on the topic has focused on post-zygotic isolation. The best-known model for explaining post-zygotic reproductive isolation at the genic level is the Bateson – Dobzhansky – Muller incompatibility model. In the simplest case, the BDM model describes how allelic divergence at two loci can generate incompatible genotypes between populations. Briefly, two genes interact to perform a vital cellular function and when populations

diverge, alleles at the two loci may evolve differently in separate populations. When the two diverged populations come into contact again and hybridize, the diverged alleles at the two loci cannot interact to function properly, thereby causing a detrimental effect, including offspring inviability (Dobzhansky, 1936; Muller, 1942; Orr, 1996). BDM incompatibilities may also involve additional regulatory loci, resulting in complex epistatic interactions among alleles at multiple loci (Alcázar *et al.*, 2009; Moyle and Nakazato, 2009). Despite the large theoretical background supporting the presence of such incompatibility loci (IL), few such IL pairs have been described genetically and mechanistically (Maheshwari and Barbash, 2011). The largest body of evidence has come from crosses between sister species within the model genus *Drosophila*, which have identified gene interactions related to sex-specific sterility or lethality (Orr, 2005). In fungi, the only known BDM incompatibilities are related to nuclear–mitochondrial pairs of loci associated with spore viability and fertility between baker's yeast, *Saccharomyces cerevisiae*, and its close relatives (Presgraves, 2010).

Along with BDM incompatibility, the roles of chromosomal structural rearrangements on reproductive isolation have also been investigated (Delneri *et al.*, 2003; Noor *et al.*, 2001). Recent studies have identified multiple fixed chromosomal rearrangements between the two varieties of *C. neoformans*, including large-scale translocations and inversions (Sun and Xu, 2009). Indeed, recombination rates were found to be lower in an intervarietal cross than in intravarietal crosses, and the rearranged regions showed significantly lower recombination rates than syntenic regions (Sun and Xu, 2007, 2009). The lower recombination rates suggested that these rearrangements likely negatively impacted basidiospore viability. However, the statistical significance

of individual rearrangements on hybrid basidiospore viability remains unknown.

The objective of this study is to identify whether there is any evidence for BDM incompatibility affecting post-zygotic reproductive isolation in *C. neoformans*. In *C. neoformans*, because complete heterozygotes derived from the fusion of haploid strains of var. *neoformans* and var. *grubii* are viable (Sun and Xu, 2007), if BDM incompatibilities exist, we expect such allelic combinations to be functionally recessive. Thus, our analyses will be focused on searching for homozygous–homozygous incompatible loci. Through analyzing 206 progeny genotypes at 55 co-dominant molecular markers, we identified five putative pairs of genomic regions that were consistent with BDM incompatibility in our major cross. Among these five pairs, two pairs were refuted based on data from two additional crosses. We discuss the implications of this study on the evolution of *C. neoformans* and on fungal speciation research.

3.3 Materials and methods

3.3.1 Strains

Four model laboratory strains and a clinical isolate were used as parents in the hybrid crosses in this study. The four model lab strains were as follows: JEC20, JEC21, KN99a, and KN99α. JEC20 (serotype D, MATa) and JEC21 (var. *neoformans* or serotype D, MATα) are isogenic strains, differing only at the MAT locus (Heitman *et al.*, 1999). Similarly, KN99a (serotype A, MATa) and KN99α (var. *grubii* or serotype A, MATα) are also isogenic, with the exception of the MAT locus (Nielsen *et al.*, 2003). The clinical isolate used in our main hybrid cross was CDC15 (serotype

A, MATα). CDC15 has been used to construct a hybrid linkage map (Sun and Xu, 2007) and shown to be more representative of the chromosome structures of serotype A strains than strain H99 (Sun and Xu, 2009). H99 is the type strain of var. *grubii* and was used to construct the isogenic pair KN99a and KN99α. Both CDC15 and natural hybrids were from a national surveillance conducted by the US Center for Disease Control and Prevention (Brandt *et al.*, 2001).

3.3.2 Hybrid crosses

Methods for mating, basidiospore microdissection, basidiospore germination, DNA extraction, and PCR–RFLP genotyping followed those described in Vogan *et al.* (2013). Briefly, parental strains JEC20 and CDC15 were mated on V8 agar media. After 4 weeks, basidiospores were collected and individual basidiospores were plated on YEPD agar media using a micromanipulator. Basidiospores were incubated for 2 weeks at 23 °C, after which DNA was extracted from the colonies and genotyping was performed using co-dominant PCR–RFLP markers. A total of 55 co-dominant markers were used, 51 of the markers were from those reported previously (Sun and Xu, 2007; Vogan *et al.*, 2013) and the additional four markers were designed with the software Prifi (Fredslund *et al.*, 2005). The detailed information regarding all primers, including their chromosomal locations is provided in the supplementary data, Table 3.S1. Twenty-five of the 55 markers were on chromosome 1, spaced approximately one every 100 kb. The remaining 30 markers were distributed over the other 13 chromosomes.

While our main focus was on JEC20 × CDC15, to further validate the potential BDM incompatibilities, progeny from the following two additional crosses were examined: KN99a × JEC21 (KaJ) and KN99α × JEC20 (KlJ). The objective of these crosses was to examine whether the identified BDM incompatibilities were specific to the examined parental strains and whether the varietal backgrounds of the mating types influence the potential BDM incompatibilities. The methods for mating, basidiospore isolation, DNA extraction, and genotyping for these two crosses followed the methods described above. However, only markers identified as potential BDM loci in JEC20 × CDC15 were examined in the two additional crosses. In addition, to examine potential incompatibilities between nuclear loci and the mitochondrial genome, the mitochondrial genotypes of all KaJ and KlJ progeny were examined using primers for ND5, which contains a length polymorphism between var. *neoformans* and var. *grubii* (Xu *et al.*, 2009).

Different from the typical crosses in diploid species of plants and animals, in *C. neoformans*, the parental strains are haploid and the mating products are heterokaryotic or diploid. In intravarietal crosses, these heterokaryotic–diploid mating products undergo meiosis to generate recombinant haploid basidiospores. The basidiospores germinate, undergo asexual reproduction, and form visible colonies from which DNA was extracted for our genotyping analyses. However, in the intervarietal hybrid crosses, the basidiospores are often diploid or aneuploid and these were directly analyzed in our study (Sun and Xu, 2007; Vogan *et al.*, 2013). Thus, all progeny analyzed in this study were the first generation of sexual progeny, but they are genetically analogous to the F2 individuals commonly used in hybrid crosses of plants or animals. Because most progeny were heterozygous for at least one locus, we treated these progeny as

diploid (though many were likely aneuploid) in our calculations of allele frequencies in the progeny population, with the null distribution of 50 % of the alleles coming from each parent at each locus.

3.3.3 Screening for BDM incompatibility between alleles at pairs of loci

In our crosses, the parental strains are all haploid, containing one allele at each locus (Heitman *et al.*, 1999; Nielsen *et al.*, 2003; Sun and Xu, 2007, 2009). However, most hybrid progeny are heterozygous in at least one of the loci (Sun and Xu, 2007, 2009; Vogan *et al.*, 2013). Since completely heterozygous progeny are known to be viable (Xu *et al.*, 2000b) and by default allelic pairs at different loci from the same parent are also genetically compatible, our search for BDM incompatibility was focused on pairs of loci with one being homozygous for the allele from one parent and the other locus with the allele from the alternative parent. If both homozygous recombinant genotypes at the two loci are found in the viable progeny population that we genotyped, the alleles and loci located close to the analyzed marker loci are deemed genetically compatible with each other for basidiospore viability. However, if either one or both homozygous recombinant genotypes were not found, then one or more alleles and loci around those marker regions would be considered consistent with BDM incompatibility.

Specifically, to detect genetic incompatibilities, genotypes of the hybrid progeny were used to first construct a pairwise matrix where counts represent the number of progeny that were homozygous for one allele at a given marker and homozygous for the

other allele from the alternate parent at all other markers (supplementary data, Table 3.S2). Potential BDM incompatibility loci were classified as either bidirectionally incompatible or unidirectionally incompatible. Bidirectionally incompatible loci were defined as marker pairs for which no homozygous recombinant genotypes for the two loci were observed in the progeny. Specifically, assume the two marker loci are named "1" and "2" and at each locus, the allele from var. *grubii* is represented by "A" (representing serotype A) and that from var. *neoformans* is represented by "D" (representing serotype D), the two loci are considered bidirectionally incompatible if neither genotype 1A2D nor 1D2A are found in the viable progeny. Unidirectionally incoempatible loci were defined as pairs of marker loci where one combination (e.g., 1A2D) was never observed in the hybrid progeny, while the other (e.g., 1D2A) was found in the progeny.

In addition to analyzing the number of progeny with reciprocal homozygous recombinant genotypes, we used two additional criteria to identify potential BDM incompatibility regions. In the first, we excluded marker loci pairs that were located close to each other on the same chromosome with reference to sequenced strains of both parents. In the second, for those that were located far apart from each other, but still showed either bidirectional or unidirectional incompatibility, we conducted a two-tailed binomial test with the null hypothesis that reciprocal combinations of homozygous alleles (i.e., 1A2D and 1D2A) for the same marker pair should be observed in equal proportions. In these tests, to correct for multiple tests and minimize false positives, we applied a false discovery rate (FDR) correction with $\alpha = 0.05$ (Benjamini and Hochberg, 1995).

3.3.4 Analyses of allelic combinations at three loci

Marker pairs that showed significantly (FDR adjusted q < 0.013) different reciprocal genotype counts (i.e., between genotypes 1A2D and 1D2A) between pairs of loci were further analyzed to assess if a third marker (3) could be identified, which together could represent a three-locus genetic incompatibility. The same principle used for identifying two-locus genetic incompatibility was used here to identify three-locus genetic incompatibility. Specifically, markers were determined to be participating in a three-locus BDM genetic incompatibility interaction if a given allele at the third marker was never observed with the markers of interest at two other loci in any viable progeny. For example, the absence of allelic combinations 1A2D3A and 1A2D3AD or 1D2A3D and 1D2A3AD in the progeny population would be consistent with three-locus genetic incompatibility. To rule out potential false positives due to the low numbers of individuals possessing a given combination of alleles at markers 1 and 2, the expected counts were generated for each three-marker combination based on the allele frequencies at the markers of interest. The expected counts for statistical analyses were calculated as follows: (percent of homozygous A alleles at marker 1) × (percent of homozygous D alleles at marker 2) × (percent of homozygous A alleles at marker 3 + percent of heterozygous alleles at marker 3) or f1A × f2D × (f3AD + f3A). To eliminate the effect of linkage on our tests and to be conservative in our analyses, interacting markers (either 1 and 2 or 2 and 3) which occurred on the same chromosome were excluded. For three loci combinations that met the above criteria, a two-tailed binomial test was used to determine the significance for each of the aforementioned comparisons. In these tests, we corrected for multiple tests to minimize false positives using a false discovery rate of $\alpha = 0.05$ and empirically adjusted the

statistical significance threshold to $q < 0.0032$ for each putatively incompatible three loci combinations based on the recommendations by Benjamini and Hochberg (1995). The expected count for each combination was determined using a custom C++ script.

3.4 Results

3.4.1 Hybrid basidiospore viability

A total of 1358 basidiospores were microdissected from JEC20 × CDC15. Among these, 865 never germinated and remained at the one-cell stage; 263 (19.4 %) germinated but after a couple of cell divisions, failed to divide further to form visible colonies on petri dishes; the remaining 230 germinated and formed fully visible colonies. Figure 3.1 shows examples of basidiospores that only underwent a couple of cell divisions. Interestingly, though there was no further cell division, these cells continued to expand to diameters up to ∼5 times greater than normal cells (Figure 3.1).

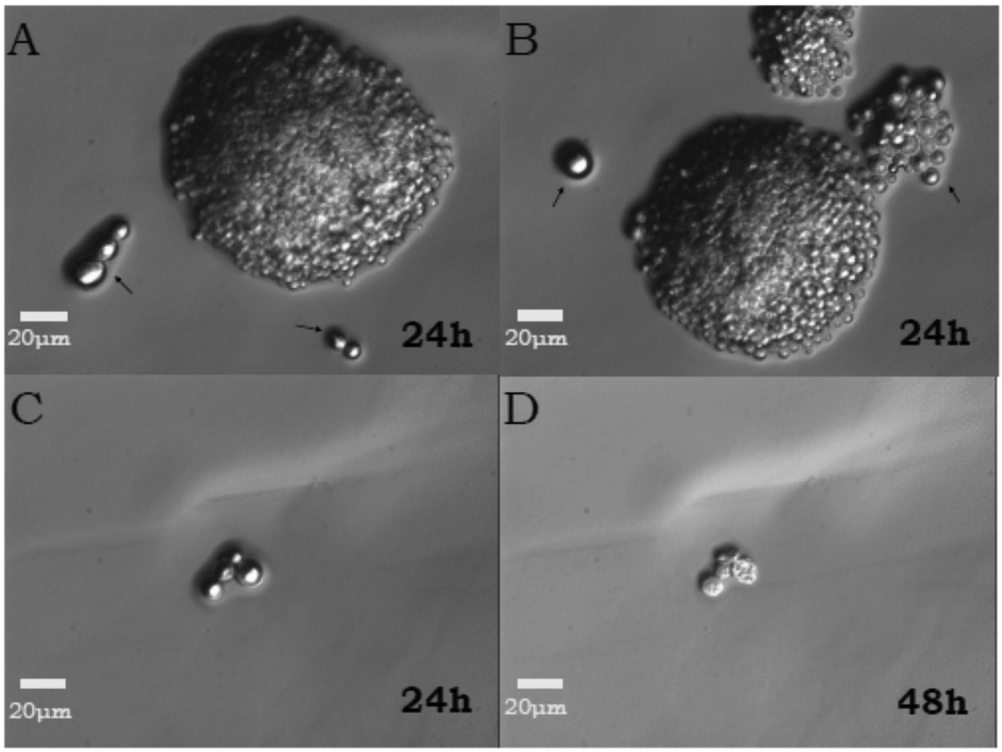

Figure 3.1: Hybrid colonies germinated on YEPD media showing enlarged cell phenotypes. (A) Arrows indicate cells with enlarged phenotype, 24 h after basidiospores were dissected and incubated. (B) Left arrow indicates an enlarged cell; the right arrow indicates a colony with cells of larger size than normal cells but smaller than the one indicated by the left, and which can form viable colonies. (C–D) The same colony at 24 and 48 h after incubation on YEPD medium, wherein the cells appear to lyse.

The 230 viable progeny were genotyped at all 55 co-dominant markers. Individual progeny from the same basidia and with identical genotypes at all markers represented clonal elements in our sample. Therefore, only one representative progeny was included for subsequent analyses for each genotype from each basidium. A total of 206 unique progeny were analyzed from the cross between JEC20 and CDC15. In addition, a further 93 germinated progeny were analyzed from the KlJ cross and 115 from the KaJ cross (supplementary data, Table 3.S2).

3.4.2 Segregation distortion

All markers showed significant deviation from expected Mendelian ratios (Figure 3.2). In general, heterozygotes were under-represented, while homozygotes for the parental serotype D allele were over-represented. With the exception of markers A7470, D510, D2010, RUM1, I70, and I4370, all other 49 markers differed significantly from the 1:1 ratio between A and D alleles. Skewed ratios ranged from 1:1.24 to 1:2.68 (A:D), with a mean value of 1:1.75. Chromosomes 4 and 9 were the only ones to show no significant segregation distortion. This result suggested that ∼85 % of the genome (12 of the 14 chromosomes) likely experienced significant segregation distortion. Additionally, all distorted markers were skewed towards the JEC20 parental alleles, indicating that the distortion is biased to the "maternal" (MATa) parent (all progeny inherited the MATa mitochondrial DNA). Interestingly, the MATa-biased segregation distortion was not obvious in the two isogenic crosses for the small number of loci that we genotyped (supplementary data, Table 3.S3). However, the smaller progeny sizes and limited number of markers assayed in the two isogenic crosses made it difficult to draw statistically robust conclusions about whether there was MATa-biased segregation

distortion at other loci in these two crosses (supplementary data, Table 3.S3).

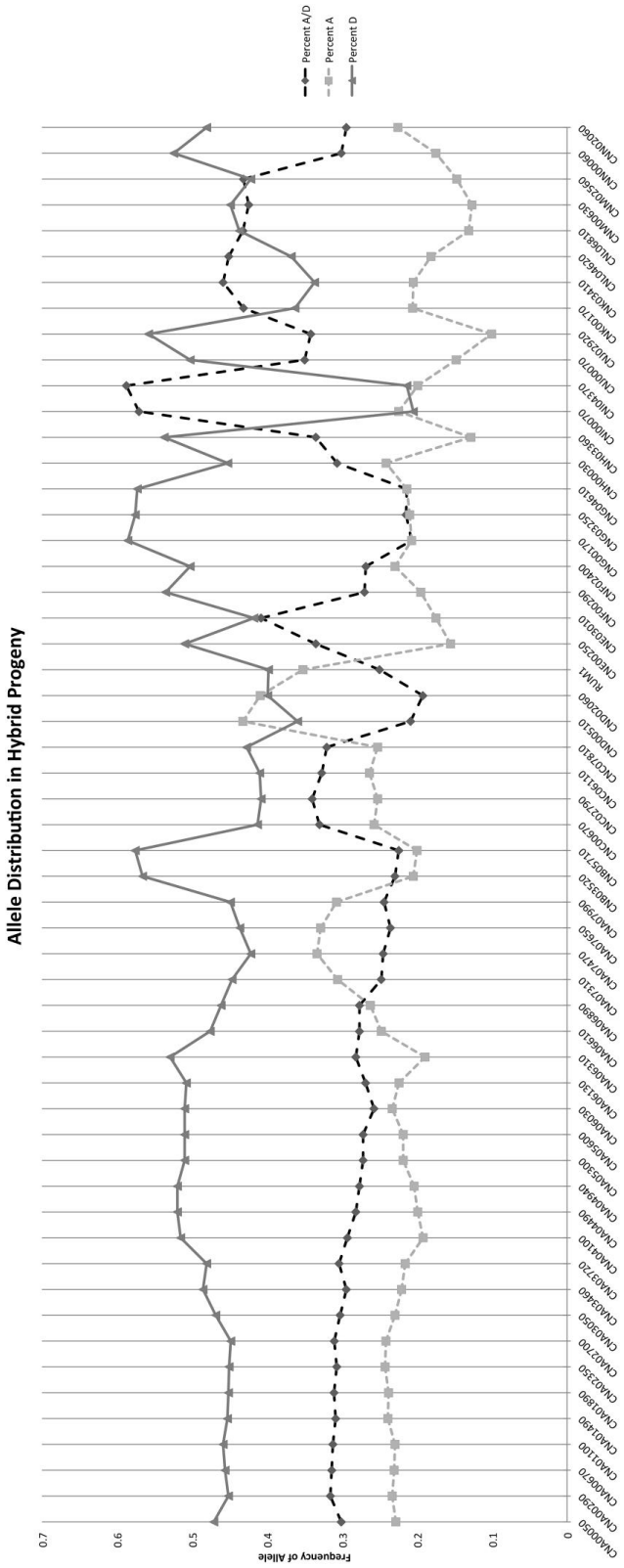

Figure 3.2: A graph of allele frequencies in the hybrid progeny from CDC15 × JEC20 across all markers genotyped in this study.

3.4.3 Evidence for BDM incompatibilities between pairs of loci

The number of times an individual possesses a homozygous A allele at one marker given that the allele of a second marker is homozygous for the D allele and vice versa was tallied (supplementary data, Table 3.S2). Reciprocal recombinant genotypes with counts of zero signify that the divergent alleles from the two parents at the two loci were not found in any of the hybrid progeny, indicating a pair of putative bidirectionally incompatible loci. Using the genome of JEC21 as a reference, we found that of the three pairs of loci showing potential bidirectional incompatibility, only one pair (markers C670 and C6110) was not located next to each other in the JEC21 genome. This putative bidirectionally incompatible pair of loci is named incompatible pair 1 or IP1 (Figure 3.3).

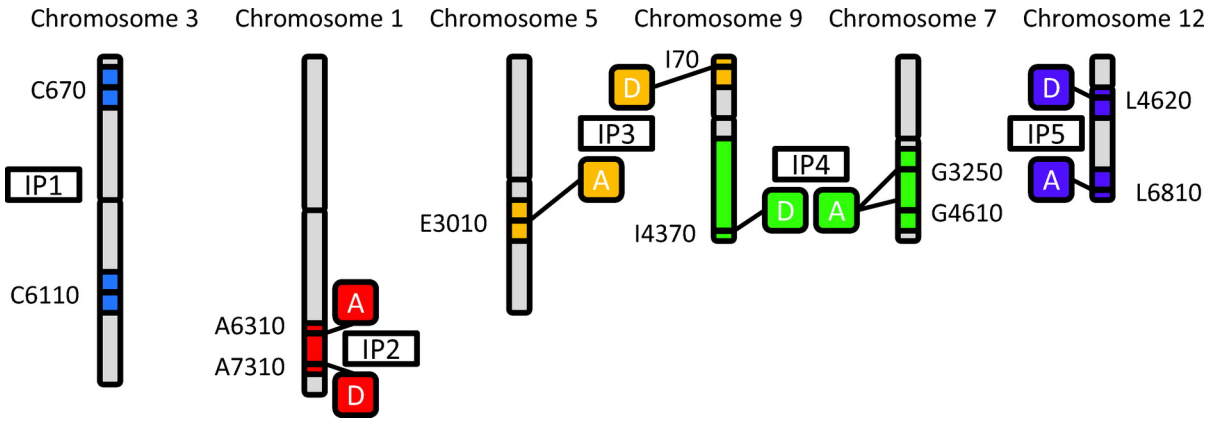

Figure 3.3: A schematic representation of the incompatible pairs (IPs) of loci identified in JEC20 × CDC15. For unidirectional pairs, relevant alleles are identified.

Homozygous allelic combinations between pairs of loci where one combination was not observed (i.e., value = 0) while the other was significantly different from zero ($q < 0.013$) indicated a unidirectional incompatibility. Four pairs of loci showed this pattern (Figure 3.3). In the first unidirectional incompatible pair (IP2), the serotype A alleles from markers A6310 to A6610 were observed to be incompatible with serotype D alleles from markers A6610 to A7310. In the second unidirectional incompatible pair (IP3), the serotype A allele at marker E3010 was observed to be incompatible with the serotype D allele at marker I70. In the third unidirectional incompatible pair (IP4), the serotype A alleles from markers G3250 and G4610 were observed to be incompatible with the serotype D allele at marker I4370. In the fourth unidirectional incompatible pair (IP5), the serotype A allele at marker L6810 was observed to be incompatible with the serotype D allele at marker L4620 (Figure 3.3). Among these four incompatible pairs, IP2 and IP3 were further confirmed as unidirectionally incompatible in the two additional isogenic crosses and in natural hybrids. In contrast, regions IP4 and IP5 were found to be compatible in the two isogenic crosses (supplementary data, Table 3.S2).

We also conducted an additional test to determine the number of pairs of loci that would be expected to have a statistically nonzero number of the homozygous recombinant 1A2D or 1D2A genotypes. Here, the observed allele frequencies in homozygous states were used to calculate the expected recombinant genotype counts. Of the 2970 (55×54) possible homozygous recombinant genotypes between pairs of loci, only 8 ($\sim 0.27\,\%$) were expected to have their counts not significantly different from zero. None of these eight was identified as part of the aforementioned IPs.

While the above analyses focused on potentially lethal (basidiospore inviability) allelic combinations, other non-lethal reproduction-related genetic incompatibilities were likely to be present. Such incompatibilities could be partially inferred based on the significant differences in the counts of reciprocal recombinant genotypes in the progeny. Indeed, many such putative loci pairs were identified. Out of the 1485 ($55 \times 54/2$) pairwise loci comparisons, 412 pairs ($\sim$28 %) were significantly skewed towards one allelic combination over the other (supplementary data, Table 3.S2). Some of these skewed genotype ratios may be the result of complex epistasis with a third marker, which will be described below.

3.4.4 Three-locus incompatibility

Here, the locus pairs that showed incompatibilities in the above two-locus analyses were excluded in the three-locus analyses. Instead, locus pairs that showed significant skews in reciprocal recombinant genotypes in the two-locus pairwise comparisons (as identified above) were chosen as the first two markers. The third marker represented an additional locus where no hybrid progeny was observed to possess the given allelic combination when the allele is from the same parent as that indicated by the first marker, in either the homozygous or heterozygous state. An additional criterion was that the expected values should be observed significantly more often than zero (i.e., FDR, $q < 0.0032$). A total of 13 putative three-locus incompatibilities were observed, with 12 showing the 1A2D3A incompatibilities while 1 showing the 1D2A3D incompatibility (supplementary data, Table 3.S4). These are called incompatibile trios (ITs). Because of their chromosomal locations and linkage relationships, these 13 putative ITs could be combined into three groups, with each involving three

loci/genomic regions. These three ITs are shown in Figure 3.4.

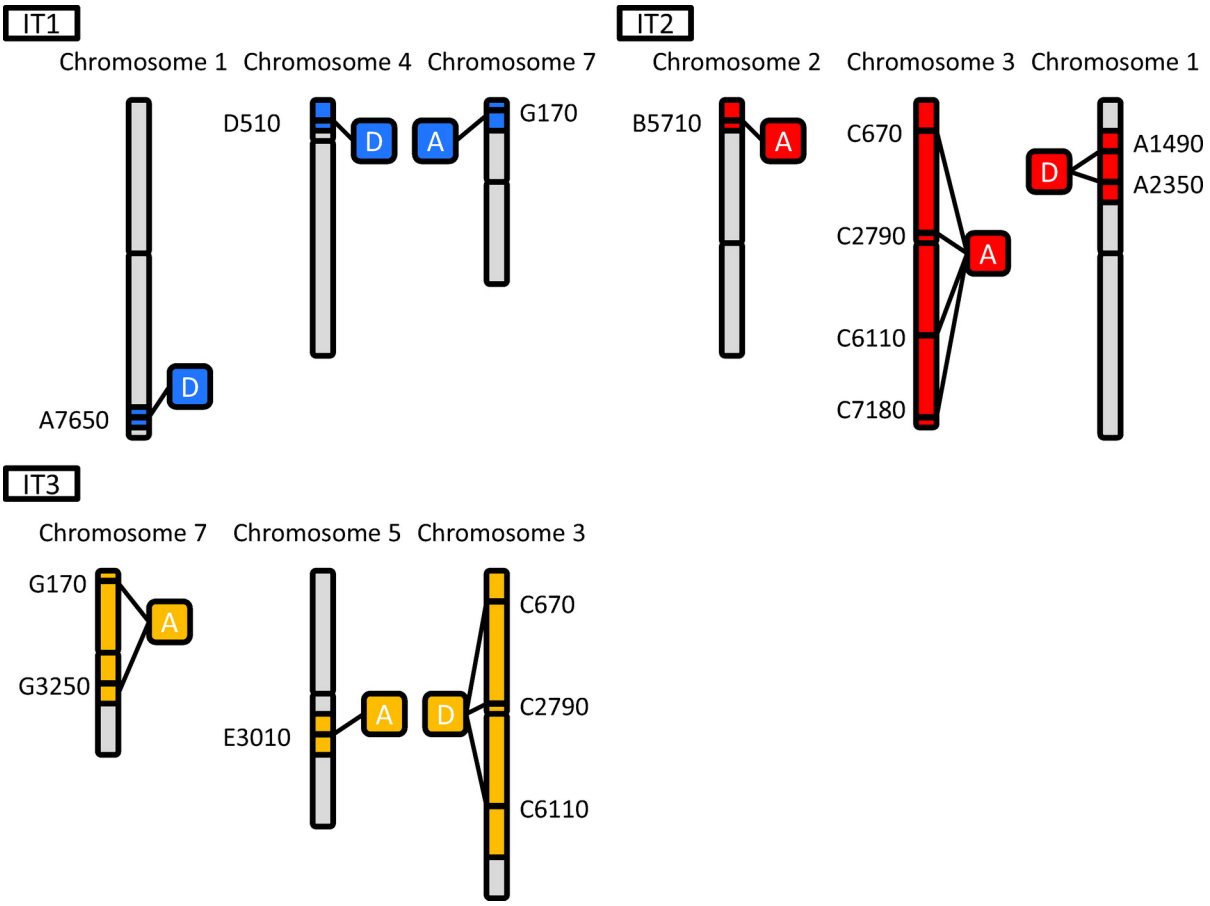

Figure 3.4: A schematic representation of the incompatible trios (ITs) identified in JEC20 × CDC15. Relevant alleles are identified.

3.5 Discussion

In this study, we examined the germination of basidiospores from a hybrid cross between the two divergent lineages in *C. neoformans* and investigated potential BDM genetic incompatibility using 55 co-dominant markers. Our results revealed two types of inviable basidiospores, identified significant, genome-wide allelic skews towards the serotype D parent, and located putative BDM incompatibilities. To our knowledge, this is the first evidence for nuclear–nuclear BDM incompatibility in fungi. Below we discuss the relevance of our results to previous studies and the potential implications.

3.5.1 Hybrid progeny viability

In this study, two types of inviable hybrid basidiospores were found: (i) those unable to germinate at all and (ii) those capable of germinating but which failed to divide further after 1 to 2 cell divisions. These two types of basidiospore inviabilities likely reflect different underlying processes. Even in the intravarietal cross, not all basidiospores germinate (Wickes, 2002), and in monokaryotic fruiting, germination rates <30 % have been observed (Lin *et al.*, 2005). Consequently, it is likely that many of the basidiospores that failed to germinate did so for reasons beyond hybrid incompatibility. In contrast, those which germinated but stopped dividing after one to two divisions have not been reported previously. These basidiospores likely had normal DNA replication and active metabolism (including nutrient and water uptake) but were unable to undergo proper cell divisions. As was observed, the second type inviable cells were much larger in size than normal cells.

In *C. neoformans*, artificially synthesized diploid strains have enlarged nuclei and greater cell size over haploid parental strains (Sia *et al.*, 2000). Similar observations have also been made in both *S. cerevisiae* and a nonmodel brewer's yeast *Torulaspora delbrueckii* where cells with higher ploidies are typically larger (Jorgensen *et al.*, 2002; Sasaki and Ohshima, 1987; Torres *et al.*, 2007). If true, the second type of inviability was likely due to BDM genetic incompatibility affecting cell division and not due to faulty DNA replication. This hypothesis was further supported by results from a whole-genome knock-out study in *S. cerevisiae* (Jorgensen *et al.*, 2002). In this study, multiple single gene deletions caused cells to have enlarged cell volumes and many of the genes were found to be involved in cell cycle procession. If cytoplasmic contents accumulate faster than cell division in these cells, these cells would expand and eventually burst due to osmotic stress.

3.5.2 Segregation distortion

Our analyses identified a significant segregation distortion among the hybrid progeny for markers located on 12 of the 14 chromosomes, in favor of the serotype D alleles. Our results differed from those reported previously that showed little allele skews in either the intravarietal cross between two serotype D strains (Marra *et al.*, 2004) or the intervarietal cross (Sun and Xu, 2007) in *C. neoformans*. The earlier study by Sun and Xu (2007) used identical parental strains as the current study, but only chromosomes 2 and 14 showed segregation distortion in the earlier study. These observed differences might be related to how the progeny populations were obtained. Specifically, basidiospores from the Sun and Xu (2007) study were germinated at 37 °C for only 4 d, whereas in this study basidiospores were allowed to germinate for

2 weeks at 23 °C. Many of the colonies analyzed in this study showed no visible colony after 4 d (detailed observations not recorded), though they formed normal colonies by 14 d. Strains of serotype D including JEC20 are known to be less tolerant to high temperature than strains of serotype A (Shahid *et al.*, 2008). Although the effect of temperature on hybrid progeny germination has not been critically examined in *C. neoformans*, one of the 230 progeny analyzed here was not able to grow at 37 °C (data not shown). We also examined whether including all 230 hybrid progeny instead of the 206 would eliminate the observed segregation distortion. However, our analyses found no obvious difference in the allelic ratios between the samples of all 230 progeny versus the 206 progeny.

Segregation distortion has been observed across a large variety of taxa, including: angiosperms (Fishman *et al.*, 2001), bryophytes (McDaniel *et al.*, 2007), insects (Tan *et al.*, 2001), and fish (Rogers *et al.*, 2007). However, what is unusual about the distortion in our hybrid cross were its scale and unidirectionality. To our knowledge, only one interspecific cross in sunflowers *Helianthus* had a greater proportion of its genome (∼90 % of loci) showing segregation distortion (Rieseberg *et al.*, 2000) than ours (∼85 %). Interestingly, the only two chromosomes not showing segregation distortions in our cross also had low levels of recombination. Of these two chromosomes, chromosome 4 contains the MAT locus (∼100 kb) and chromosome 9 has a large inversion (∼400 kb).

In our estimates of allele frequencies in the progeny, we assumed that each progeny was a diploid and each homozygous locus contained two copies of the same allele. However, this assumption might not hold true for all the homozygous loci. A previous

study has shown evidence of aneuploidy in hybrid progeny (Lengeler *et al.*, 2001). As such, it may be the case that for some homozygous sites, only one copy of the allele is present instead of two. This may result in a perceived skew in the allele frequencies that is not actually present. However, our recent results showed that aneuploidy in the hybrid progeny were likely the result of mitotic recombination within the basidia, preceding the germination process (Vogan *et al.*, 2013). Thus, even if the assumption about the allele frequencies was false, the skewed allele ratios should still reflect the genetic and genome structural incompatibility between the parental genomes.

3.5.3 Cytonuclear interaction, basidiospore viability, and segregation distortion

In *C. neoformans*, mitochondria are uniparentally inherited from the MATa parent (Xu *et al.*, 2000a), which is JEC20 in our main cross. Because all of the markers that showed segregation distortion were skewed towards JEC20, it is possible that the observed segregation distortion may be due to cytonuclear incompatibility between JEC20 mitochondrial loci and CDC15 nuclear loci. Indeed, an example of cytonuclear incompatibility has been described in the hybrid cross between *S. cerevisiae* and *Saccharomyces bayanus* (Chou *et al.*, 2010; Chou and Leu, 2010; Lee *et al.*, 2008). Similar to the two varieties in *C. neoformans*, *S. cerevisiae* and *S. bayanus* are estimated to have diverged for ∼20 million years (Scannell *et al.*, 2011). On average, 12.5 % of hybrid spore pairings between *S. cerevisiae* and *S. bayanus* formed zygotes, though few of the spores from these hybrids were able to generate viable F2 offspring, and the viable ones were usually sterile (Sebastiani *et al.*, 2002). This hybrid sterility and inviability was linked to nuclear–mitochondrial incompatibility

(Chou *et al.*, 2010; Chou and Leu, 2010; Lee *et al.*, 2008), whereby the nuclear loci from *S. bayanus* were incompatible with the mitochondrial loci from *S. cerevisiae*. While none of these studies reported any significant segregation distortion for nuclear loci between the two parental species, this is not to be expected, as mitochondrial inheritance in *Saccharomyces* is biparental (Strausberg and Perlman, 1978).

Fully viable and fertile progeny containing the haploid var. *neoformans* nuclear and the var. *grubii* mitochondrial genomes have been reported (Yan and Xu, 2003), but strains containing the haploid var. *grubii* nuclear genome and the var. *neoformans* mitochondrial genome have not been reported. All progeny from JEC20 × CDC15 had the var. *neoformans* mitochondria (Xu *et al.*, 2000b; Yan and Xu, 2003). As a result, we cannot exclude the possibility that there is BDM unidirectional incompatibility between the haploid var. *grubii* nuclear genome and the var. *neoformans* mitochondrial genome. However, there is no evidence for cytonuclear incompatibility between the 55 analyzed marker loci in the var. *grubii* nuclear genome and the var. *neoformans* mitochondrial genome because each of the 55 analyzed marker loci had at least one homozygous serotype A allele in our progeny population (Figure 3.2). The widespread nature of the segregation distortion suggests that any cytonuclear incompatibility would involve complex epistatic interactions with multiple nuclear loci.

3.5.4 Two-locus BDM incompatibility

Our analyses identified one candidate incompatibility region involved in a bidirectional BDM incompatibility and four candidate incompatibility regions involved in unidirectional BDM incompatibility. Here, we further discuss these regions.

While IP1 appears to involve nonadjacent loci according to the published genome of JEC21, based on the published genome sequence of the var. *grubii* type strain H99, the two markers are actually located very close to each other, only ∼40 kb apart in H99. The different organizations in the genomes of these two varieties were due to multiple translocations between var. *grubii* and var. *neoformans* (Sun and Xu, 2009). While the physical distances between the two markers are unknown in the CDC15 genome, the lack of observed viable recombinant genotypes between these two loci could be due to their close physical distance. Therefore, we believe the evidence for bidirectional BDM incompatibility affecting basidiospore germination in our cross is very tentative and awaits further confirmation.

The markers located in IP2 are within ∼150 kb of each other (Figure 3.3). However, despite their relatively close physical distance, this region likely contains true BDM incompatible genes. Specifically, the genetic linkage map distance between the two loci A6130 and A7310 is >8.0 cM (Vogan *et al.*, 2013) and the observed counts of the reciprocal recombinant genotypes for markers bordering the region were significantly higher than zero ($p = 0.000\,002$). Furthermore, this region was confirmed in the reciprocal crosses involving isogenic parents. Interestingly, this region contained an inversion between the two varieties, identified as SI(1)C by Sun and Xu (2009) and that our marker A6890 resided within the inversion. The inverted region contains only three genes, which Liu et al. (2008) successfully knocked out individually without noticing any obvious phenotypic defects. Furthermore, these three genes shared a higher sequence identity between the two varieties (∼95 %) than those of their surrounding regions in the genome (∼90 %) (supplementary data, Table 3.S5). Therefore, it is highly unlikely that the three genes within the inverted region are

causing the observed genetic incompatibility. Instead, genes outside of the inversion were likely responsible for the observed unidirectional incompatibility. Of the 122 annotated genes flanked by IP2 markers, 50 have no known function, 14 are part of known subunits of larger protein complexes, and 36 are known enzymes (supplementary data, Table 3.S5). Because the functions of known BDM incompatibility genes in other organisms vary greatly (Maheshwari and Barbash, 2011), we are unable to use prior findings to speculate which of these 122 genes might be the specific BDM incompatibility genes. Targeted gene knock-outs in this region would be required to reveal the precise genes that are interacting to cause the observed incompatibility.

In IP3, marker I70 was one of the few markers showing no segregation distortion and relatively few individuals were homozygous for this marker. However, because only two markers were genotyped from each of chromosome 5 and chromosome 9, the candidate incompatibility gene pool is very large and a true IP may be present nearby our marker loci. For IP4 and IP5, the missing allelic combinations were found in one of the isogenic crosses. Therefore, IP4 and IP5 were not variety-specific acBDM incompatibility regions but might represent strain-specific BDM incompatibilities.

The high basidiospore inviability in our hybrid cross would have suggested a potentially large number of loci involved in BDM incompatibility. However, the limited number of observed pairwise BDM incompatibility was not completely unexpected. Indeed, similar analyses in other organisms have found no pairwise incompatibilities associated with complete lethality between nuclear loci (Kao *et al.*, 2010; Moyle and Nakazato, 2009; Willett, 2011). In contrast, evidence for asymmetry has been reported for cytonuclear incompatibility in *S. cerevisiae* (Chou *et al.*, 2010; Chou and

Leu, 2010). We would also like to point out that our study only surveyed chromosome 1 somewhat in depth; other potential BDM incompatibilities located on other chromosomes could have been missed in our analyses. Furthermore, many of the biased distributions of reciprocal recombinant genotypes identified here could have contributed quantitatively to basidiospore viability, which together cause incompatibility. However, this type of multiple-locus synergistic interaction (known as complex heterospecific epistasis) affecting spore viability was rarely observed in *Saccharomyces* (Jasnos and Korona, 2007) and has not been demonstrated in other fungi.

3.5.5 Three-locus incompatibility

As an alternative explanation for the biased allelic combinations in the hybrid progeny, more than two loci may interact epistatically to result in complex BDM incompatibilities (Turelli and Orr, 2000). Examples of multi-locus interactions associated with a lethal or sterile phenotype have been identified in *Drosophila* (Cabot *et al.*, 1994; Davis *et al.*, 1994; Orr and Irving, 2001). Our analyses identified three such combinations related to basidiospore viabilities. However, more three-way incompatible combinations might exist between var. *neoformans* and var. *grubii* than what we inferred here. Specifically, due to the relatively low number of germinated and analyzed progeny, our calculated expected values are too low to be statistically different from zero for many of the recombinant genotypes. For example, based on our sample size (206 individuals) and the overall observed homozygosity, for the 1D2A3D incompatible genotype, our data lacked power to obtain statistical significance for ∼2.5 % of allelic combinations. For the 1A2D3A incompatible genotype, significance could not be obtained for ∼16.1 % of the allelic combinations.

3.5.6 Contributors to basidiospore viability

In our analyses, the overall basidiospore inviability was 83 %. If we assume that reciprocal allelic combinations should be present in equal numbers, our putatively identified IPs could explain about 60 out of the 1128 (∼5 %) inviable basidiospores. By a similar reasoning, the putatively identified ITs could explain about 134 (∼12 %) of the inviable basidiospores. Given that basidiospore inviability is about 20 % in intravarietal crosses in *C. neoformans* due to unknown factors, if we assume a similar rate for intervariety crosses, about 60 % of the inviable basidiospores still remain unaccounted for. However, chromosomal structural differences are known to impact the viability of sexual gametes (Orr, 2005). In *C. neoformans*, the two varieties have 32 chromosomal structural differences, including inversions, complex rearrangements, and a large translocation between chromosome 8 and chromosome 12 (Sun and Xu, 2009). All these rearrangements could have contributed significantly to hybrid basidiospore viability. The contribution of each of these chromosomal rearrangements to hybrid basidiospore viability remains to be examined.

3.5.7 Impact on evolution

Hybridization is a common evolutionary process and natural hybrids have been found in a variety of taxa (Arnold *et al.*, 2012). Natural hybrids of fungal pathogens have shown increased pathogenicity and (or) host range (Newcombe *et al.*, 2000; Olson and Stenlid, 2002; Park *et al.*, 1999). For example, in the oomycete *Phytophthora*, the hybrids have killed off large stands of Alder in the United Kingdom (Brasier *et al.*, 1999). Similarly, common plant fungal pathogens in the *Gibberella fujikuroi* complex were likely derived from an ancient hybridization event (O'Donnell and Cigelnik,

1997). In *C. neoformans*, hybrids have been found in a variety of geographic areas and these hybrids share striking genetic similarities with many of the hybrid plant fungal pathogens, including aneuploidization or polyploidization, and resistance to environmental stresses.

Population genetic studies have identified signatures of both recent (Xu *et al.*, 2000a, 2002) and potentially ancient (Kavanaugh *et al.*, 2006) hybridizations in *C. neoformans*. These hybrids have been isolated not only from patients but also in a variety of natural environments (Litvintseva *et al.*, 2005; Network, 2002; Nishikawa *et al.*, 2003). In many plants and animals where sexual reproduction is obligatory, hybrid sterility represents the end of their reproductive contribution to the next generation. However, because fungi can reproduce asexually, hybrid sterility does not lead to their extinction. Indeed, over the short term, some of these sterile hybrids may have superior asexual fitness than fertile parental strains capable of both sexual and asexual reproduction (Shahid *et al.*, 2008). However, over long evolutionary time, the lack of sexual reproduction ability may lead to the extinction of these hybrids (Xu, 2012).

In conclusion, we have identified the first candidate regions for BDM incompatibility associated with reproductive isolation between two varieties in *C. neoformans*, var. *neoformans*, and var. *grubii*. Our study should help future investigations to identify the precise genes controlling this incompatibility and if possible, link these genes to the observed abortive phenotype in basidiospore germination and other life history traits. If successful, these would represent the first genes involved in nuclear–nuclear BDM incompatibility to be identified in fungi. Furthermore, we have also identified

candidate regions for complex epistatic incompatible interactions and provided support for the hypothesis that this type of interaction may be more common than the simple two-locus incompatibility.

There are many different modes through which BDM incompatibilities can manifest, and some of these mechanisms may be unique to a given group of organisms. So far, most research on post-zygotic reproductive isolation has focused on models in mammals, insects, and flowering plants, with *Saccharomyces* being the only fungal group. Our results suggest that *C. neoformans* can be used as not only a model organism for studying fungal pathogenesis, but also for speciation research.

3.6 Supplementary Data

Table 3.S1: Primers and marker names used for genotyping in this study.

ID	Name	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Chromosome (JEC21)	Size D (bp)	Enzyme
CNA00050F	A50	AGCAATTCCAAACCGACCCC	31347	1440	17738	1	1436	HaeIII
CNA00050R		TTACGCCCACACCAAGGCAT	29908		19173			
CNA00290F	A290	AAACTCGCGGTTGCCAATCC	120407	1170	95921	1	1205	SacI
CNA00290R		TTGGAGGATGCAGGCGGTTT	121576		97125			
CNA00670F	A670	TTGGGCCAGGAGTGAGTGAT	213956	1316	192305	1	1309	HaeIII
CNA00670R		AAGGCAGAAAGAGCCGCGTT	215271		193613			
CNA01100F	A1100	GCAAAGACTGACACCAGCGACC	322838	1060	298164	1	1061	HaeIII
CNA01100R		CTGCCTCTTTTCCAAGCACAGC	323897		299224			
CNA01490F	A1490	AGGCAGGCGCGTCAGCTTTTTG	438028	1303	401640	1	1320	HaeIII
CNA01490R		AAACAGGCGACCATTGCGGGAG	439330		402959			
CNA01890F	A1890	ATCACGCAACCCGTTGCCAT	543126	1227	508335	1	1230	SacI
CNA01890R		TGCCTTGGCGTTGGCTCTTT	544352		509564			
CNA02350F	A2350	CGAGGCTTTGAGAGGATGGGG	643257	1156	613955	1	1167	HindIII
CNA02350R		CCGCTCGAACATGTACTCACCC	644412		615121			
CNA02700F	A2700	TTGGATGTTGTCCATCCCGC	729033	1368	703078	1	1368	EcoRI
CNA02700R		TCAAGACCGGCATTGTCGCA	730400		704445			
CNA03050F	A3050	CCCCCATTGTCGCCAAACAT	819061	1183	795229	1	1178	HaeIII
CNA03050R		AAGCAATTCCCCACGGCGAT	820243		796406			
CNA03460F	A3460	CCACGGTTGGGCATGAACA	911508	1667	900629	1	1667	HaeIII
CNA03460R		TTGCCTGGCTATCCATTC	909842		902295			

ID	Name	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Chromosome (JEC21)	Size D (bp)	Enzyme
CNA03720F	A3720	GCTGAGAGGTGCCCAGTTGAG	1008429	1177	999753	1	1170	HinfI
CNA03720R		ACAGCTTGATCGTGCCATCAGG	1009605		1000922			
CNA04100F	A4100	CCACCAGCGCAAAAACGAC	1109343	1726	1100766	1	1748	HindIII
CNA04100R		TGATGCGCCGCCTTTCTCTT	1111068		1102513			
CNA04490F	A4490	TGCCCGACCTCCAAAAGCAGAC	1211780	1225	1203345	1	1222	HindIII
CNA04490R		CCAGATGCCATCACGATCCACG	1213004		1204566			
CNA04940F	A4940	GCTCAAAAAGACCAGCCGCTTC	1305670	1023	1307790	1	1026	PvuII
CNA04940R		GGACCTGCTAGCGTTGTGAGAG	1306692		1308815			
CNA05300F	A5300	TCGGACAGCAAGTGAGTCCAGG	1399036	1097	1406625	1	1096	HaeIII
CNA05300R		TAAGCCTGTTGAGCGAATCGGG	1400132		1407720			
CNA05600F	A5600	TGGGTTTGGCTTCAGGCAG	1484100	1068	1500087	1	1068	HaeIII
CNA05600R		CCGCTCTTCACATGCAGCAA	1485167		1501154			
CNA06030F	A6030	GGGTTACCAGCGGTGGCGATC	1596113	1099	1612358	1	1096	PstI
CNA06030R		GTGACGGCCAACCCTGAAGGAG	1597211		1613453			
CNA06130F	A6130	AGTTTCCTCCGATCCGGCGTC	1636495	1093	1656370	1	1102	HinfI
CNA06130R		CTGCCTCGATGACTTGGCTG	1637587		1657471			
CNA06310F	A6310	CGTATCCAGCGGCTGACCAC	1696056	1100	1715148	1	1105	PvuII
CNA06310R		AATGGTCGCTAGACTGGACGGG	1697155		1716252			
CNA06610F	A6610	GCCTCAATTCACGCCCAGACCC	1776337	1126	1803026	1	1123	SacI
CNA06610R		CTCCTTCGCTCCCTTCTTCGTC	1777462		1804148			
CNA06890F	A6890	CGTGCCCTTCCCCAAACAAT	1853447	1453	1879077	1	1455	BamHI
CNA06890R		TCATTGTGCGAGATGCCTGC	1851995		1880531			
CNA07310F	A7310	ACACCCCAAATTCCCCAACC	1973202	975	2000368	1	975	PstI
CNA07310R		ACATCCCAAACGAACCCAC	1974176		2001342			
CNA07470F	A7470	TCCGGCATGCATGATCCGAA	2018541	1345	2044802	1	1331	HinfI
CNA07470R		AATCGCCTGCAACTGCGCAA	2019885		2046132			
CNA07650F	A7650	AGAAGAGCATGGCAGCGGAA	2073146	1344	2098757	1	1352	HinfI
CNA07650R		ATGTCGTCGTCGTTTCGCCC	2074489		2100108			
CNA07990F	A7990	TCCAATGGACGAGGACGATG	2177600	1430	2200900	1	1424	HinfI
CNA07990R		TGACCGGTGTGGGTTGCAAT	2179029		2202323			
CNB03520F	B3520	TTTCGGGAGTTGGGAGCACA	1036775	1060	1051980	2	1061	HaeIII
CNB03520R		TTGGCTGCGGTACTGGCATTCTT	1037834		1053040			
CNB05710F	B5710	TTGGACAACGCAAGACCCCAG	1598321	1262	1601790	2	1263	HindIII
CNB05710R		TGTTGCAAGCAACGATGCC	1599582		1603052			
CNC00670F	C670	TGTGCGGCTTTGGGATTGGT	195225	1110	191360	3	1108	HaeIII
CNC00670R		TTGCAGGTATGGCCGAATGG	196334		192467			
CNC02790F	C2790	GATGAAATGGCGAGGACGCA	866572	1469	799711	3	1470	EcoRI
CNC02790R		TTCCGCGTTGCAACACAACC	868040		801180			
CNC06110F	C6110	AAGGGCGTTGATCCGGCAAT	235559	1404	1795499	3	1403	HaeIII
CNC06110R		TTCCCCAGGTCGTTTGGGAT	236962		1796901			
CNC07180F	C7180	TGGAGGCGTTGGGCGAAATAGAG	15726	1316	2100876	3	1309	HaeIII
CNC07180R		TTCAGCCGTCGCCTTTACCACAA	17041		2102184			
CND00510F	D510	CGGTGCCGCTTTATTTGTGGC	1650153	1325	150300	4	1327	HinfI
CND00510R		TCTAGCGCCAAAGCGTGCAAG	1651477		151626			
CND02060F	D2060	TCAGGTTCAAACCGCCAGCA	1261617	1223	553800	4	1222	HaeIII
CND02060R		AGTTTTGCCCCGCTTCGCTTGC	1262839		555021			
RUM1F	RUM1	TGAAGATTTTGGATTCGAAGAAGGTGACG	258645	1345	59083	4	1340	SacI

ID	Name	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Chromosome (JEC21)	Size D (bp)	Enzyme
RUM1R		CAAGTGCAGAGCTGATCGGCATGGG	259989		57744			
CNE00250F	E250	TGGCGTCTCTTTGAACGCGATC	44253	1118	55046	5	1118	HaeIII
CNE00250R		ATGGCGGAATGTCCGGCTTT	45370		56163			
CNE03010F	E3010	TTGCTGGCAACCACCAGCTT	822857	1111	855287	5	1117	HaeIII
CNE03010R		TTTCCGCACCGGTTGATGCT	823967		856403			
CNF00290F	F290	TCATGCCCCTTCGCCTTCAT	1307533	1457	96744	6	1455	HaeIII
CNF00290R		TTCTCCTTCTCCCCATCCCA	1308989		98198			
CNF02400F	F2400	TCAAAGCTTCCGCCCGTGTT	726889	1277	701994	6	1262	HindIII
CNF02400R		CAGCCGCCTCAAATCACGAA	728165		703255			
CNG00170F	G170	TTTCTTCCGCCGCTTCTCAC	1348500	1245	42764	7	1232	HaeIII
CNG00170R		ACAGCGCGTTGAGTTTCGGT	1349744		43995			
CNG03250F	G3250	TTGCCAATAACGTGGCACGG	444807	1424	916601	7	1423	HindIII
CNG03250R		AAAGGGAGGCGGCTGATGATA	446230		918023			
CNG04610F	G4610	TGTTTCCCACAGGCCAAGGACT	59766	1466	1308769	7	1514	HinfI
CNG04610R		CGTGCGCGAATGCATCGATAT	61231		1310282			
CNH00030F	H30	TGTCGATGTGCTTCTCGGCA	12634	1272	1186643	8	1274	PstI
CNH00030R		CTCCCTCCCATCCCAAAACAC	13905		1187916			
CNH03360F	H3360	CGGGGCTATTTGGAGCGAAA	968260	1396	150788	8	1380	HaeIII
CNH03360R		ATGATGGGGCCTCTGGATTG	969655		152167			
CNI00070F	I70	CCGCCTGCACACCTTTCTT	1142613	1214	20265	9	1200	XhoI
CNI00070R		TGTCTTCGGTTTGGATGGG	1143826		21464			
CNI04370F	I4370	AGCGGCTACAGCAAAAGCGA	30620	1078	1166708	9	1084	HinfI
CNI04370R		AACATGTCCGCCTCACCCAA	31697		1167791			
CNJ00070F	J70	ATGGCGGAAGAGGCGTATGA	1016363	1153	14214	10	1135	HinfI
CNJ00070R		CCTGTCCAGTGCGCATTTCG	1017515		15348			
CNJ02920F	J2920	TGGGGGAGAAAGGACATTGG	175236	1555	904610	10	1568	HinfI
CNJ02920R		CAAATGCCGAGCTCCCTTC	176790		906177			
CNK00170F	K170	AACATGGCATCTCCCCCCAA	1500435	1108	54380	11	1111	HinfI
CNK00170R		TCGTGCTGACCATGCGGTTT	1501542		55490			
CNK03410F	K3410	TTTCGCCGCACCCCCTTTTT	1513810	1131	1002531	11	1125	HinfI
CNK03410R		CCTCGCCGCCCAATAATTCA	1514940		1003655			
CNL04620F	L4620	TTCGTGGCGACAGGTTTTGGG	595892	1321	289852	12	1321	HindIII
CNL04620R		TTCAGCGATGGGTTGAGGCA	597212		291172			
CNL06810F	L6810	TTAATGGACTGGGCAGATGCTCGTC	18019	868	894746	12	879	SacI
CNL06810R		ATGTCTTCTCCCGCCCTTTTTGCC	18886		895624			
CNM00630F	M630	TGCCAATTGCAAGGGTGGCT	183577	1133	195041	13	1134	PstI
CNM00630R		TGCGTTGAACAACGCGACCT	184709		196174			
CNM02560F	M2560	ATGGACGCTCTCACATTACCTTGC	757906	1491	776201	13	1493	AccI
CNM02560R		ACGCTGCCCTCTCCCACAGTC	759396		777693			
CNN00060F	N60	CCCAACCTCATCCCACCTC	40214	1223	18763	14	1228	XhoI
CNN00060R		ACAGAACCCATTGAGCCCGA	41436		19990			
CNN02060F	N2060	TTGGAACAGGCCACTCGGAA	632500	1432	644695	14	1433	HaeIII
CNN02060R		ACCGCCAAGGATTCTTGCGA	633931		646127			
ND5F	ND5	CTATTGGTGTTACAGGAGCTCAC	12806	438	NA	Mitochondria	750	NA
ND5R		GAGCCTTCATACCCTGCCTTATTTGC	13243		NA			

Table 3.S2: Pairwise comparison of all markers genotyped in this study for the 206 genotypically unique progeny from the JEC20 × CDC15 cross.

		A																																																				
		CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07180	CND00510	CND02060	RUM1	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060
	CNA00050		3	3	4	6	6	6	6	6	6	7	9	8	9	10	10	11	10	13	25	27	29	32	31	31	10	10	16	14	17	15	45*	42*	34*	10	12	11	15	10*	8	11	18	6	20*	12	9	4*	14	16	12	10	9	7	10	5
	CNA00290	2		1	1	3	3	5	6	6	5	5	7	6	7	7	7	8	7	8	20	22	24	27	26	26	7*	7	15	13	16	14	42*	39*	33*	9	11	12	15	8*	6	9	17	6	19*	11	8*	4*	12	12	8	9	9	7	8*	7
	CNA00670	2	0		0	2	2	4	5	5	4	4	6	5	6	6	6	7	6	7*	19	21	23	26	26	26	7*	7	15	13	16	14	41*	38*	33	10	11	12	15	8*	6	9	17	6	19*	11	8	4*	12	12	8	9	9	7	8*	7
	CNA01100	3	1	1		2	2	4	5	5	4	4	6	5	6	6	6	8	7	8	20	22	24	27	27	27	7*	7	16	14	17	15	41*	38*	33	10	11	12	15	9*	7	10	17	6*	19*	11	8	4*	12	12	9	9	9	7	8*	7
	CNA01490	4	2	2	1		0	2	3	3	2	2	4	5	6	6	6	8	7	8	19	21	23	26	26	26	6*	7*	14	12	15	12	38*	35*	30	10	10	11	15	9*	7*	10	15	5*	19*	11	8*	4*	11	10	8	8	8	7	8*	7
	CNA01890	5	3	3	2	1		2	3	3	2	2	4	5	6	6	6	8	7	8	20	22	24	27	27	27	7*	8	14	12	15	13	39*	36*	31	9	11	12	16	9*	7	10	16	5*	19*	11	8*	4*	12	10	9	9	9	7	8*	7
	CNA02350	4	4	4	3	2	1		1	1	2	2	4	5	6	6	6	8	7	8	20	22	24	27	27	27	7*	8*	15	12	15	13	39*	36*	31	10	11	11	16	9*	7*	10	16	5*	19*	11	8*	3*	13	11	9	7	9	7	7*	4*
	CNA02700	6	6	6	5	4	3	2		0	1	1	3	4	5	5	5	7	6	7	19	21	23	26	27	27	7*	9*	15	12	15	13	40*	36*	31*	9*	11	11	16	9*	7*	10	16	5*	19*	11	8*	3*	12	11	9	8	9	6	7*	4
	CNA03050	9	10	10	9	8	7	6	3		1	1	3	4	5	6	6	8	7	8	20	22	27	30*	31	31*	11	10	18	14	17	15	41*	37*	31*	11	12	11	17	9*	7	10	19	7	20*	15*	9	4*	13	12	11	10	10	7	8	4
	CNA03460	12	11	11	10	9	8	9	6	3		0	2	3	4	5	5	7	6	7	19	21	26	29*	30*	31*	12	12	18	14	17	15	42*	38*	32*	11	12	12	17	8	6	9	19	7	20*	15*	9	4*	14	13	10	10	10	7	8	6
	CNA03720	12	10	10	9	8	7	8	6	3	0		2	3	4	4	4	6	5	6	18	20	25	28*	29*	30*	11	11	18	14	17	15	41*	37*	32*	10	11	12	17	8	6	9	18	7	19*	14*	8	4*	14	13	9	9	10	7	8	6
	CNA04100	17	16	16	15	14	13	14	11	8	5	4		1	2	4	4	7	6	8	20	22	27*	29*	30*	31*	13	15	19	14	18	16	44*	41*	31*	11	12	14	20	8	10	13	19	7	18*	15*	10	5*	12	12	10	10	9	7	9	11
	CNA04490	17	16	16	15	16	15	16	13	10	7	6	2		1	3	3	6	5	8	20	22	27*	32*	33*	34*	13	15	21	16	20	18	45*	42*	34*	11	12	14	20	8	10	13	19	8	21*	16*	9	4*	13	15	13	10	8	6	9	10
	CNA04940	17	16	16	15	16	15	16	13	10	7	6	2	0		2	2	5	5	7	19	21	26*	31*	32*	33*	13	15	21	16	20	18	46*	43*	34*	11	12	13	19	8	10	13	19	8	21*	16*	9	4*	13	15	12	10	8	6	9	11
	CNA05300	16	14	14	13	14	13	14	12	9	6	5	2	0	0		0	3	3	5	17	19	24*	29*	30*	31*	11	13	20	15	19	17	45*	41*	34*	10	11	13	19	8	10	13	18	8	20*	15*	7	3*	13	15	11	9	8	5	8	10
	CNA05600	16	14	14	13	14	13	14	12	9	6	5	2	0	0	0		3	3	5	17	19	24*	29*	30*	31*	11	13	20	15	19	17	45*	41*	34*	10	11	13	19	8	10	13	18	8	20*	15*	7	3*	13	15	11	9	8	5	8	10
	CNA06030	17	15	15	15	16	15	16	14	11	8	7	5	3	3	3	3		0	2	14	17	22*	27*	28*	29*	12	13	19	15	18	16	44*	39*	32*	9*	10	12	18	7	9	12	19	9	20*	15*	7	3*	15	17	9	9	9	6	8*	10
	CNA06130	17	15	15	15	16	15	16	14	11	8	7	5	3	3	3	3	0		2	14	17	22*	25*	26*	27*	12	13	18	14	17	16	44*	39*	32*	9*	10	12	17	7	9	12	18	9	19*	14*	7	3*	15	17	8	9	9	6	8	10
	CNA06310	22	20	20*	20	21	20	20	19	16	13	12	10	10	10	10	10	7	7		12*	15*	20*	26*	27*	28*	14	15	21	19	20	18	49*	45*	36*	11	13	15	21	9	12	15	22	10	20*	16*	9	6	20	22	9	10	10	7	12	14
	CNA06610	22	20	20	20	21	20	20	19	16	13	12	10	10	10	10	10	7	7	0*		3	8*	14*	16*	16*	12	13	19	18	18	16	43*	39*	29*	9*	8	12	18	9*	12*	12	21	10	19*	15	9*	7*	20	22	10	11*	9	7	13	15
	CNA06890	21	19	19	19	20	19	19	18	15	12	11	9	9	9	9	9	7	7	0*	0		5	11	13	13	12	13	16	15	15	13	43*	39*	29*	9*	8	12	18	9*	12*	12	21	9*	19	15	8*	7*	19	21	10	11*	9	7	13	15
	CNA07310	18	16	16	16	17	16	16	15	15	12	11	9*	9*	9*	9*	9*	7*	7*	0*	0*	0		6	8	8	10*	13*	14	13	13	11	41*	37*	29	10*	6*	12*	16	9*	12*	12*	19	7*	17	12	8*	7*	16	18	9	10*	8*	6*	13*	16
	CNA07470	17	15	15	15	16	15	15	14	14*	11*	10*	8*	8*	9*	9*	9*	8*	8*	2*	2*	2	2		2	2	9*	13*	14	13	13	12*	41	37	29	11*	6*	12*	15	10*	13*	13*	16	7*	14	10	8*	7*	16	17	9*	11*	8*	6*	13*	16
	CNA07650	17	15	15	15	16	15	15	15	15	12*	11*	9*	9*	10*	10*	10*	9*	9*	3*	3*	3	3	1		0	10*	14*	12	13	11	11*	44	39	28	11*	6*	13*	15	9*	13*	13*	17	7*	14	10	8*	7*	18	18	9*	11*	8*	6*	12*	16
	CNA07990	16	14	14	14	15	14	14	14	14*	12*	12*	10*	10*	11*	11*	11*	10*	10*	4*	4*	4	4	2	1		10*	14*	12	13	11	11	45*	39*	29	11*	6	13*	15	10*	13*	13*	17	7*	14	10	8*	7*	18	18	9*	11*	8*	6*	12*	15
	CNB03520	20	20*	20*	20*	21*	20*	21*	21*	21	18	18	17	16	17	17	17	18	17	17	22	23	25*	30*	30*	29*		8	28	24	28	27	51*	45*	38*	13	13	18	21	15	16	17	22	9	26*	17*	11	7	21	23*	13	9	13	11	17	19
	CNB05710	19	19	19	19	21*	21	22*	26*	24	22	22	20	20	21	21	21	21	20	15	20	21	30*	37*	37*	32*	10		29	25	29	29	48*	42*	36*	10	14	19	23	19	20	19	27	14	27*	21*	12	9	24*	26*	17*	13	17	16	18	24
D	CNC00670	9	8	8	8	7	7	9	11	10	8	8	8	8	10	11	11	11	10	9	14	14	19	23	22	19	15	15		1	0	4	33	30	21	10	13	13	16	6*	6*	8*	16	6*	19	14	6*	3*	17	11	9	8	7*	6	7	10
	CNC02790	11	10	11	11	10	9	11	13	12	10	10	9	9	10	11	11	12	12	9	14	14	19	23	22	19	15	15	3		2	6	33*	29	22	9	12	13	16	7*	7*	9*	15	7	18	14	6*	4*	16	11	9	8	6	6	8	10
	CNC06110	9	8	8	8	7	6	8	10	9	7	7	8	8	9	10	10	10	10	8	13	13	18	22	21	18	15	15	0	1		4	32	29	21	10	13	11	14	6*	5*	7*	15	6*	19	14	6*	3*	17	11	8	8	7	6	7	9
	CNC07180	13	12	12	12	11	10	12	13	12	10	10	10	10	12	13	13	13	12	11	19	19	24	29*	27*	25	16	16	4	5	4		40*	37	29	11	11	11	15	8*	7*	9	16	7	20*	14	7*	4*	19	12	10	7	7	7	8	10
	CND00510	15*	14*	14*	13*	12*	11*	11*	14*	12*	10*	10*	8*	10*	11*	12*	12*	12*	11*	6*	11*	12*	19*	24	25	21*	13*	11*	18	16*	20	20*		3	13	6*	9*	10*	15*	10*	10*	10*	14*	6*	11	14	9*	6*	14*	13*	14*	9*	13*	12*	8*	15*
	CND02060	15*	14*	14*	13*	12*	11*	11*	14*	12*	10*	9*	9*	10*	11*	11*	11*	10*	9*	5*	10*	11*	18*	23	24	20*	13*	11*	19	16	20	20	10		14	5*	8*	10*	14*	11*	10*	10*	15*	6*	13	16	7*	4*	14*	13	14	9*	13*	11*	8*	14*
	RUM1	16*	16*	17	17	17	16	16	14*	11*	10*	9*	7*	7*	8*	10*	10*	8*	7*	7*	11*	12*	17	22	21	21	11*	10*	14	14	14	16	26	20		6*	9*	8*	10*	7*	9*	9*	14	3*	14	14	9*	6*	16	15	8	5*	8*	8*	8*	12*
	CNE00250	22	20	20	20	22	22	22	25*	22	21	21	18	21	21	22	22	23*	23*	17	27*	28*	37*	37*	34*	30*	20	17	24	22	24	24	40*	37*	31*		13	13	18	12	13	11	16	7	20*	20*	11	9	22*	22*	13	7	14	13	7	14
	CNE03010	14	12	13	13	15	14	14	17	15	14	14	13	14	14	15	15	14	14	9	16	17	24*	24*	21*	17	14	11	18	16	18	15	35*	33*	29*	6		5	9	12	13	10	16	7	11*	13*	9	8	19	20	9	8	8	8	6	13
	CNF00290	18	21	21	21	22	21	19	19	16	15	16	16	15	15	17	17	16	16	16	24	25	29*	31*	29*	29*	19	16	26	24	25	21	49*	45*	35*	11	9		6	14	13	13	20	10	22*	18*	15	9	25*	24	15	12	10	10	13	12
	CNF02400	19	21	21	21	22	21	20	20	17	15	16	16	16	16	18	18	17	17	16	24	25	28	31	29	29	17	13	23	22	23	19	49*	43*	32*	8	7	3		14	12	12	21	9	24*	20*	16	8	23	20	12	10	9	9	10	11
	CNG00170	26*	24*	24*	25*	25*	25*	25*	26*	23*	21	19	13	15	17	18	18	18	17	19	29*	30*	34*	39*	37*	38*	18	20	25*	25*	27*	24*	53*	47*	37*	8	14	13	16		6	10	19*	7	23*	16*	13	8	19	19	17	9	7	9	10	18
	CNG03250	20	18	18	19	20*	19	20*	20*	18	15	15	14	14	16	17	17	17	16	19	31*	32*	36*	41*	39*	40*	16	20	23*	24*	25*	25*	53*	47*	40*	12	17	15	17	4		4	21*	8	25*	18*	11	7	21	20	15	9	8	9	11	15
	CNG04610	19	17	17	18	18	18	19	19	16	14	14	12	13	14	15	15	15	14	17	24	25	29*	35*	33*	34*	16	18	22*	23*	24*	22	51*	45*	38*	9	12	13	14	4	0		19	7	25*	17*	11	7	20	20	16	8	8	9	10	14
	CNH00030	17	15	15	15	14	14	15	16	15	14	13	9	12	13	14	14	14	14	12	19	22	25	27	27	26	15	15	19	18	19	19	39*	34*	28	7	14	13	14	6*	7*	10		1	21	17	9	6*	14	11	8	3	9	9	6	13
	CNH03360	18	18	18	19*	19*	18*	19*	20*	19	16	16	13	14	14	15	15	15	15	14	23	24*	29*	33*	32*	30*	10	11	20*	17	19*	17	45*	42*	31*	6	10	10	14	14	15	17	8		20*	12	11	9	17	18*	3	3	8	11	10	15
	CNI00070	5*	5*	5*	5*	5*	5*	5*	6*	4*	4*	4*	3*	2*	3*	4*	4*	4*	3*	5*	5*	7	9	9	9	9	7*	6*	7	7	7	7*	17	15	8	2*	0*	4*	4*	3*	2*	2*	11	6*		3	6*	3*	10	9	2	3*	3*	4*	4*	4*
	CNI04370	4	4	4	4	4	3	3	3	3*	2*	2*	2*	1*	2*	3*	3*	3*	2*	4*	4	6	8	8	9	9	4*	7*	6	6	6	6	21	20	13	1*	2*	3*	6*	2*	0*	0*	11	4	10		7*	3*	8	9	2	3	2	2	4*	3*
	CNJ00070	20	22*	21	21	23*	24*	23*	22*	18	19	19	17	16	16	16	16	16	15	15	23*	23*	28*	34*	35*	34*	15	14	21*	19*	22*	23*	52*	44*	31*	10	17	19	19	11	12	15	19	9	29*	24*		4	21	28*	16	8	11	9	15	13
	CNJ02920	22*	24*	23*	23*	24*	24*	23*	24*	21*	21*	21*	18*	18*	19*	19*	19*	18*	17*	15	24*	25*	34*	38*	40*	35*	18	16	22*	20*	22*	22*	53*	45*	36*	11	15	16	18	16	17	19	21*	9	29*	23*	4		27*	29*	17*	11	12	10	12	17*
	CNK00170	17	16	17	16	17	16	18	15	15	14	13	9	8	8	10	10	13	13	15	16	16	16	17	17	16	9	9*	15	11	14	14	33*	30*	25	5*	8	10*	11	7	11	11	16	7	18	11	8	4*		9	10	7	4*	5*	7	12
	CNK03410	15	14	15	15	14	13	15	12	12	11	10	7	8	8	10	10	12	12	12	14	15	15	16	16	15	5*	6*	12	9	11	12	29*	26	21	5*	9	10	11	7	10	10	11	5*	15	10	8*	4*	4		6	7	5*	5*	8	12
	CNL04620	14	14	13	14	14	13	15	15	15	13	13	11	12	12	12	12	12	12	11	18	19	20	24*	24*	23*	4	4*	15	12	14	11	30*	28	19	5	5	9	8	10	11	12	8	0	11	7	10	5*	12	13		0*	10	9	7	11
	CNL06810	18	18	16	16	16	16	18	18	17	16	16	13	16	17	17	17	18	17	18	26*	27*	28*	34*	34*	33*	12	12	20	17	19	16	36*	34*	21*	5	10	15	14	11	12	14	9	0	15*	12	10	6	14	16	11*		10	9	6	10
	CNM00630	20	22	21	21	21	21	19	19	18	17	17	12	12	12	13	13	14	14	14	20	21	24*	29*	28*	28*	12	13	21*	17	19	18	47*	45*	34*	11	10	11	18	12	14	14	13	4	20*	9	11	8	17*	19*	9	7		7	13	16
	CNM02560	16	18	17	17	17	17	16	14	13	12	12	9	8	10	10	10	11	10	12	18	19	22*	26*	25*	25*	9	9	14	12	13	10	41*	37*	28*	7	7	10	15	10	11	12	13	5	16*	9	7	4	17*	20*	9	6	4		9	10
	CNN00060	24	23*	23*	22*	22*	22*	22*	23*	19	18	17	13	17	18	19	19	22*	21	19	25	25	29*	33*	32*	30*	21	17	18	18	19	20	39*	37*	29*	8	14	17	17	10	12	15	18	5	22*	17*	11	5	17	13	16	11	11	10		13
	CNN02060	15	18	17	17	17	17	16*	15	11	11	12	12	15	16	17	17	20	19	18	22	22	25	30	29	28	18	16	14	14	15	16	39*	37*	28*	5	12	15	17	8	6	7	18	6	21*	15*	9	3*	16	13	14	9	9	7	6	

1, Values represent the number of times a given allelic combination was observed. Reciprocal recombinant genotype pairs that differ significantly are marked by * (q¡0.013). Statistically significant reciprocal loci pair combinations involved in BDM incompatibilities are highlighted in yellow. Statistically significant reciprocal loci trio combinations are highlighted in orange.

2, The black bars across the diagonal of the table represent self-self combinations and are all known to be genetically compatible (see text for details).

Table 3.S3: Allele frequencies of the hybrid progeny at all markers. Chi sq is the chi squared statistic testing the ratio of serotype A alleles to serotype D alleles. Chi sq Mendel is the chi squared statistic testing whether allele frequencies differ from expected Mendelian ratios (1:2:1).

		CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710
JEC20 × CDC15	Total A/D	62	65	64	64	62	64	62	63	62	60	62	58	58	57	56	56	53	54	58	57	57	51	50	48	50	47	46
	Total A	47	48	47	47	48	49	49	49	47	45	44	38	41	42	45	45	48	45	39	51	54	63	68	67	63	42	41
	Total D	97	93	93	94	91	93	91	91	96	99	98	102	107	107	105	105	105	102	109	98	95	92	86	89	92	116	118
	Total	206	206	204	205	201	206	202	203	205	204	204	198	206	206	206	206	206	201	206	206	206	206	204	204	205	205	205
	Percent A/D	0.30	0.32	0.31	0.31	0.31	0.31	0.31	0.31	0.30	0.29	0.30	0.29	0.28	0.28	0.27	0.27	0.26	0.27	0.28	0.28	0.28	0.25	0.25	0.24	0.24	0.23	0.22
	Percent A	0.23	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.23	0.22	0.22	0.19	0.20	0.20	0.22	0.22	0.23	0.22	0.19	0.25	0.26	0.31	0.33	0.33	0.31	0.20	0.20
	Percent D	0.47	0.45	0.46	0.46	0.45	0.45	0.45	0.45	0.47	0.49	0.48	0.52	0.52	0.52	0.51	0.51	0.51	0.51	0.53	0.48	0.46	0.45	0.42	0.44	0.45	0.57	0.58
	Proportion A	0.38	0.39	0.39	0.39	0.39	0.39	0.40	0.40	0.38	0.37	0.37	0.34	0.34	0.34	0.35	0.35	0.36	0.36	0.33	0.39	0.40	0.43	0.46	0.45	0.43	0.32	0.31
	Proportion D	0.62	0.61	0.61	0.61	0.61	0.61	0.60	0.60	0.62	0.63	0.63	0.66	0.66	0.66	0.65	0.65	0.64	0.64	0.67	0.61	0.60	0.57	0.54	0.55	0.57	0.68	0.69
	chi sq Mendel	56.91	47.70	49.06	50.47	47.90	48.33	47.58	46.59	55.43	63.18	59.96	75.33	81.61	82.11	77.84	77.84	80.09	75.36	86.89	62.53	57.41	60.67	56.20	61.92	61.99	113.53	120.13
	chi sq	24.27	19.66	20.75	21.55	18.40	18.80	17.47	17.38	23.42	28.59	28.59	41.37	42.29	41.02	34.95	34.95	31.54	32.33	47.57	21.45	16.32	8.17	3.18	4.75	8.20	53.42	57.84
KaJ	Total A/D																			33	48	48	51					
	Total A																			69	56	55	53					
	Total D																			13	10	10	10					
	Total																			115	114	113	114					
	Percent A/D																			18.80	20.45	21.21	20.61					
	Percent A																			45.11	43.94	43.18	43.51					
	Percent D																			36.09	35.61	35.61	35.88					
	Proportion A																			0.74	0.70	0.70	0.69					
	Proportion D																			0.26	0.30	0.30	0.31					
	chi sq Mendel																			75.42	39.96	38.40	33.70					
	chi sq																			54.54	37.12	35.84	32.44					
KlJ	Total A/D																			25	27	28	27					
	Total A																			23	22	21	21					
	Total D																			45	44	44	44					
	Total																			93	93	93	92					
	Percent A/D																			0.25	0.24	0.23	0.23					
	Percent A																			0.48	0.47	0.47	0.48					
	Percent D																			0.27	0.29	0.30	0.29					
	Proportion A																			0.38	0.38	0.38	0.38					
	Proportion D																			0.62	0.62	0.62	0.63					
	chi sq Mendel																			30.29	26.76	26.10	27.20					
	chi sq																			10.41	10.41	11.38	11.50					

		CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060
JEC20 × CDC15	Total A/D	68	70	67	66	43	39	49	69	84	54	55	40	44	41	61	68	117	121	71	68	88	94	92	89	87	88	62	60
	Total A	53	52	54	52	89	83	69	32	36	39	47	40	43	41	48	26	46	41	30	20	42	42	37	27	26	30	36	46
	Total D	85	84	84	88	74	81	78	105	86	107	103	113	118	110	90	109	42	44	102	111	74	69	75	90	92	86	108	98
	Total	206	206	205	206	206	203	196	206	206	200	205	193	205	192	199	203	205	206	203	199	204	205	204	206	205	204	206	204
	Percent A/D	0.33	0.34	0.33	0.32	0.21	0.19	0.25	0.33	0.41	0.27	0.27	0.21	0.21	0.21	0.31	0.33	0.57	0.59	0.35	0.34	0.43	0.46	0.45	0.43	0.42	0.43	0.30	0.29
	Percent A	0.26	0.25	0.26	0.25	0.43	0.41	0.35	0.16	0.17	0.20	0.23	0.21	0.21	0.21	0.24	0.13	0.22	0.20	0.15	0.10	0.21	0.20	0.18	0.13	0.13	0.15	0.17	0.23
	Percent D	0.41	0.41	0.41	0.43	0.36	0.40	0.40	0.51	0.42	0.54	0.50	0.59	0.58	0.57	0.45	0.54	0.20	0.21	0.50	0.56	0.36	0.34	0.37	0.44	0.45	0.42	0.52	0.48
	Proportion A	0.42	0.42	0.43	0.41	0.54	0.50	0.48	0.32	0.38	0.33	0.36	0.31	0.32	0.32	0.39	0.30	0.51	0.49	0.32	0.27	0.42	0.43	0.41	0.35	0.34	0.36	0.33	0.37
	Proportion D	0.58	0.58	0.57	0.59	0.46	0.50	0.52	0.68	0.62	0.67	0.64	0.69	0.68	0.68	0.61	0.70	0.49	0.51	0.68	0.73	0.58	0.57	0.59	0.65	0.66	0.64	0.67	0.63
	chi sq Mendel	33.73	31.09	33.37	39.17	72.09	77.01	49.83	74.18	31.28	88.56	74.62	121.38	121.65	112.61	47.52	89.99	4.26	6.38	69.40	103.17	13.88	8.52	16.12	42.34	47.19	34.59	82.97	61.10
	chi sq	9.94	9.94	8.78	12.58	2.18	0.04	0.83	51.74	24.27	46.24	30.60	55.22	54.88	49.59	17.73	67.87	0.16	0.09	51.07	83.23	10.04	7.11	14.16	38.53	42.50	30.75	50.33	26.51
KaJ	Total A/D								73					56	28			72	63					42	61				
	Total A								38					48	41			37	36					63	43				
	Total D								1					3	3			1	10					2	7				
	Total								112					107	72			110	109					107	111				
	Percent A/D								0.34					0.45	0.57			0.34	0.33					0.59	0.39				
	Percent A								0.01					0.03	0.04			0.01	0.09					0.02	0.06				
	Percent D								0.65					0.52	0.39			0.65	0.58					0.39	0.55				
	Proportion A								0.67					0.71	0.76			0.66	0.62					0.79	0.66				
	Proportion D								0.33					0.29	0.24			0.34	0.38					0.21	0.34				
	chi sq Mendel								34.77					38.08	43.67			34.07	15.06					74.50	24.44				
	chi sq								24.45					37.85	40.11			23.56	12.40					69.55	23.35				
KlJ	Total A/D								45					28	24			68	66					45	32				
	Total A								18					21	18			17	25					9	8				
	Total D								25					7	23			5	2					10	9				
	Total								88					56	65			90	93					64	49				
	Percent A/D								0.20					0.38	0.28			0.19	0.27					0.14	0.16				
	Percent A								0.28					0.13	0.35			0.06	0.02					0.16	0.18				
	Percent D								0.51					0.50	0.37			0.76	0.71					0.70	0.65				
	Proportion A								0.46					0.63	0.46			0.57	0.62					0.49	0.49				
	Proportion D								0.54					0.38	0.54			0.43	0.38					0.51	0.51				
	chi sq Mendel								1.16					7.00	5.22			26.71	27.73					10.59	4.63				
	chi sq								1.11					7.00	0.77			3.20	11.38					0.03	0.04				

Table 3.S4: Three-locus allelic combinations of markers not observed in the hybrid progeny.

D	A	D/AD
A7650	G170	D510

A	D	A/AD
B5710	A1490, A2350	C670 – C7810
G170	C670 – C6110	E3010
G3250	C6110	E3010

Table 3.S5: Comparison of genes within IR2.

JEC21	H99	DNA Identity (%)	A.A. Identity (%)	Gene
CNA06140	CNAG_00634	92	93	hypothetical protein
CNA06150	CNAG_00635	90	93	cytoplasmic protein
CNA06160	CNAG_00636	90	91	CDC7 protein kinase
CNA06170	CNAG_00637	89	94	cystathionine beta-synthase
CNA06180	CNAG_00638	90	86	GTPase
CNA06190	CNAG_00639	89	96	hypothetical protein
CNA06200	CNAG_00640	94	100	small subunit ribosomal protein S4-A
CNA06210	CNAG_00641	93	97	Pol II transcription elongation factor
CNA06220	CNAG_00642	88	81	hypothetical protein
CNA06230	CNAG_00643	91	93	hypothetical protein
CNA06240	CNAG_00644	91	94	sphingolipid delta-4 desaturase
CNA06250	CNAG_00645	94	91	2,5-diamino-6-(ribosylamino)-4(3H)-pyrimidinone 5'-phosphate reductase
CNA06260	CNAG_07959	87	92	hypothetical protein
CNA06270	CNAG_00647	90	99	hypothetical protein
CNA06280	CNAG_00648	89	90	esterase/lipase
CNA06290	CNAG_00649	93	99	tryptophan synthase, beta subunit
CNA06300	CNAG_00650	91	87	GRAM domain-containing protein
CNA06310	CNAG_00651	91	94	ATP-binding cassette, subfamily D (ALD), peroxisomal long-chain fatty acid import protein
CNA06320	CNAG_00652	87	89	hypothetical protein
CNA06330	CNAG_00653	83	76	hypothetical protein
CNA06340	CNAG_00654	92	95	sulfiredoxin
CNA06350	CNAG_00655	95	99	large subunit acidic ribosomal protein P1
CNA06360	CNAG_00656	94	99	large subunit ribosomal protein L7e
-	CNAG_00657	92	-	hypothetical protein
CNA06370	CNAG_00658	88	89	hypothetical protein
CNA06380	CNAG_00659	89	85	TFIIH basal transcription factor complex TTD-A subunit
CNA06390	CNAG_00660	85	88	histone deacetylase
CNA06400	CNAG_00661	86	84	hypothetical protein
CNA06410	CNAG_00662	88	97	carboxymethylenebutenolidase
CNA06420	CNAG_00663	90	92	hypothetical protein
-	CNAG_00664	86	-	hypothetical protein
CNA06430	CNAG_00665	92	95	DNA topoisomerase 2-associated protein PAT1
CNA06440	CNAG_00666	91	89	nucleoporin nsp1
CNA06450	CNAG_00667	89	89	hypothetical protein
CNA06460	CNAG_00668	88	88	hypothetical protein
CNA06470	CNAG_00669	93	89	hypothetical protein
CNA06480	CNAG_00670	86	84	hypothetical protein
CNA06490	CNAG_00671	90	92	cytoplasmic protein
CNA06500	CNAG_00672	93	100	small subunit ribosomal protein S11
CNA06510	CNAG_00673	93	92	cytoplasmic protein
CNA06520	CNAG_00674	88	86	hypothetical protein
CNA06530	CNAG_00675	87	91	carnitine/acyl carnitine carrier
CNA06540	CNAG_00676	94	95	ESCRT-I complex subunit VPS28
CNA06550	CNAG_00677	89	96	E3 ubiquitin-protein ligase UHRF1
CNA06560	CNAG_00678	89	95	urease accessory protein UreG
CNA06570	CNAG_00679	87	97	hypothetical protein
CNA06580	CNAG_00680	93	96	kinetochore protein Nuf2

JEC21	H99	DNA Identity (%)	A.A. Identity (%)	Gene
CNA06590	CNAG_00681	92	96	condensin complex subunit 3
CNA06600	-	-	-	hypothetical protein
CNA06610	CNAG_00682	90	93	kinesin
CNA06620	CNAG_00683	87	88	CMGC/CLK protein kinase
CNA06630	CNAG_00684	89	98	ribulose-phosphate 3-epimerase
CNA06640	CNAG_00685	94	97	pre-mRNA-splicing ATP-dependent RNA helicase PRP28
CNA06650	CNAG_00686	92	95	hypothetical protein
CNA06660	CNAG_00687	88	81	hypothetical protein
-	CNAG_00688	90	-	hypothetical protein
CNA06670	CNAG_00689	91	92	beta-1,4-mannosyltransferase
CNA06680	CNAG_00690	91	93	hypothetical protein
CNA06690	CNAG_00691	89	88	hypothetical protein
CNA06700	CNAG_00692	91	95	FAD dependent oxidoreductase
CNA06710	CNAG_00693	91	90	F-box and WD-40 domain-containing protein CDC4
CNA06720	-	100	-	t-RNA - Ala
CNA06730	CNAG_00694	94	100	pre-mRNA-splicing factor CLF1
CNA06740	CNAG_00695	92	94	hypothetical protein
CNA06750	CNAG_00696	92	94	alpha-mannosidase
CNA06760	CNAG_00697	91	97	UDP-glucose 4-epimerase
CNA06770	CNAG_00698	89	88	hypothetical protein
CNA06780	CNAG_00699	90	85	transmembrane receptor
CNA06790	CNAG_00700	94	99	phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
CNA06800	CNAG_00701	88	80	hypothetical protein *(CAP64 gene-product)
CNA06810	CNAG_00702	84	78	hypothetical protein
CNA06820	CNAG_00703	90	100	large subunit ribosomal protein L31e
CNA06830	CNAG_00704	86	78	hypothetical protein
CNA06840	CNAG_00705	93	99	RuvB-like helicase 2
CNA06850	CNAG_00706	92	94	RNA-binding protein rnp24
CNA06860	CNAG_00707	89	94	protein SYM1
CNA06870	CNAG_00710	90	98	glutamine synthetase
CNA06880	CNAG_00709	94	95	uracil-DNA glycosylase
CNA06890	CNAG_00708	94	99	pre-mRNA-splicing factor SLT11
CNA06900	CNAG_00711	91	99	hypothetical protein
CNA06910	CNAG_00712	93	95	hypothetical protein
CNA06920	CNAG_00713	91	98	V-type H+-transporting ATPase 54 kDa subunit
CNA06930	CNAG_00714	91	90	hypothetical protein
CNA06940	CNAG_00715	92	-	hypothetical protein
CNA06950	CNAG_00716	90	100	cytochrome c
CNA06960	CNAG_00717	90	93	hypothetical protein
CNA06970	CNAG_00718	92	97	chromatin assembly factor 1 subunit B
CNA06980	CNAG_00719	88	94	ribosome biogenesis ATPase
CNA06990	CNAG_00720	85	94	DNA repair protein RAD51
CNA07000	CNAG_00721	93	97	alpha-1,3-mannosyltransferase
CNA07010	CNAG_00722	93	96	large subunit ribosomal protein L27
CNA07020	CNAG_00723	90	92	hypothetical protein
CNA07030	CNAG_07381	91	95	ATP-dependent RNA helicase prh1
CNA07040	CNAG_07382	94	98	hypothetical protein
CNA07050	CNAG_00726	89	94	hypothetical protein
CNA07060	CNAG_00727	89	91	mitochondrial protein with role in iron accumulation

JEC21	H99	DNA Identity (%)	A.A. Identity (%)	Gene
CNA07070	CNAG_00728	91	86	dityrosine transporter
CNA07080	CNAG_00729	83*	100	hypothetical protein
CNA07090	CNAG_00730	92	97	ATP-binding cassette transporter
CNA07100	CNAG_00732	86	83	hypothetical protein
CNA07110	CNAG_00733	89	90	hypothetical protein
CNA07120	CNAG_00734	93	96	dihydroorotase, homodimeric type
CNA07130	CNAG_00735	91	93	aldehyde dehydrogenase family 7 member A1
CNA07140	CNAG_00736	91	89	exocyst protein
CNA07150	CNAG_00737	91	86	hypothetical protein
CNA07160	-	100	-	tRNA-Val
CNA07170	CNAG_00738	89	70	hypothetical protein
CNA07180	CNAG_00739	89	89	hypothetical protein
CNA07190	CNAG_00740	92	93	hypothetical protein
CNA07200	CNAG_00741	92	99	ATP-dependent RNA helicase SUB2
CNA07210	CNAG_00742	92	98	serine palmitoyltransferase
CNA07220	CNAG_00743	93	97	imidazoleglycerol phosphate synthase, cyclase subunit
CNA07230	CNAG_00744	91	91	alpha 1,6-mannosyltransferase
CNA07240	CNAG_00745	90	90	HAL protein kinase
CNA07250	CNAG_00746	92	96	hypothetical protein
CNA07260	CNAG_00747	93	98	succinyl-CoA synthetase beta subunit
-	CNAG_00748	92	-	hypothetical protein
CNA07270	-	81	-	hypothetical protein
CNA07280	CNAG_00749	91	94	alternative sulfate transporter
CNA07290	CNAG_00750	87	93	hypothetical protein
-	CNAG_00751	88	-	hypothetical protein
CNA07300	CNAG_04652	92*	67	enoyl reductase

Identities are calculated from best BLAST hits for either the genomic sequence (DNA) or the protein (A.A.) sequence.
Loci highlighted in yellow indicate markers genotyped in this study.Loci highlighted in red indicate loci that were identified as non-lethal when knocked out by Liu *et al.* (2008).

Table 3.S6: Genotypes of hybrid progeny from all crosses and natural isolates.

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
2	JK1	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	D	D	D	D	A	D	A/D
2	JK2	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	D	D	D	D	A/D	?	D
3	JK3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D	A	A	A/D
3	JK4	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	D	D	D	D	A	A	A/D
3	JK5	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D
7	JK6	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	A/D	A/D	A	D	D
6	JK7	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D
6	JK8	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D
13	JK9	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
13	JK10	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
13	JK11	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
13	JK12	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
3	JK13	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A	A	D
10	JK14	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A/D	A/D	A/D
17	JK15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
28	JK16	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
28	JK17	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
28	JK18	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
17	JK19	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A	A	A
14	JK20	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	D	A
17	JK21	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A	A	A	A	A	A	A
17	JK22	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	A	A	A	A	A	A
58	JK23	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
28	JK24	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
28	JK25	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
28	JK26	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
30	JK27	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	D	A	A	A
30	JK28	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	D	A	A	A
30	JK29	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	D	A	A	A
30	JK30	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	?	A	A/D	A/D	A/D	A/D	A/D	D	A	A	A
30	JK31	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A/D	A/D	A	A	A	D	A	A	A
30	JK32	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	A	A	A	D	A	A	A

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
34	JK33	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A/D	A	A/D	D	D	A
34	JK34	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D	D	?
35	JK35	D	A/D	A/D	A/D	A/D	A/D	D	D	D	A/D	A/D	A/D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D
79	JK36	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D	D	D	D	D	D	D
19	JK37	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	A/D	A/D	A/D
22	JK38	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	A	A	A/D	A	A/D	A/D	A	A	A
22	JK39	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	A	A	A/D	A	A/D	A/D	A	A	A
22	JK40	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	A	A	A/D	A	A/D	A/D	A	A	A
23	JK41	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
23	JK42	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A	A	A	A	D	D	A
23	JK43	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D
23	JK44	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
24	JK45	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A	D	D	D	D	D	D	D	D
24	JK46	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	A
27	JK47	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
27	JK48	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	A	A
58	JK49	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	?
58	JK50	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D
58	JK51	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A
58	JK52	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A
58	JK53	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A
58	JK54	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D
59	JK55	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
59	JK56	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
59	JK57	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	A/D	A/D	A/D	A/D	D	D	A/D
59	JK58	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A/D
46	JK59	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A/D	A/D	?
46	JK60	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A
48	JK61	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A/D
51	JK62	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
51	JK63	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A/D	A/D	A/D
51	JK64	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	D	A/D	A/D	A/D	A/D
51	JK65	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
51	JK66	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
51	JK67	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
51	JK68	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	A/D
53	JK69	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A/D	A/D	A/D	A	A/D	A/D	A
53	JK70	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
2	JK71	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	D	D	D
37	JK72	A	A	A	A	A	A	A	A	A	A	A	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	D
37	JK73	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	A	A	A
37	JK74	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	A	A	A
40	JK75	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	?	D	D	D	D	A	A	A	D	D	A	A	A	D	A/D	A/D	A/D
42	JK76	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	?	A	A/D	A/D	A/D	A/D	A/D	A/D	?
44	JK77	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	A	D	D
45	JK78	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	A	A	D
45	JK79	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	A	A	D
10	JK80	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A
18	JK81	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
74	JK82	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	D
74	JK83	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A/D	A/D	A/D	A/D	A/D	A/D
74	JK84	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	A	D	A	A	D	D	A
75	JK85	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
76	JK86	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A
76	JK87	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	?	D	D	D	D	D	D	D	A	A	A
76	JK88	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A
79	JK89	D	D	D	D	D	D	D	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D	D	D	D	D	D	D
79	JK90	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
81	JK91	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	A/D	A	A	A	D	D	D
90	JK92	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	D	A	A	D
90	JK93	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	D	D	A	A	D
90	JK94	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A
24	JK95	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A	A
83	JK96	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D
83	JK97	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D
30	JK98	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	D

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
84	JK99	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	A	A	A	D	D	A
84	JK100	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D
85	JK101	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A/D	A/D	A	A	A	A	A/D	A/D	A/D
85	JK102	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	D	D	D	D	D	D	D
85	JK103	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
85	JK104	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A/D	A/D	A	A	A	A	A/D	A/D	A/D
86	JK105	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A/D	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	?	?
86	JK106	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	D	D	D	A/D	A/D	D	D	D	D	A/D	A/D	A/D
87	JK107	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	D	D
87	JK108	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	D	D
90	JK109	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	D	D	D
100	JK110	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A
100	JK111	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A
101	JK112	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A	A	A	A	A	A	A
101	JK113	A	A	A	A	A	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A	A	A	A	A	A	A
101	JK114	A	A	A	A	A	A	A	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A	A	A	A	A	A	A
104	JK115	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	D	D	D	D	D	D	D	A/D
75	JK116	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D
79	JK117	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	D	D	D	D	D	D	D	D	D
46	JK118	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A	A	A	A	A/D	A/D	A/D
77	JK119	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	A	A/D	A	A/D	D	D	D
56	JK120	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D
56	JK121	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D
56	JK122	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D
57	JK123	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D
59	JK124	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	D	A/D	D
60	JK125	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D
62	JK126	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	A	D	A/D
63	JK127	D	D	D	D	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A
63	JK128	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	D	D	D	D	D	D	D
63	JK129	D	D	D	D	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A
91	JK130	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
92	JK131	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	A/D	A/D	A/D

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
92	JK132	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	A/D	A/D	A/D
92	JK133	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	D	A	A	D	D	D	D	A	A	A
92	JK134	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D
93	JK135	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A
93	JK136	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	D	A/D	A/D	A/D	A/D	A	A	A
93	JK137	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A
93	JK138	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
93	JK139	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A
93	JK140	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	A	A	A	A	A	A
94	JK141	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	D	D
95	JK142	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D	D	A/D
95	JK143	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	A	A	D
96	JK144	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A
96	JK145	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A	A	A	A	A	A	D
99	JK146	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	D	A	A	A
64	JK147	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A/D
28	JK148	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A	A/D	A/D	A/D	A/D	A	A	A
29	JK149	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D
64	JK150	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A/D
65	JK151	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A
65	JK152	D	D	D	D	D	D	D	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A
65	JK153	D	D	D	D	D	D	D	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A
67	JK154	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	A	A	A	D	D	?
67	JK155	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A	A	A	A	A	D	D	D
68	JK156	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	A/D	D	D	D
68	JK157	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	D	D
69	JK158	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A	A	A
69	JK159	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	A
69	JK160	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	?	A	A	A	A	A	A	A	D	D	D	A/D	A/D	D	D	D	D
118	JK161	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A/D	A/D	A/D	A/D	D	D	D
118	JK162	A	?	A	A	?	A	?	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A/D	A/D	A/D	A/D	D	D	D
118	JK163	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	A/D	A/D	D
121	JK164	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	D	D	A

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
122	JK165	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	D	D
123	JK166	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	D
126	JK167	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	D	A	A	A	A	D	D	D
126	JK168	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	D	A	A	A	A	D	D	D
126	JK169	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	D	D	D	D	D	D	D	D
111	JK170	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	D	A	A	A
111	JK171	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D
111	JK172	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	D	D	A/D	D	A/D	A/D	A	A	D
113	JK173	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D	D	D
113	JK174	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D	D	D
114	JK175	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	A	A	A
114	JK176	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D	D	A	D	D	D
114	JK177	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D	D	A	D	D	D
114	JK178	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D	D	A	A/D	A/D	A/D
132	JK179	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A	A	A	A	A	D	D	A	A	A	A	D	D	D
134	JK180	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D	A	A	A
134	JK181	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D	A	A	A
134	JK182	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D	A	A	A
135	JK183	A	D	D	D	D	D	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
135	JK184	A	D	D	D	D	D	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D
157	JK185	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D
157	JK186	D	D	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D
159	JK187	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	A	A	A	D	D	D
159	JK188	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	A	A	A	D	D	D
162	JK189	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	A/D	A/D	A	A/D	D	D	A
146	JK190	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A	A	A	A/D	A/D	D	A/D	A/D	A/D	A/D	D	D	A/D
146	JK191	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A	A	A	A/D	A/D	D	D	D	D	D	D	D	A
149	JK192	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	A	A	A
149	JK193	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	A	A	A
149	JK194	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	A	A	D
151	JK195	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A/D	A	A	A	D	A	A	A
152	JK196	A/D	A/D	D	D	D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	?	D	D	D	D	A/D	A/D	A/D
136	JK197	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
137	JK198	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	?	D	D	D	D	A	A	A	D	D	D	D	D	D	D	D	D
137	JK199	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	D	D	A	A	A	A	D	D	?
137	JK200	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	D	D	A	A	A	A	D	D	D
141	JK201	D	D	D	D	D	D	?	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	D	A/D	D	A/D	A/D	?	D
146	JK202	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A	A	A	?	A	A	A	A	A	A	A/D	A/D	A/D	A	A	A	A/D	A/D	D	A/D	A/D	A/D	A/D	D	D	A/D
146	JK203	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A	A	A	A/D	A	D	D	D	D	D	D	D	A
167	AV1	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	D	D	D	D	A/D	A	A	A	A	A	A	A
176	AV2	D	D	D	D	D	D	D	D	D	D	D	?	A/D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	A/D	D	A/D	A	A	A/D
176	AV3	D	D	?	D	D	D	D	D	D	D	D	D	A/D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	A/D	D	A/D	A	A	A/D
176	AV4	D	D	D	D	?	D	D	D	D	D	D	D	A/D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	A/D	D	A/D	A	A	A/D
176	AV5	D	D	D	D	D	D	D	D	D	D	D	D	A/D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	A/D	D	A/D	A	A	A/D
177	AV6	A	A	A	A	A	A	A	A	A	A	?	D	D	D	D	D	D	D	D	D	D	D	D	D	?	D	D	A/D	A/D	A/D	A/D	A	A	D
177	AV7	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	D
177	AV8	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	?	D	A	A	D
177	AV9	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A	A	D
179	AV10	A	A	A	A	A	A	?	A	A	A	A	?	A	A	A	A	A	A	D	D	D	D	D	D	D	A/D	A/D	A	A	A	A	D	A	A/D
180	AV11	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A/D
187	AV12	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D
187	AV13	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D
187	AV14	D	D	D	D	D	D	D	A	A	A	?	?	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	D	D
189	AV15	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	A	A	A
189	AV16	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	D
190	AV17	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	D	D	D	D	D	A	D	A	A	D	D	A
194	AV18	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	?
194	AV19	A	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	?
194	AV20	A	A	A	A	A	A	A	A	A	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
194	AV21	A	A	A	A	?	A	A	A	?	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A	A	A
196	AV22	?	A	A	A	A	A	A	?	?	D	?	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A	A	?
196	AV23	D	A	A	A	A	A	A	?	D	D	?	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	D	D	A	A	D
197	AV24	D	A	A	A	A	A	A	A	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A	A	A	A	A	A	A
197	AV25	D	A	A	A	A	A	A	A	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A	A	A	A	A	A	A
197	AV26	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A	A	A	A	A	A	?
197	AV27	D	A	?	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	D	A/D	A/D	A/D	A/D	A	A	?

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
a3	KaJ1																			A	A	A	A/D												
a11	KaJ2																			A	A	A	A												
a11	KaJ3																			A	A	A	A												
a11	KaJ4																			A	A	A	A												
a11	KaJ5																			A	A	A	A												
a11	KaJ6																			A	A	A	A												
a11	KaJ7																			A	A	A	A												
a11	KaJ8																			A	A	A	A												
a11	KaJ9																			A	A	A	A												
a11	KaJ10																			A	A	A	A												
a11	KaJ11																			A	A	A	A												
a11	KaJ12																			A	A	A	A												
a11	KaJ13																			A	A	A	A												
a11	KaJ14																			A	A	A	A												
a11	KaJ15																			A	A	A	A												
a11	KaJ16																			A	A	A	A												
a13	KaJ17																			A/D	A/D	A/D	A/D												
a13	KaJ18																			A/D	A/D	A/D	A/D												
a13	KaJ19																			A/D	A/D	A/D	A/D												
a13	KaJ20																			A/D	A/D	A/D	A/D												
a13	KaJ21																			A/D	A/D	A/D	A/D												
a13	KaJ22																			A/D	A/D	A/D	A/D												
a16	KaJ23																			D	A/D	A/D	A/D												
a16	KaJ24																			A	A/D	A/D	A/D												
a16	KaJ25																			A	A/D	A/D	A/D												
a16	KaJ26																			D	A/D	A/D	A/D												
a16	KaJ27																			A	A/D	A/D	A/D												
a16	KaJ28																			A	A/D	A/D	A/D												
a20	KaJ29																			A/D	A/D	A/D	A/D												
a20	KaJ30																			A/D	A/D	A/D	A/D												
a20	KaJ31																			A/D	A/D	A/D	A/D												
a20	KaJ32																			A/D	A/D	A/D	A/D												
a20	KaJ33																			A/D	A/D	A/D	A/D												

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
a20	KaJ34																			A/D	A/D	A/D	A/D												
a20	KaJ35																			A/D	A/D	A/D	A/D												
a20	KaJ36																			A/D	A/D	A/D	A/D												
a20	KaJ37																			A/D	A/D	A/D	A/D												
a20	KaJ38																			A/D	A/D	A/D	A/D												
a20	KaJ39																			A/D	A/D	A/D	A/D												
a11	KaJ41																			D	D	D	D												
a11	KaJ42																			D	D	D	D												
a11	KaJ43																			D	D	D	D												
a11	KaJ48																			A	A	A	A												
a14	KaJ49																			A	A	A	A												
a14	KaJ50																			A	A	A	A												
a14	KaJ51																			A	A	A	A												
a14	KaJ52																			A	A	A	A												
a14	KaJ53																			A	A	A	A												
a14	KaJ54																			A	A	A	A												
a14	KaJ55																			A	A	A	A												
a14	KaJ56																			A	?	?	?												
a14	KaJ57																			A	A	A	A												
a14	KaJ58																			A	A	A	A												
a14	KaJ59																			A	A	A	A												
a14	KaJ60																			A	A	A	A												
a14	KaJ61																			A	A	A	A												
a14	KaJ62																			A	A	A	A												
a14	KaJ63																			A	A	A	A												
a14	KaJ64																			A	A	A	A												
a14	KaJ65																			A	A	A	A												
a14	KaJ66																			A	A	A	A												
a14	KaJ67																			A	A	A	A												
a14	KaJ68																			A	A	A	A												
a14	KaJ69																			A	A	A	A												
a15	KaJ70																			A/D	A/D	A/D	A/D												
a15	KaJ71																			A	A	A	A												

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
a15	KaJ72																			A/D	A/D	A/D	A/D												
a15	KaJ73																			A	A	A	A												
a15	KaJ74																			A/D	A/D	A/D	A/D												
a15	KaJ75																			A	A	A	A												
a15	KaJ76																			A	A	A	A												
a15	KaJ77																			A/D	A/D	A/D	A/D												
a15	KaJ78																			A/D	A/D	A/D	A/D												
a15	KaJ79																			A/D	A/D	A/D	A/D												
a15	KaJ80																			A/D	A/D	A/D	A/D												
a15	KaJ81																			A/D	A/D	A/D	A/D												
a15	KaJ82																			A/D	A/D	A/D	A/D												
a15	KaJ83																			A/D	A/D	A/D	A/D												
a15	KaJ84																			A	A	A	A/D												
a15	KaJ85																			A/D	A/D	A/D	A/D												
a16	KaJ86																			A	A/D	A/D	A/D												
a19	KaJ87																			A	A/D	A/D	A/D												
a19	KaJ88																			A	A/D	A/D	A/D												
a19	KaJ89																			A	A/D	A/D	A/D												
a19	KaJ90																			A	A	?	A/D												
a19	KaJ91																			D	A/D	A/D	A/D												
a19	KaJ92																			A	A/D	A/D	A/D												
a19	KaJ93																			A	A/D	A/D	A/D												
a19	KaJ94																			A	A/D	A/D	A/D												
a19	KaJ95																			A	A/D	A/D	A/D												
a21	KaJ96																			D	D	D	D												
a21	KaJ97																			A	A	A	A												
a21	KaJ98																			A	A	A	A												
a21	KaJ99																			D	D	D	D												
a21	KaJ100																			A/D	A/D	A/D	A/D												
a21	KaJ101																			A/D	A/D	A/D	A/D												
a21	KaJ102																			A	A	A	A												
a21	KaJ103																			D	D	D	D												
a21	KaJ104																			A	A	A	A												

Individual	Basidium	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNC00670	CNC02790	CNC06110	CNC07810	CNB03520	CNB05710	CND00510	CND02060	RUM1
KaJ105	a21																			A	A	A	A												
KaJ106	a21																			A	A	A	A												
KaJ107	a21																			A	A	A	A												
KaJ108	a21																			A	A	A	A												
KaJ109	a21																			D	D	D	D												
KaJ110	a21																			A/D	A/D	A/D	A/D												
KaJ111	a21																			A	A	A	A												
KaJ112	a21																			A	A	A	A												
KaJ113	a21																			A/D	A/D	A/D	A/D												
KaJ114	a21																			A	A	A	A												
KaJ115	a21																			A/D	A/D	A/D	A/D												
KaJ116	a21																			A	A	A	A												
KaJ117	a21																			D	D	D	D												
KaJ118	a21																			D	D	D	D												
KaJ119	a21																			D	D	D	D												
KaJ120	a22																			A	A	A	A												
KlJ1	l1																			A/D	A/D	A/D	A/D												
KlJ2	l1																			A/D	A/D	A/D	A/D												
KlJ3	l1																			A	A/D	A/D	A/D												
KlJ4	l1																			A/D	A/D	A/D	A/D												
KlJ5	l1																			A/D	A/D	A/D	A/D												
KlJ6	l1																			A/D	A/D	A/D	A/D												
KlJ7	l1																			A/D	A/D	A/D	A/D												
KlJ8	l3																			A/D	A/D	A/D	A/D												
KlJ9	l3																			A/D	A/D	A/D	A/D												
KlJ10	l3																			A/D	A/D	A/D	A/D												
KlJ11	l3																			A	A	A/D	A												
KlJ12	l3																			A/D	A/D	A/D	A/D												
KlJ13	l3																			A	A	A	A												
KlJ14	l3																			A	A	A	A												
KlJ15	l3																			A/D	A/D	A/D	A/D												
KlJ16	l4																			A	A	A	A												
KlJ17	l4																			A	A	A	A												

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
l4	KlJ18																			D	D	D	D												
l12	KlJ19																			D	D	D	D												
l12	KlJ20																			D	D	D	D												
l12	KlJ21																			D	D	D	D												
l8	KlJ22																			D	A/D	A/D	A/D												
l9	KlJ23																			D	D	D	D												
l9	KlJ24																			D	D	D	D												
l9	KlJ25																			D	D	D	D												
l9	KlJ26																			D	D	D	D												
l9	KlJ27																			D	D	D	D												
l9	KlJ28																			D	D	D	D												
l10	KlJ29																			D	D	D	D												
l10	KlJ30																			A/D	A/D	A/D	A/D												
l10	KlJ31																			A/D	A/D	A/D	A/D												
l10	KlJ32																			A/D	A/D	A/D	A/D												
l11	KlJ33																			A/D	A/D	A/D	A/D												
l11	KlJ34																			A/D	A/D	A/D	A/D												
l14	KlJ35																			A/D	A/D	A/D	A/D												
l14	KlJ36																			A/D	A/D	A/D	A/D												
l14	KlJ37																			A/D	A/D	A/D	A/D												
l14	KlJ38																			A/D	A/D	A/D	A/D												
l14	KlJ39																			A/D	A/D	A/D	A/D												
l14	KlJ40																			A/D	A/D	A/D	A/D												
l14	KlJ41																			A/D	A/D	A/D	A/D												
l14	KlJ42																			A/D	A/D	A/D	A/D												
l15	KlJ43																			A	A	A	A												
l15	KlJ44																			A	A	A	A												
l15	KlJ45																			D	D	D	D												
l15	KlJ46																			A	A	A	A												
l15	KlJ47																			A	A	A	A												
l15	KlJ48																			A	A	A	A												
l15	KlJ49																			A	A	A	A												
l15	KlJ50																			A	A	A	A												

Basidium	Individual	CNA00050	CNA00290	CNA00670	CNA01100	CNA01490	CNA01890	CNA02350	CNA02700	CNA03050	CNA03460	CNA03720	CNA04100	CNA04490	CNA04940	CNA05300	CNA05600	CNA06030	CNA06130	CNA06310	CNA06610	CNA06890	CNA07310	CNA07470	CNA07650	CNA07990	CNB03520	CNB05710	CNC00670	CNC02790	CNC06110	CNC07810	CND00510	CND02060	RUM1
115	KlJ51																			A	A	A	A												
115	KlJ52																			A	A	A	A												
115	KlJ53																			A	A	A	A												
115	KlJ54																			A	A	A	A												
115	KlJ55																			A	A	A	A												
118	KlJ56																			A	A	A	A												
119	KlJ57																			D	D	D	D												
119	KlJ58																			D	D	D	D												
119	KlJ59																			D	D	D	D												
119	KlJ60																			D	D	D	D												
119	KlJ61																			A/D	A/D	A/D	A/D												
119	KlJ62																			D	D	D	D												
119	KlJ63																			D	D	D	D												
119	KlJ64																			D	D	D	D												
119	KlJ65																			D	D	D	D												
119	KlJ66																			D	D	D	D												
119	KlJ67																			D	D	D	D												
119	KlJ68																			D	D	D	D												
119	KlJ69																			D	D	D	D												
119	KlJ70																			D	D	D	D												
119	KlJ71																			D	D	D	D												
120	KlJ72																			D	D	D	D												
120	KlJ73																			D	D	D	D												
120	KlJ74																			D	D	D	D												
120	KlJ75																			D	D	D	D												
120	KlJ76																			D	D	D	D												
120	KlJ77																			D	D	D	D												
120	KlJ78																			D	D	D	D												
120	KlJ79																			D	D	D	D												
120	KlJ80																			D	D	D	D												
126	KlJ81																			A	A	A	D												
126	KlJ82																			A	A	A	A												
126	KlJ83																			D	D	D	D												

Individual	Basidium	RUM1	CND02060	CND00510	CNC07810	CNC06110	CNC02790	CNC00670	CNB05710	CNB03520	CNA07990	CNA07650	CNA07470	CNA07310	CNA06890	CNA06610	CNA06310	CNA06130	CNA06030	CNA05600	CNA05300	CNA04940	CNA04490	CNA04100	CNA03720	CNA03460	CNA03050	CNA02700	CNA02350	CNA01890	CNA01490	CNA01100	CNA00670	CNA00290	CNA00050
KlJ84	l26													D	D	D	D																			
KlJ85	l26													D	A	A	A																			
KlJ86	l26													A	A	A	D																			
KlJ87	l26													D	D	D	D																			
KlJ88	l26													D	D	D	D																			
KlJ89	l26													A	A	A	A																			
KlJ90	l26													D	D	D	D																			
KlJ91	l26													D	D	D	D																			
KlJ92	l26													D	D	D	D																			
KlJ93	l26													D	D	D	D																			
CDC-26	Natural Hybrids													?			?																			
CDC-46														A			?																			
CDC-47														A			A																			
CDC-62														A			?																			
CDC-66														A			A																			
CDC-74														A			A																			
CDC-174														AD			?																			
CDC-190														AD			AD																			
CDC-228														A			A																			
CDC-280														A			A																			
CDC-304														AD			?																			
CDC-328														A			A																			
CDC-354														A			?																			
CDC-355														A			A																			

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
2	JK1	D	A/D	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	A/D	A/D	D	D	D	D	
2	JK2	D	A/D	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	A/D	A/D	D	D	D	D	
3	JK3	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A	A	D	D	A	D	
3	JK4	A/D	A/D	A	A	A/D	A/D	A/D	A	A	A	A	D	D	D	D	A	A	A	A	A	D	
3	JK5	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	A/D	A/D	D	D	A	A	D	A	
7	JK6	D	D	D	D	A	A	A	A/D	D	A/D	A/D	D	D	D	D	A/D	A/D	D	D	A/D	A/D	
6	JK7	A	A	D	D	D	D	A	D	D	A/D	A	D	D	A/D	A/D	A/D	D	A/D	A/D	D	A/D	
6	JK8	A	A	A/D	A/D	D	D	A	D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	
13	JK9	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
13	JK10	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	
13	JK11	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
13	JK12	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
3	JK13	D	D	A	A	D	D	D	D	A/D	D	D	D	D	A/D	A/D	A/D	D	D	D	D	D	
10	JK14	A/D	A/D	A/D	A/D	A	A	A	A/D	D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	
17	JK15	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A	A	A	A	
28	JK16	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
28	JK17	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
28	JK18	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
17	JK19	A	A	D	A/D	A	A	?	A	A	A/D	A/D	A/D	A/D	A	A	A	A	A	A	A	A	
14	JK20	A/D	A/D	A/D	A/D	D	A/D	A/D	D	A/D	A/D	A/D	A	A/D	A/D	A/D	A	A	A/D	A/D	A	A	
17	JK21	A	A	D	D	A	A	A	A	A	A	A/D	A	A	A	A	A	A	A/D	A	A	A	
17	JK22	A/D	A/D	D	D	D	D	D	A	A	A	A	A	A/D	D	D	A/D	A/D	A/D	A/D	A	A	
58	JK23	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
28	JK24	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
28	JK25	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
28	JK26	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
30	JK27	D	D	D	D	D	D	D	A/D	D	A/D	A/D	D	D	A/D	A/D	D	D	D	D	A/D	A/D	
30	JK28	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	A/D	A/D	D	D	D	D	A/D	A/D	
30	JK29	D	D	D	D	D	D	D	A/D	D	A	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	
30	JK30	D	D	D	D	D	D	D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	
30	JK31	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	
30	JK32	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	D	A/D	A/D	D	D	D	D	A/D	A/D	
34	JK33	D	D	A/D	A/D	D	A	A	D	D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	A/D	A/D	

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
34	JK34	A/D	A/D	D	A	D	D	D	D	A/D	D	D	A/D	A/D	D	D	A/D	A/D	D	D	A/D	A/D	
35	JK35	A/D	A/D	D	A/D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	D	A	D	A/D	A/D	A/D	A/D	
79	JK36	A/D	A/D	A/D	A/D	D	D	D	D	D	A/D	A/D	D	D	A/D	A/D	D	D	D	D	D	D	
19	JK37	A/D	A/D	D	D	A/D	A/D	A/D	A	A/D	A/D	A/D	D	D	A	A	A/D	A/D	D	D	A/D	A/D	
22	JK38	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A	A	
22	JK39	A	A	A/D	A/D	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A	A	
22	JK40	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A	A	
23	JK41	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	D	A	A	A	
23	JK42	A	A	A	A	D	D	D	A	A	A/D	A/D	D	D	A/D	A/D	A	A	A	A/D	A/D	A/D	
23	JK43	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	D	A	A/D	A	
23	JK44	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	D	A	A	A	
24	JK45	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	A	
24	JK46	D	D	D	D	D	D	D	D	A/D	A	D	D	D	D	D	A/D	A/D	D	D	D	D	
27	JK47	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
27	JK48	A	A	A	A	D	D	D	D	D	A/D	A/D	A	A/D	D	D	D	D	D	D	D	D	
58	JK49	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
58	JK50	A	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
58	JK51	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
58	JK52	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
58	JK53	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
58	JK54	A	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
59	JK55	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	
59	JK56	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	
59	JK57	D	D	D	D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	D	D	D	
59	JK58	D	A/D	D	D	?	A/D	?	D	A/D	A/D	A/D	A	?	A/D	A/D	D	D	A	D	D	D	
46	JK59	A/D	A/D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	
46	JK60	A	A	A	A	A	A	A	A	A/D	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A	A	D	A/D	
48	JK61	A/D	A/D	D	D	D	D	D	A	A/D	A/D	D	A	D	A/D	A/D	A	A	A/D	A/D	D	D	
51	JK62	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	
51	JK63	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	
51	JK64	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
51	JK65	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A/D	A/D	
51	JK66	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
51	JK67	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
51	JK68	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
53	JK69	D	A	D	D	D	D	D	D	D	A	A	D	D	D	D	D	D	A/D	A/D	D	D	
53	JK70	D	D	D	A/D	D	D	D	D	D	A/D	A/D	D	A	A	D	A/D	A/D	D	D	A	A/D	
2	JK71	D	A	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	A/D	D	D	D	D	D	
37	JK72	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	A/D	A/D	D	A	A	A	
37	JK73	A	D	A	A	A	A	A	A	A	A	A	D	D	A	A	A/D	A/D	A	D	A	D	
37	JK74	A	D	A	A	A	A	A	A	A	A	A	D	D	A	A	A/D	A/D	A	D	A	D	
40	JK75	A/D	A/D	A/D	A	D	D	D	A	D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	D	D	
42	JK76	D	D	?	D	?	A	?	A/D	?	A	A	?	A	?	A/D	?	A/D	A/D	A/D	A/D	A/D	
44	JK77	D	D	A/D	D	D	D	D	A	A/D	A	A/D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	
45	JK78	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	
45	JK79	D	D	D	D	D	D	D	D	D	D	A/D	D	D	A/D	A/D	D	D	D	D	D	D	
10	JK80	A/D	A/D	A/D	A	A/D	A/D	A/D	A/D	D	A	D	A	A	A/D	A/D	A/D	A/D	D	D	A/D	A/D	
18	JK81	A	A	D	D	D	D	D	A	A	A	A	?	D	A	A	A	A	A/D	A/D	A	A	
74	JK82	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A	D	A/D	D	A/D	A/D	D	D	
74	JK83	A	A/D	D	A	A/D	A/D	A/D	A/D	D	A/D	A/D	A/D	A	A/D	A/D	D	D	D	A/D	A	A/D	
74	JK84	A/D	A/D	A/D	A/D	D	D	D	D	D	A/D	A/D	D	D	D	D	D	D	D	A	D	D	
75	JK85	D	D	A/D	A/D	A/D	A/D	A/D	A	D	A/D	A/D	D	D	D	A	A/D	A/D	A/D	D	A/D	D	
76	JK86	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	
76	JK87	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	
76	JK88	D	D	D	D	D	D	D	D	D	D	A/D	D	D	A	A	D	D	D	D	D	D	
79	JK89	A/D	A/D	A/D	A/D	D	D	D	D	D	A	A/D	D	D	A/D	A/D	D	D	D	D	D	D	
79	JK90	A/D	A/D	D	D	D	D	D	A/D	D	D	D	A/D	A/D	D	D	D	D	D	D	D	D	
81	JK91	A/D	A/D	A/D	A/D	D	D	D	A/D	D	A/D	A/D	D	A	A/D	A/D	A	A	A/D	A/D	A	A	
90	JK92	D	A	D	A	D	D	D	D	D	A	D	D	D	A	A	A/D	A/D	D	D	A/D	A/D	
90	JK93	D	A	A/D	A	D	D	D	D	D	A	D	D	D	A	A	A/D	A/D	D	D	A/D	A/D	
90	JK94	A/D	A/D	A	D	A	D	D	A/D	D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	D	D	
24	JK95	D	D	D	D	A/D	A/D	A/D	A/D	D	A	A	D	D	D	D	A/D	A/D	A/D	A/D	D	D	
83	JK96	D	D	A/D	A/D	D	D	D	A/D	D	A/D	A/D	D	D	D	A	A	D	A/D	A/D	A/D	A/D	
83	JK97	D	D	A/D	A/D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	A	A	D	A/D	A/D	A/D	A/D	
30	JK98	D	D	D	D	D	D	D	A	?	D	D	A/D	D	A/D	A/D	D	D	D	D	A/D	A/D	
84	JK99	A	A/D	A	A/D	A	D	D	D	A/D	A	A	D	D	A	A	A	A	D	D	D	A	

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
84	JK100	D	A/D	D	D	D	D	D	D	D	D	D	D	D	A	D	D	D	D	D	A	A	
85	JK101	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
85	JK102	D	A/D	D	D	D	D	D	D	D	A	A	D	D	D	D	A/D	A/D	D	D	D	D	
85	JK103	D	D	D	D	D	D	D	A	D	D	A/D	D	D	A	A	D	D	A/D	A/D	D	D	
85	JK104	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	D	D	A/D	A/D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	
86	JK105	A/D	A/D	?	A	?	A	?	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	?	?	A/D	?	
86	JK106	A/D	A/D	A/D	A/D	D	D	A/D	D	A/D	A/D	A/D	A/D	D	A	A/D	A/D	A/D	A/D	D	D	D	
87	JK107	D	D	D	D	D	D	D	D	A/D	A/D	A	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	
87	JK108	D	D	D	D	D	D	D	D	A/D	A/D	A	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	
90	JK109	D	D	D	A/D	A/D	A/D	A/D	D	D	D	A/D	D	D	A/D	A	D	D	D	D	D	D	
100	JK110	D	D	D	A	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	D	D	D	D	
100	JK111	D	D	D	A	D	D	D	A/D	D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	D	D	D	D	
101	JK112	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A	A	A	A	
101	JK113	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	
101	JK114	A/D	A/D	A	A	A	A	A	A	A	A/D	A/D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	
104	JK115	D	D	D	D	A/D	A/D	A/D	A/D	D	A/D	A/D	A/D	A/D	D	A/D	D	D	A/D	A/D	A/D	D	
75	JK116	A/D	D	D	D	D	D	D	A	A	D	D	A/D	A/D	D	D	A/D	A	A/D	A/D	D	D	
79	JK117	A/D	A/D	A/D	A/D	D	D	D	D	D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	
46	JK118	A/D	A/D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	
77	JK119	A/D	A/D	D	D	A	A/D	A/D	A/D	D	D	D	D	D	A/D	A/D	D	D	A/D	A/D	A	A/D	
56	JK120	A/D	A/D	D	D	D	D	D	A	D	A	D	D	D	D	A	D	D	D	D	D	D	
56	JK121	D	D	D	D	A/D	A/D	A/D	A	D	A	A/D	D	D	D	A	D	D	D	D	A/D	A/D	
56	JK122	D	D	D	D	D	D	D	A	D	A	D	D	D	D	A	D	D	D	D	D	D	
57	JK123	D	D	D	D	D	D	D	A	D	D	D	A	A/D	D	D	D	D	D	D	D	D	
59	JK124	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	D	D	D	D	D	D	D	A/D	D	D	
60	JK125	D	D	A/D	A/D	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	D	A/D	A/D	A/D	
62	JK126	A/D	A/D	A	D	D	D	D	A	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	
63	JK127	D	D	D	D	D	D	D	A	A	D	D	D	D	D	A	A/D	A/D	D	D	A/D	A/D	
63	JK128	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D	D	D	D	D	
63	JK129	D	D	D	A/D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	A	A/D	A/D	A/D	A/D	A/D	A/D	
91	JK130	D	D	D	D	A/D	A/D	D	A/D	A/D	D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
92	JK131	D	D	D	D	A	A	A	A/D	D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	
92	JK132	D	D	D	D	A	A	A	A/D	D	A/D	A/D	D	D	D	D	D	D	D	D	D	D	

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
92	JK133	D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	D	
92	JK134	D	A/D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	D	D	D	?	
93	JK135	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A	A	A	A	A	A	
93	JK136	A	A	A	A	A	A	A	A	A	A/D	A/D	A	A	A	A	A	A	A	A	A	A	
93	JK137	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A	A	A	A	A	A	
93	JK138	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A	A	A	A	A/D	A/D	A/D	A/D	
93	JK139	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	A	A/D	A/D	A/D	A/D	A	A	A	A	A	A	
93	JK140	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
94	JK141	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	A	A	A/D	A/D	D	D	D	D	
95	JK142	D	A/D	D	D	D	D	D	A/D	D	A/D	D	A/D	D	A/D	A/D	A/D	A/D	D	D	D	D	
95	JK143	A	D	D	D	A	A	A	D	D	D	A	A	A	A/D	A/D	D	D	A/D	A/D	A/D	A/D	
96	JK144	D	D	A/D	A/D	D	D	D	D	D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	D	A	D	D	
96	JK145	D	A/D	D	D	D	D	D	A/D	D	A/D	A/D	A	A	A/D	A/D	D	D	A	A	A	A	
99	JK146	A	A	A	A	A	A	A	A	A	A/D	A/D	A	A	A	A	A	A/D	A	A	A	A	
64	JK147	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	
28	JK148	A	D	A/D	A/D	D	D	D	A	A/D	D	D	D	D	A	A	A/D	A/D	A/D	A/D	A	A	
29	JK149	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	D	D	A	A	D	D	
64	JK150	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	
65	JK151	A/D	A/D	D	D	D	D	D	D	D	A	D	D	D	D	D	A/D	A/D	D	A/D	D	D	
65	JK152	A/D	A/D	D	D	D	D	D	D	D	A	D	D	D	D	D	A/D	A/D	D	A/D	D	D	
65	JK153	A/D	A/D	D	D	D	D	D	D	D	A	D	D	D	D	D	A/D	A/D	D	A/D	D	D	
67	JK154	D	A	A/D	A/D	A	A	A	A	A	A/D	A/D	A/D	A/D	D	D	A	A	A	A	A	A	
67	JK155	D	A	A	A	A	A	?	A	A	A	A	D	D	A	A	A	A	A	A	A	A	
68	JK156	D	D	D	D	D	D	D	D	?	A/D	A/D	A/D	D	A/D	A/D	D	D	A/D	A/D	D	D	
68	JK157	A/D	A/D	D	D	D	D	D	D	D	A/D	A/D	A/D	D	A/D	A/D	D	D	A/D	A/D	D	D	
69	JK158	A/D	A/D	D	D	D	D	D	D	D	A	D	D	D	D	D	A/D	A/D	D	A/D	D	D	
69	JK159	A/D	A/D	D	D	D	D	D	D	D	A	D	D	D	D	D	A/D	A/D	D	A/D	D	D	
69	JK160	A/D	A/D	A	A	D	D	D	A	A/D	D	D	A/D	A/D	A/D	A/D	A	D	A/D	D	A/D	A/D	
118	JK161	D	D	D	D	D	D	D	A/D	D	A/D	A/D	A	D	D	D	D	D	D	D	A/D	A/D	
118	JK162	D	D	?	D	?	D	?	A/D	D	A/D	A/D	?	D	D	D	D	D	D	D	A/D	A/D	
118	JK163	D	A/D	?	D	?	A/D	?	D	D	D	D	A	A/D	A/D	A/D	D	D	A/D	A/D	D	D	
121	JK164	A/D	A/D	A/D	D	?	A/D	?	D	A/D	A	A	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	
122	JK165	D	D	D	D	D	D	D	D	D	A/D	D	A/D	D	D	D	D	D	A/D	A/D	D	D	

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
123	JK166	D	D	A/D	D	A	D	D	A/D	D	A/D	A/D	A/D	A/D	A	D	D	D	D	D	D	D	
126	JK167	D	D	D	D	D	D	D	A/D	D	A/D	A	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
126	JK168	D	D	D	D	A/D	A/D	A/D	A	A	D	A	D	D	A	A	A/D	A/D	A	A	D	D	
126	JK169	D	D	D	D	D	D	D	A	A	D	A	D	D	A/D	A/D	A	A	D	D	D	D	
111	JK170	A	A	D	D	D	D	D	A/D	D	A	A	D	D	D	D	D	D	D	D	A/D	A/D	
111	JK171	D	D	D	D	A	A	A/D	D	D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	D	D	
111	JK172	D	D	D	D	D	D	D	D	D	A/D	A/D	D	A/D	D	D	A/D	A/D	A/D	A/D	D	D	
113	JK173	A/D	A/D	D	A	?	D	?	D	D	A/D	A/D	D	?	A/D	A/D	D	D	D	?	D	D	
113	JK174	A/D	A/D	D	A/D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	D	D	D	D	
114	JK175	A	A	A	A	?	D	D	A/D	A/D	A	A	D	A/D	A/D	A/D	A/D	A/D	D	D	A	D	
114	JK176	D	A	A	A	?	D	?	D	D	A/D	A/D	D	D	D	D	D	D	A/D	A/D	D	D	
114	JK177	D	A	A	A	?	D	?	A	A/D	A/D	A/D	D	D	A/D	D	A/D	A/D	A	A/D	D	D	
114	JK178	D	A	A	A	D	D	A	D	D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
132	JK179	D	D	D	D	D	D	D	D	D	A/D	A/D	A	D	A	A	D	D	D	D	A/D	A/D	
134	JK180	D	D	D	D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	D	A	A/D	A	A	A/D	A/D	D	D	
134	JK181	D	D	D	D	D	D	D	A	A	D	D	A	D	A	A/D	A/D	A/D	A/D	A/D	D	D	
134	JK182	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	A/D	D	A	A/D	A	A	A/D	A/D	D	D	
135	JK183	D	D	A/D	A/D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	D	A	
135	JK184	D	D	A	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	D	D	A/D	A/D	D	A	
157	JK185	A/D	A/D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A/D	
157	JK186	A/D	A/D	D	D	D	D	D	D	D	D	A/D	D	D	A/D	A/D	D	D	D	D	A/D	A/D	
159	JK187	D	A/D	D	D	A	A	A	D	D	A	A	D	D	A/D	A/D	D	D	A	A	A/D	A/D	
159	JK188	D	A/D	A	A/D	D	D	D	D	D	A	A	D	D	A	A	D	D	A	A	A/D	A/D	
162	JK189	D	D	D	D	D	D	D	A/D	A/D	A/D	A/D	D	D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
146	JK190	D	D	A/D	A	A	A	A	A/D	D	A/D	A/D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	
146	JK191	D	D	A	A	A	A	A	D	D	A	A	A/D	D	A/D	A/D	D	D	A	A	D	A	
149	JK192	A	A	?	A/D	?	D	?	D	D	A	A	D	D	D	D	D	A/D	D	D	A	A	
149	JK193	A/D	A/D	?	A/D	?	D	?	A	A	A/D	A/D	D	?	D	D	A/D	A/D	D	D	A/D	A/D	
149	JK194	D	D	A/D	D	D	D	D	D	D	D	D	D	?	D	D	D	D	A/D	A/D	D	D	
151	JK195	D	A/D	D	D	D	D	A	D	D	A/D	A/D	D	D	D	D	D	D	A/D	D	D	D	
152	JK196	A	D	D	D	?	D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	D	D	
136	JK197	D	D	D	D	A	D	D	D	D	D	D	D	D	A	A	D	D	D	D	A/D	A/D	
137	JK198	A/D	A/D	D	D	D	D	D	?	A/D	A	A	D	D	A/D	A/D	A	D	D	D	D	D	

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
137	JK199	A/D	A/D	D	D	A	A	A	?	A/D	A/D	A/D	D	D	D	?	A	D	D	D	D	D	
137	JK200	A/D	D	D	D	D	D	D	?	A/D	A/D	A/D	D	D	A	A	A	D	D	D	D	D	
141	JK201	A/D	D	?	?	?	D	?	?	D	D	D	?	?	?	D	?	A	D	D	D	D	
146	JK202	D	D	A/D	A	A	A	A	?	A/D	A/D	A/D	A/D	D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	
146	JK203	D	D	A/D	A	A	A	?	?	A/D	A	A	A/D	D	A	A/D	A/D	A/D	A/D	A/D	A/D	A	
167	AV1	A	D	D	A/D	A	A	A	A/D	A/D	A/D	A/D	A/D	D	D	D	A	A	D	D	D	A	
176	AV2	A/D	A/D	A/D	A/D	A/D	D	D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	
176	AV3	A/D	A/D	A/D	A/D	A/D	D	D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	
176	AV4	A/D	A/D	A/D	A/D	A/D	D	D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	
176	AV5	A/D	A/D	A/D	A/D	A/D	D	D	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A	
177	AV6	A/D	A/D	A/D	A/D	D	A	A	A/D	D	A/D	A/D	D	D	D	D	D	D	D	D	D	A	
177	AV7	A/D	A/D	A	A	D	A	A	A/D	D	A/D	A/D	D	D	D	D	D	D	D	D	D	A	
177	AV8	A/D	A/D	A	A	D	A	A	D	D	D	A/D	D	D	D	D	D	D	D	D	D	A	
177	AV9	A/D	A/D	A/D	A/D	D	A	A	D	D	A/D	A/D	D	D	D	D	D	D	D	D	D	A	
179	AV10	D	D	D	D	D	A	A	A	A/D	A/D	A/D	A	A	A	A	A	A	D	A/D	D	A	
180	AV11	D	D	A/D	A/D	D	D	D	D	D	A/D	A/D	A	A	D	D	D	D	A	A	D	D	
187	AV12	D	D	D	D	D	D	D	A/D	D	?	A/D	A/D	A/D	A	A/D	D	D	D	A	D	D	
187	AV13	D	D	D	D	D	D	D	A/D	D	D	A/D	A/D	A/D	A	A/D	D	D	D	A	D	D	
187	AV14	D	D	D	D	D	?	D	A/D	D	D	A/D	A/D	A/D	A	A/D	D	D	D	A	D	D	
189	AV15	D	D	D	D	D	D	D	D	D	D	D	A/D	A/D	D	D	D	D	D	D	D	D	
189	AV16	A	D	D	D	D	D	D	D	A/D	A	A	D	D	A/D	A/D	A/D	A/D	D	A/D	A	A	
190	AV17	D	D	A	A	A/D	A/D	A/D	A	A	?	A	D	A	D	A	A	A	D	D	A	A	
194	AV18	D	A	A	A	D	D	D	D	A/D	A	A	D	A	A/D	A/D	A	D	A/D	A/D	D	D	
194	AV19	D	A	A	A	A/D	A/D	A/D	D	A/D	A	A	D	A/D	A/D	A/D	A	D	A/D	A/D	D	D	
194	AV20	D	A	A	A	D	D	D	D	A/D	A	A	A/D	A/D	A/D	A/D	A	D	A/D	A/D	D	D	
194	AV21	D	A	A	A	D	?	D	D	A/D	A	A	D	D	A/D	A/D	A	D	A/D	A/D	D	D	
196	AV22	A	A	D	D	A/D	A/D	A/D	A	A/D	A	A	A	A	D	D	A	A	A/D	A/D	D	D	
196	AV23	A	A	D	D	A/D	A/D	A/D	A	A/D	A	A	A	?	D	D	A	A	A/D	A/D	D	D	
197	AV24	A/D	A/D	D	D	A	A	A	A/D	?	A/D	A/D	D	D	A	A	D	D	D	D	A	D	
197	AV25	A/D	A/D	D	D	A	A	A	A/D	D	A/D	A/D	D	D	A	A	D	D	D	D	A	D	
197	AV26	A/D	A/D	D	D	A	A	A	A/D	D	A/D	A/D	D	D	A	A	D	D	D	D	A	D	
197	AV27	A/D	A/D	D	D	A	A	A	A/D	D	A/D	A/D	D	D	A	A	D	D	D	D	A	D	
a3	KaJ1	A				A/D		?			A/D	A/D					A/D	A/D					A

ND5	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
CNN02060																																	
CNN00060																																	
CNM02560																																	
CNM00630																																	
CNL06810	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
CNL04620	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A
CNK03410																																	
CNK00170																																	
CNJ02920																																	
CNJ00070																																	
CNI04370	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
CNI00070	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
CNH03360																																	
CNH00030																																	
CNG04610	A	A	A	A	?	?	A	A	A	A	A	A	?	?	A	A	A	A/D	A/D	A/D	?	?	?	?	?	?	?	?	A/D	A/D	A/D	A/D	A/D	A/D
CNG03250	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
CNG00170																																	
CNF02400																																	
CNF00290																																	
CNE03010	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	?	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D	A/D
CNE00250																																	
Individual	KaJ2	KaJ3	KaJ4	KaJ5	KaJ6	KaJ7	KaJ8	KaJ9	KaJ10	KaJ11	KaJ12	KaJ13	KaJ14	KaJ15	KaJ16	KaJ17	KaJ18	KaJ19	KaJ20	KaJ21	KaJ22	KaJ23	KaJ24	KaJ25	KaJ26	KaJ27	KaJ28	KaJ29	KaJ30	KaJ31	KaJ32	KaJ33	KaJ34
Basidium	a11	a11	a11	a11	a11	a11	a11	a11	a11	a11	a11	a11	a11	a11	a11	a13	a13	a13	a13	a13	a13	a16	a16	a16	a16	a16	a16	a20	a20	a20	a20	a20	a20

ND5	CNN02060	CNN00060	CNM02560	CNM00630	CNL06810	CNL04620	CNK03410	CNK00170	CNJ02920	CNJ00070	CNI04370	CNI00070	CNH03360	CNH00030	CNG04610	CNG03250	CNG00170	CNF02400	CNF00290	CNE03010	CNE00250	Individual	Basidium
A					A/D	A					A/D	A/D			?	A/D				A/D		KaJ35	a20
A					A/D	A					A/D	A/D			?	A/D				A/D		KaJ36	a20
A					A/D	A					A/D	A/D			A/D	A/D				A/D		KaJ37	a20
A					A/D	A					A/D	A/D			A/D	A/D				A/D		KaJ38	a20
A					A/D	A					A/D	A/D			A/D	A/D				A/D		KaJ39	a20
D					A/D	A/D					A	A			A/D	A/D				A/D		KaJ41	a11
D					A/D	A/D					A	A			A	A/D				A/D		KaJ42	a11
D					A	A					A	A			A/D	A/D				A/D		KaJ43	a11
D					A	A					A/D	A/D			A	A				A		KaJ48	a11
D					A	A					A	D			A/D	D				A/D		KaJ49	a14
D					D	D					A/D	A/D			A	?				A		KaJ50	a14
D					A	A					A/D	A/D			A	?				A/D		KaJ51	a14
D					A	A					A	D			A/D	A				A/D		KaJ52	a14
D					A	A					?	D			A/D	?				A/D		KaJ53	a14
D					A	A					?	D			A/D	?				A/D		KaJ54	a14
D					A	D					A/D	?			?	?				A		KaJ55	a14
D					?	?					A/D	?			?	?				A		KaJ56	a14
D					A	A/D					A/D	?			?	?				A		KaJ57	a14
D					A	A					A/D	A			A/D	?				A/D		KaJ58	a14
D					A/D	A/D					A	D			A/D	?				A		KaJ59	a14
D					A	A					A/D	A/D			A	A				A		KaJ60	a14
D					A/D	A/D					A	?			D	?				A		KaJ61	a14
D					A	A					A	D			A/D	A/D				A/D		KaJ62	a14
D					A/D	A/D					A/D	D			A	?				A		KaJ63	a14
D					?	A					A/D	A			?	?				A		KaJ64	a14
D					A/D	A/D					A/D	A			A/D	?				A		KaJ65	a14
D					A/D	A/D					A/D	A			A/D	?				A		KaJ66	a14
D					A	A					A	D			A/D	A				A/D		KaJ67	a14
D					?	A/D					?	A			A	?				A/D		KaJ68	a14
D					A	A					A	D			A/D	A				A/D		KaJ69	a14
A					A/D	A/D					A	A			D	?				A		KaJ70	a15
A					A/D	A/D					A/D	A/D			A/D	?				A		KaJ71	a15
A					A/D	A/D					A/D	A/D			?	D				A		KaJ72	a15

Basidium	Individual	CNE03010	CNE00250	CNF02400	CNF00290	CNG04610	CNG03250	CNG00170	CNH03360	CNH00030	CNI04370	CNI00070	CNJ02920	CNJ00070	CNK03410	CNK00170	CNL06810	CNL04620	CNM02560	CNM00630	CNN02060	CNN00060	ND5
a15	KaJ73	A				A/D	A/D				A/D	A/D					A/D	A/D					A
a15	KaJ74	A				?	A/D				?	A/D					?	?					A
a15	KaJ75	A				A	A/D				A/D	A/D					D	D					A
a15	KaJ76	A				A/D	A/D				A/D	A/D					A/D	A/D					A
a15	KaJ77	A				A/D	A/D				A/D	A/D					A/D	A/D					A
a15	KaJ78	A				A	A/D				A/D	A/D					A/D	A/D					A
a15	KaJ79	A				A/D	A/D				A/D	A/D					A/D	A/D					A
a15	KaJ80	A				A/D	A/D				A/D	A/D					A/D	A/D					A
a15	KaJ81	A				A/D	A/D				A/D	A/D					A/D	A					A
a15	KaJ82	A				A/D	A/D				A/D	A/D					A/D	A					A
a15	KaJ83	A				A/D	A/D				A/D	A/D					A/D	A/D					A
a15	KaJ84	A				D	?				A/D	A/D					D	A					A
a15	KaJ85	A				A/D	A/D				A/D	A/D					A/D	A/D					A
a16	KaJ86	A/D				?	A				D	D					A/D	A/D					A
a19	KaJ87	A/D				?	A				A/D	A/D					A/D	?					A
a19	KaJ88	A/D				A	A				A/D	A/D					A/D	A/D					A
a19	KaJ89	A/D				A	A				A/D	A/D					A/D	A/D					A
a19	KaJ90	A/D				?	?				?	?					D	?					A
a19	KaJ91	A/D				?	D				A	?					D	?					A
a19	KaJ92	A/D				A	A				A/D	A/D					A/D	A/D					A
a19	KaJ93	A/D				A	A				A/D	A/D					A/D	A/D					A
a19	KaJ94	A/D				A	A				A/D	A/D					A/D	A/D					A
a19	KaJ95	A/D				A	A				A/D	A/D					A/D	A/D					A
a21	KaJ96	A				A	A				A/D	A/D					A/D	A/D					A
a21	KaJ97	A/D				A	A				A/D	A/D					A	A					A
a21	KaJ98	A/D				A	A				A	A					A	A					A
a21	KaJ99	A				A/D	A/D				A/D	A/D					A/D	A/D					A
a21	KaJ100	A/D				A	A				A/D	A/D					A	A					A
a21	KaJ101	A/D				A	A				A/D	A/D					A	A					A
a21	KaJ102	A/D				A	A				A	A					A	A					A
a21	KaJ103	A				?	?				A/D	A/D					?	A/D					A
a21	KaJ104	A				A/D	A/D				A	A					A/D	A/D					A
a21	KaJ105	A/D				A	A				A	A					A	A					A

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
a21	KaJ106		A				A	A			A/D	A/D					A	A					A
a21	KaJ107		A/D				A	A			A	A					A	A					A
a21	KaJ108		A/D				A	A			A	A					A	A					A
a21	KaJ109		A				A/D	?			A/D	A/D					A	A/D					A
a21	KaJ110		A/D				A	A			A	A					A	A					A
a21	KaJ111		A/D				A	A			A	A					A	A					A
a21	KaJ112		A/D				A	A			A	A					A	A					A
a21	KaJ113		A/D				A	A			A/D	A/D					A	A					A
a21	KaJ114		A/D				A	A			A	A					A	A					A
a21	KaJ115		?				A	A			A/D	A/D					A	A					A
a21	KaJ116		A/D				A	A			A	A					A	A					A
a21	KaJ117		A				A/D	A/D			A/D	A/D					A	A/D					A
a21	KaJ118		A				A	?			A/D	A/D					A	A/D					A
a21	KaJ119		A				A/D	A/D			A/D	A/D					A	?					A
a22	KaJ120		D				A/D	A/D			A/D	A/D					A/D	?					A
l1	KlJ1		A/D				A/D	A/D			A/D	A/D					A/D	A/D					D
l1	KlJ2		?				A/D	?			A/D	A/D					A/D	?					D
l1	KlJ3		?				A	?			A/D	A/D					A	?					D
l1	KlJ4		?				A/D	?			A/D	A/D					A/D	?					D
l1	KlJ5		A/D				A/D	A/D			A/D	A/D					A/D	A/D					D
l1	KlJ6		A/D				A/D	A/D			A/D	A/D					A/D	A					D
l1	KlJ7		A/D				A/D	A/D			A/D	A/D					A/D	A/D					D
l3	KlJ8		?				A	A			A	A					A	A					D
l3	KlJ9		A				A/D	?			A	A					A	A					D
l3	KlJ10		A				A/D	A/D			A	A					A	A/D					D
l3	KlJ11		A				A	?			A/D	A/D					A	?					D
l3	KlJ12		A/D				A	A			A	A					A	?					D
l3	KlJ13		A				A	A			A/D	A/D					A	?					D
l3	KlJ14		A				A	A			A/D	A/D					A	?					D
l3	KlJ15		A/D				A	A			A	A					A	A					D
l4	KlJ16		A				A/D	A/D			A	A					D	D					D
l4	KlJ17		A/D				A/D	A/D			A	A					D	A					D
l4	KlJ18		A/D				A	A			A	A					D	D					D

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
l12	KlJ19		A/D				D	D			A/D	A/D					D	D					D
l12	KlJ20		A/D				D	D			A/D	A/D					D	D					D
l12	KlJ21		A/D				D	D			A/D	A/D					D	D					D
l8	KlJ22		A/D				A/D	A/D			A/D	A/D					A/D	?					D
l9	KlJ23		A/D				A/D	D			A/D	A/D					A/D	?					D
l9	KlJ24		A/D				A/D	?			?	A					?	?					D
l9	KlJ25		A/D				A/D	?			A/D	A/D					?	?					D
l9	KlJ26		A/D				A/D	?			A/D	A/D					?	?					D
l9	KlJ27		A/D				A/D	?			A/D	A/D					?	?					D
l9	KlJ28		A/D				A/D	?			A/D	A/D					?	?					D
l10	KlJ29		D				D	D			A/D	A/D					A/D	A/D					D
l10	KlJ30		D				D	D			A/D	A/D					A/D	A/D					D
l10	KlJ31		D				D	?			A/D	A/D					?	?					D
l10	KlJ32		D				D	D			A/D	A/D					A/D	A/D					D
l11	KlJ33		A/D				A/D	A/D			A/D	A/D					A/D	A/D					D
l11	KlJ34		A/D				A/D	A/D			A/D	A/D					A/D	A/D					D
l14	KlJ35		A/D				A/D	A/D			A/D	A/D					?	?					D
l14	KlJ36		A/D				A/D	A/D			A/D	A/D					?	?					D
l14	KlJ37		A/D				A/D	A/D			A/D	A/D					A/D	?					D
l14	KlJ38		A/D				A/D	?			A/D	A/D					?	?					D
l14	KlJ39		A/D				A/D	A/D			A/D	A/D					?	?					D
l14	KlJ40		A/D				A/D	A/D			A/D	A/D					?	?					D
l14	KlJ41		A/D				A/D	A/D			A/D	A/D					?	?					D
l14	KlJ42		A/D				A/D	A/D			A/D	A/D					?	?					D
l15	KlJ43		A				A	A			A/D	A/D					A/D	A					D
l15	KlJ44		A				A	?			A/D	A/D					A/D	?					D
l15	KlJ45		D				A/D	A/D			A/D	A/D					D	D					D
l15	KlJ46		A				A	A			A/D	A/D					A/D	A/D					D
l15	KlJ47		A				A	A			A/D	A/D					A/D	A/D					D
l15	KlJ48		A				A	?			A/D	A/D					?	?					D
l15	KlJ49		A				A	A			A/D	A/D					A/D	?					D
l15	KlJ50		A				A	A			A/D	A/D					A/D	?					D
l15	KlJ51		A				A	?			A/D	A/D					?	?					D

Basidium	Individual	CNE00250	CNE03010	CNF00290	CNF02400	CNG00170	CNG03250	CNG04610	CNH00030	CNH03360	CNI00070	CNI04370	CNJ00070	CNJ02920	CNK00170	CNK03410	CNL04620	CNL06810	CNM00630	CNM02560	CNN00060	CNN02060	ND5
115	KlJ52		A				A	?			A/D	A/D					?	?					D
115	KlJ53		A				A	A			A/D	A/D					?	?					D
115	KlJ54		A				A	A			A/D	A/D					?	?					D
115	KlJ55		A				A	A			A/D	A/D					?	A					D
118	KlJ56		D				A	A			A	A					?	A					D
119	KlJ57		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ58		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ59		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ60		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ61		A/D				?	?			?	A/D					?	?					D
119	KlJ62		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ63		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ64		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ65		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ66		A/D				?	?			A/D	A					A/D	A/D					D
119	KlJ67		A/D				?	?			A/D	A					A/D	A/D					D
119	KlJ68		A/D				?	?			A/D	A					A/D	A/D					D
119	KlJ69		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ70		A/D				?	D			A/D	A					A/D	A/D					D
119	KlJ71		A/D				?	?			A/D	A					?	?					D
120	KlJ72		D				?	?			?	A/D					?	?					D
120	KlJ73		D				?	?			A/D	A/D					?	A/D					D
120	KlJ74		D				?	A/D			A/D	A/D					A/D	A/D					D
120	KlJ75		D				?	?			A/D	A/D					?	?					D
120	KlJ76		D				?	?			A/D	A/D					?	?					D
120	KlJ77		D				?	A/D			A/D	A/D					A/D	A/D					D
120	KlJ78		D				?	A			A/D	A/D					A/D	A/D					D
120	KlJ79		D				?	A			A/D	A/D					A/D	D					D
120	KlJ80		D				?	?			A/D	A/D					A/D	?					D
126	KlJ81		A/D				?	D			D	D					D	D					D
126	KlJ82		D				?	D			D	A/D					A/D	A/D					D
126	KlJ83		D				?	A			A	A/D					D	D					D
126	KlJ84		D				?	A/D			A	A/D					A/D	A/D					D

ND5	CNN02060	CNN00060	CNM02560	CNM00630	CNL06810	CNL04620	CNK03410	CNK00170	CNJ02920	CNJ00070	CNI04370	CNI00070	CNH03360	CNH00030	CNG04610	CNG03250	CNG00170	CNF02400	CNF00290	CNE03010	CNE00250	Individual	Basidium
D					A/D	A/D					A/D	D			D	?				D		KlJ85	126
D					A/D	A/D					A/D	A			A/D	?				D		KlJ86	126
D					?	?					D	D			?	?				A/D		KlJ87	126
D					?	A/D					A	A			D	?				D		KlJ88	126
D					?	A/D					A/D	D			D	?				D		KlJ89	126
D					?	D					A/D	A			D	?				D		KlJ90	126
D					?	A/D					A/D	A			A/D	?				D		KlJ91	126
D					?	?					A/D	A			?	?				?		KlJ92	126
D					?	A/D					A/D	A			?	?				D		KlJ93	126
												AD								AD		CDC-26	Natural Hybrids
												?								?		CDC-46	
												?								?		CDC-47	
												AD								AD		CDC-62	
												AD								AD		CDC-66	
												?								?		CDC-74	
												AD								D		CDC-174	
												AD								AD		CDC-190	
												AD								AD		CDC-228	
												AD								A		CDC-280	
												D								D		CDC-304	
												?								?		CDC-328	
												?								?		CDC-354	
												?								?		CDC-355	

3.7 Acknowledgements

Thanks to Jordan Khankhet for assistance with DNA extraction and genotyping of the hybrid progeny. Financial support for this project was provided by the Ontario Graduate Scholarship Council (A.V.) and the Natural Sciences and Engineering Research Council (NSERC) of Canada (J.X.).

Chapter 4

Identification of QTLs associated with virulence and drug resistance traits

4.1 Preface

Three main attributes allow *Cryptococcus* to infect humans: the production melanin, the presence of a polysaccharide capsules, and growth at high temperature. However, many environmental strains cannot cause infection and even clinical strains show a large amount variance in these traits. To better understand the genetics of virulence in *Cryptococcus*, a QTL analysis of a hybrid cross was employed. This allowed us to combine phenotypic data with the genotypes of the progeny from the cross to define genomic regions which contribute to the given trait. JEC20, a laboratory strain of *C. deneoformans* and CDC15, a clinical isolate, were used as parents for the cross.

This work has been submitted to G3: Genes | Genomics | Genetics for review.

I am the primary contributor of this work. The majority of the experiments were conducted by me as well as the analyses and writing of the manuscript.

Abstract

Cryptococcus neoformans is a basidiomycete fungus capable of causing deadly meningoenchephilitis, primarily in immunocompromised individuals. Formerly, *C. neoformans* consisted of two divergent lineages, but these have recently been elevated to species status, now *C. neoformans* (formally *C. neoformans* var. *grubii*) and *C. deneoformans* (formally *C. neoformans* var. *neoformans*). While both species can cause deadly infections in humans, *C. neoformans* is much more prevalent in clinical settings than *C. deneoformans*. However, the genetic factors contributing to their significant differences in virulence remain largely unknown. QTL mapping is a powerful tool which can be used to identify genomic regions associated with given quantitative traits. Here we analyzed a hybrid cross between these two species and identified a total of 23 QTLs, including five for melanin production, six for cell size, one for cell wall thickness, five for the frequency of capsule production, three for minimal inhibitory concentration (MIC) of fluconazole in broth, and three for MIC on agar. Only two of these QTLs correspond to a gene region known to influence the expression of the specified trait, the rest represent novel regions. For the fluconazole resistance associated QTLs, three showed environment- and/or concentration-specific effects. Our results provide a large number of candidate gene regions from which to explore the genetic bases for phenotypic differences between *C. neoformans* and *C. deneoformans*.

4.2 Introduction

Human fungal pathogens are relatively unique among disease-causing organisms. Unlike many bacterial and non-fungal eukaryotic pathogens, the fungi which cause the

largest global burden of infectious disease are not obligate pathogens, but opportunistic invaders. The species which inflict the heaviest toll on human life are from the genera *Aspergillus*, *Candida*, and *Cryptococcus*, which together cause upwards of 1.6 million life threatening infections annually (Brown *et al.*, 2012). *Candida* species are generally human commensals while *Cryptococcus* and *Aspergillus* species are commonly found in the environment. While these fungal pathogens typically infect immunocompromised individuals, some strains of *Cryptococcus* have caused infections in many apparently healthy individuals (Kidd *et al.*, 2004; Chen *et al.*, 2008). The details of what influences the transition from an environmental microbe to a pathogenic one are laregly unknown and *Cryptococcus* provides an ideal system to examine this question.

Cryptococcus neoformans is a basidiomycetous yeast capable of causing various forms of disease, including deadly meningoencephilitis (Mitchell and Perfect, 1995). Recently, *C. neoformans* was divided into two separate species, *C. neoformans* (formally *C. neoformans* var. *grubii* or serotype A) and *Cryptococcus deneoformans* (formally *C. neoformans* var. *neoformans* or serotype D) (Hagen *et al.*, 2015). *C. neoformans* is much more prevalent than *C. deneoformans* among clinical isolates, commonly causes systemic infection and is more viurlent in a mouse model (Kwon-Chung *et al.*, 1992), whereas *C. deneoformans* is more likely to cause cutaneous infection (Dromer *et al.*, 2007). Both species have been isolated throughout the globe, although *C. neoformans* originated in Africa (Litvintseva and Mitchell, 2012), and *C. deneoformans* possibly originated in Europe (Boekhout *et al.*, 2001). In addition to these differences, *C. deneoformans* appears to be more sensitive to cationic stress (Cruz *et al.*, 2000) and may be more susceptible to some anti-fungal drugs (Dannaoui *et al.*,

2006), though there are conflicting reports on this topic (Thompson *et al.*, 2008).

It is estimated that the two species diverged from each other approximately 18.5 million years ago (Xu *et al.*, 2000a). Despite their long history of divergence, hybridization can occur readily between them and appears to be quite prevalent in nature. Specifically, A/D hybrids are frequently isolated from both environmental and clinical settings, and in fact the holotype for *C. neoformans* was revealed to be an hybrid (Hagen *et al.*, 2015). Phylogenetic analyses revealed recent and ongoing hybridizations (Xu *et al.*, 2002; Litvintseva *et al.*, 2007), although there is evidence of ancient hybridization events as well (Kavanaugh *et al.*, 2006). Investigations into natural and laboratory hybrids have revealed a number of anomalies in the hybrids, including aneuploidy, abortive germination and suppressed recombination (Lengeler *et al.*, 2001; Sun and Xu, 2007; Vogan *et al.*, 2013; Vogan and Xu, 2014).

The production of melanin and capsule, and the ability to grow at high temperatures are necessary for virulence in *Cryptococcus* (Wang *et al.*, 1995; Kwon-Chung and Rhodes, 1986; Steenbergen *et al.*, 2001; Odom *et al.*, 1997). Additionally, the mating type may contribute significantly to the severity of infection (Barchiesi *et al.*, 2005), with the specifics differing between the species (Chang *et al.*, 2000; Yue *et al.*, 1999; Wang *et al.*, 2002). Since both species possess and require the same virulence factors, the presence of conserved virulence factors are not sufficient to explain the different clinical prevalence of the two species. However, *C. neoformans* and *C. deneoformans* may differ quantitatively in these traits. Understanding the phenotypic differences between strains of the two species and the genetic bases for such differences could contribute significantly to our understanding of their evolution. QTL mapping is a

powerful approach for identifying the genomic regions that contribute to a phenotype of interest. For example, QTL mapping has been used to elucidate the genetics of pathogen resistance in crops and in addition, has been successfully applied to the study of the pathogens themselves (Lin *et al.*, 2006; Lind *et al.*, 2007; Li *et al.*, 2011; Morrison *et al.*, 2009; Christians *et al.*, 2011; Saeij *et al.*, 2006).

Here, we have conducted a cross between *C. neoformans* and *C. deneoformans*, and collected 230 hybrid offspring. We have genotyped the progeny using 73 co-dominant markers and constructed a linkage map. We have measured quantitative traits for the known virulence factors of melanin and capsule production, as well as cell wall thickness and cell size. Since the two parental strains differ in their susceptibility towards the antifungal drug Fluconazole, we have also evaluated the growth of the progeny at a variety of Fluconazole concentrations on both agar plates and in liquid media. The aim of this study is to identify genomic regions which contribute to the differences in virulence between *C. neoformans* and *C. deneoformans*. Ultimately this will help guide the way to a better understanding of the quantitative nature of virulence factors in *Cryptococcus*.

Methods

Mapping Population

Parental strains JEC20 (*C. deneoformans*, MATa) and CDC15 (*C. neoformans*, MATα) were mated on V8 agar for $\sim$4 weeks. Basidiospores were dissected onto YEPD agar using a Singer MSM 300 micromanipulator. Basidiospores were allowed to germinate

over the following two weeks at room temperature. 230 colonies were collected to generate the mapping population in this study (Vogan *et al.*, 2013; Vogan and Xu, 2014).

Melanin Production

Strains were grown on YEPD agar for 2 d to 7 d at 30 °C. Fresh cells were collected to generate cell suspensions with $\sim 1 \times 10^6$ cells/ml. Suspensions were then inoculated onto caffeic acid agar (Hopfer and Blank, 1975) with four replicates per progeny per plate and allowed to grow for three days at 30 °C. Melanin production was approximated by light emission using the Spot Densitometry function of a FluoroChem 8900. Darker colonies with more melanin reflect less light than lighter colonies. To minimize batch effects, parental strains were used as references in each of our assays to help standardize the data for analyses.

Cell Size, Cell Wall, and Capsule Measurements

Strains were grown in Sabouraud dextrose (SD) broth for 1 day at 30 °C. 10 µl of the liquid culture were diluted into 190 µl of 10 % SD (inducing medium (Liu *et al.*, 2008)), buffered with 0.165 M MOPS to pH 7.3 and allowed to grow for 2 d. Slides were made with 5 µl of cell suspension and 9 µl of nigrosin counterstain (in lieu of india ink). Images were taken at 1600× magnification on an Olympus IX81 Microscope. Measurements of cell area, cell wall area, and capsule were made by manually fitting ellipses to the cells using the software ImageJ (Abramoff *et al.*, 2004). Approximately 25 cells were measured per strain.

Fluconazole Assays

Strains were grown on YEPD agar for 2 d at 30 °C and then transferred to either YEPD agar or RPMI for solid and liquid assays respectively. Concentrations of 0 µg/ml, 0.5 µg/ml, 1.0 µg/ml, 2.0 µg/ml, 4.0 µg/ml, 8.0 µg/ml, 16.0 µg/ml, 32.0 µg/ml and 64.0 µg/ml of Fluconazole were evaluated. For solid medium assays, Fluconzole was filter sterilized and added to media prior to pouring of the plates. Growth was determined by measuring the diameter of ∼10 colonies per strain after 2 d of growth at 30 °C. For liquid medium assays, strains were grown in 200 µl of RPMI in 96-well plates. The OD_{600} of wells was measured at the beginning of the experiment and at 72 h, the difference was taken to determine growth over this time period. The MIC for growth on agar was determined as the minimum concentration of Fluconazole at which no colonies were observed by the naked eye. The MIC for growth in broth was determined as the concentration of Fluconazole where a 90 % reduction in OD_{600} (as compared to a control with no drug) was observed. An additional concentration of 128 µg/ml was evaluated for this purpose due to a high degree of Fluconazole resistance.

Genotyping

DNA was extracted from strains using standard chloroform - isoamyl alcohol methodology (Xu *et al.*, 2000a). Seventy-four co-dominant PCR-RFLP markers were used to determine genotypes for all progeny. Markers were either taken from (Sun and Xu, 2007; Vogan *et al.*, 2013; Vogan and Xu, 2014), or designed using Prifi (Fredslund *et al.*, 2005) (Supplementary Table 4.S1).

QTL Analysis

The R package R/qtl was utilized for all QTL analyses(Broman *et al.*, 2003). A linkage map was created with markers assigned chromosomes based on their location in the reference genome JEC21 (Loftus *et al.*, 2005). One marker on Chromosome 8 was given its own linkage group since it is within a translocation between JEC20 and CDC15 (Sun and Xu, 2009). Multiple interval mapping (MIM) was used to evaluate the presence of QTLs. Haley-Knott regression was employed with a step size of 0.2 cM. A LOD significance threshold of α=0.05 was determined using 1000 permutations. MQM was conducted to determine QTLs at specific concentrations of Fluconazole (see Fluconazole Assay for details). Cofactor significance was set to 0.002 (As recommended in the manual for conservative analysis) and LOD threshold values were calculated with 1000 permutations. For both MIM and MQM, models were built using the commands `fitqtl` and `refineqtl` to refine the positions of QTLs and determine their significance to the model. Composite interval mapping (CIM) was also explored and found to largely be in agreement with the results from MIM.

4.3 Results

All phenotypes were shown to be quantitative traits with at least one QTL contributing to the trait value differences between the two parental strains. (Figure 4.1). The only exception was capsule size/thickness as we were unable to identify any significant QTLs affecting this trait. However, multiple QTLs were identified for the frequency of capsule production. A summary of all QTLs identified in this study are listed in Table 4.1. Five QTLs were identified for melanin production, six for cell size, one for

cell wall size, five for the frequency of capsule production, three for MIC in broth, and three for MIC on agar for a total of 23 QTLs. For simplicity, CDC15 alleles are annotated as A alleles (representing serotype A) and JEC20 alleles as D alleles (representing serotype D).

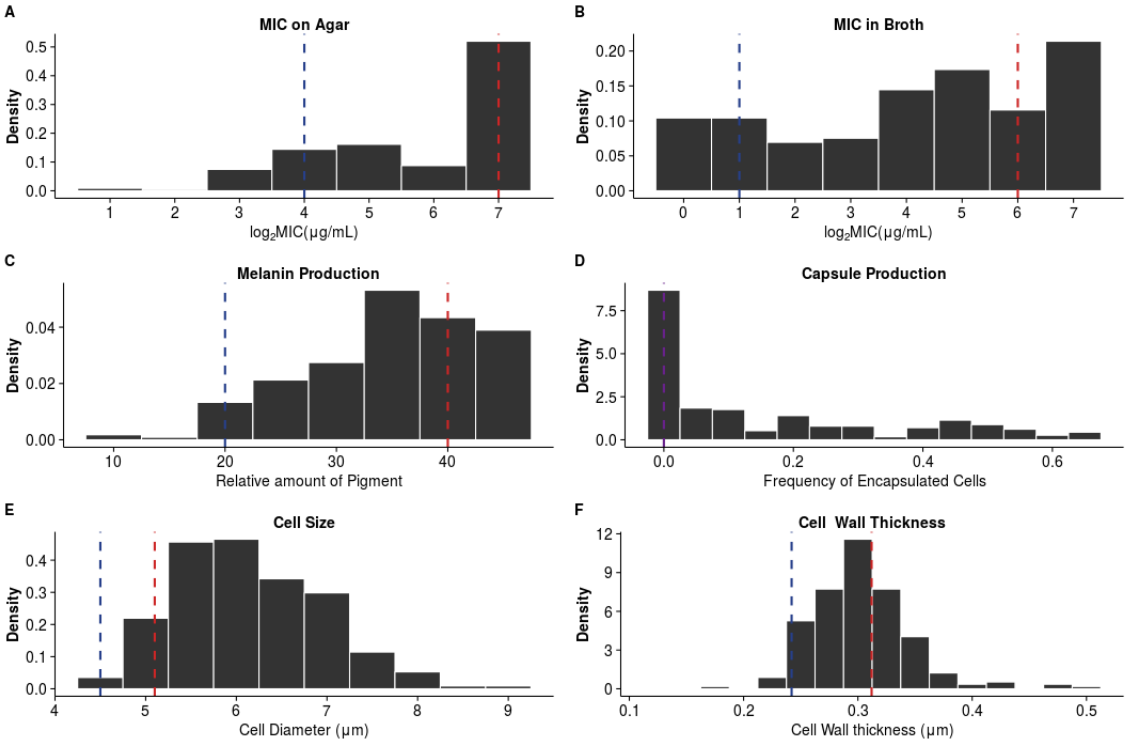

Figure 4.1: Distribution of virulence traits. Dashed lines represent the means of the parental strains JEC20 (Blue) and CDC15 (Red) or both (Purple).(A) MIC on YEPD agar. (B) MIC in liquid RPMI media. (C) Amount of melanin produced after 3 days. (D) Percent of cells which produce capsule after 2 d in inducing media. (E) Cell diameter (μm). (F) Cell wall thickness (μm).

Table 4.1: Location of QTLs identified in this study and the amount of phenotypic variance they explain.

Trait	Chromosome	Position (cM)	Nearest Marker	% PVE*	Total % PVE*
Melanin	A	15.6	CNA03020	15	49
	B	15.0	CNB03520	16	
	G	3.6	CNG01240	10	
	L	0.0	CNL03990	13	
	L	9.4	CNL06810	12	
Cell Size	A	35.7	CNA06130	8	51
	A	54.2	CNA073100	8	
	A	64.0	CNA07990	14	
	D	41.6	RUM1	5	
	F	1.2	CNF00290	13	
	I	2.6	CNI01350	8	
Cell Wall Size	A	14.6	CNA02700	8	8
Capsule	A	0.0	CNA00050	5	43
	B	0.6	CNB00360	9	
	D	41.9	CND06160	11	
	H	0.0	CNH00030	7	
	L	2.2	CNL04620	3	
MIC on agar	A	4.2	CNA00290	36	46
	E	7.8	CNE01630	3	
	N	15.0	CNN02060	4	
MIC in Broth	A	4.2	CNA00290	14	43
	A	59.8	CNA07470	11	
	C	9.4	CNC06110	4	

*PVE = Percent of phenotypic variance explained

4.3.1 Melanin

Average melanin production of JEC20 was significantly lower than that of CDC15 after 7 days ($p=1.4 \times 10^{-6}$) (Figure 4.1). Among the progeny, both hybrid vigour and hybrid depression were observed, with five strains appearing to be amelanotic. Five QTLs were identified for the production of melanin around markers CNA03050, CNB03520, CNG01240, CNL03390, and CNL06810, which together explain 49 % of the phenotypic variance. For the QTL at marker CNG01240, strains which are homozygous for A alleles or heterozygous produce more melanin than strains which are homozygous for the D allele. The QTLs at markers CNA03050 and CNB03520 exhibit an interaction effects whereby strains which are heterozygous at both markers produce more melanin than all other allelic combinations. However, strains which are heterozygous at CNA03050, but homozygous A at CNB03520 show the lowest amount of melanin production. QTLs by markers CNL03390 and CNL06810 also show an interaction effect, however all allelic combinations display roughly equal amounts of melanin production except for strains that are heterozygous at CNL03390 and homozygous A at CNL06810. A closer inspection of the data reveals that only one strain has the aforementioned allelic combination, thus this result may be a false positive (Figure 4.2).

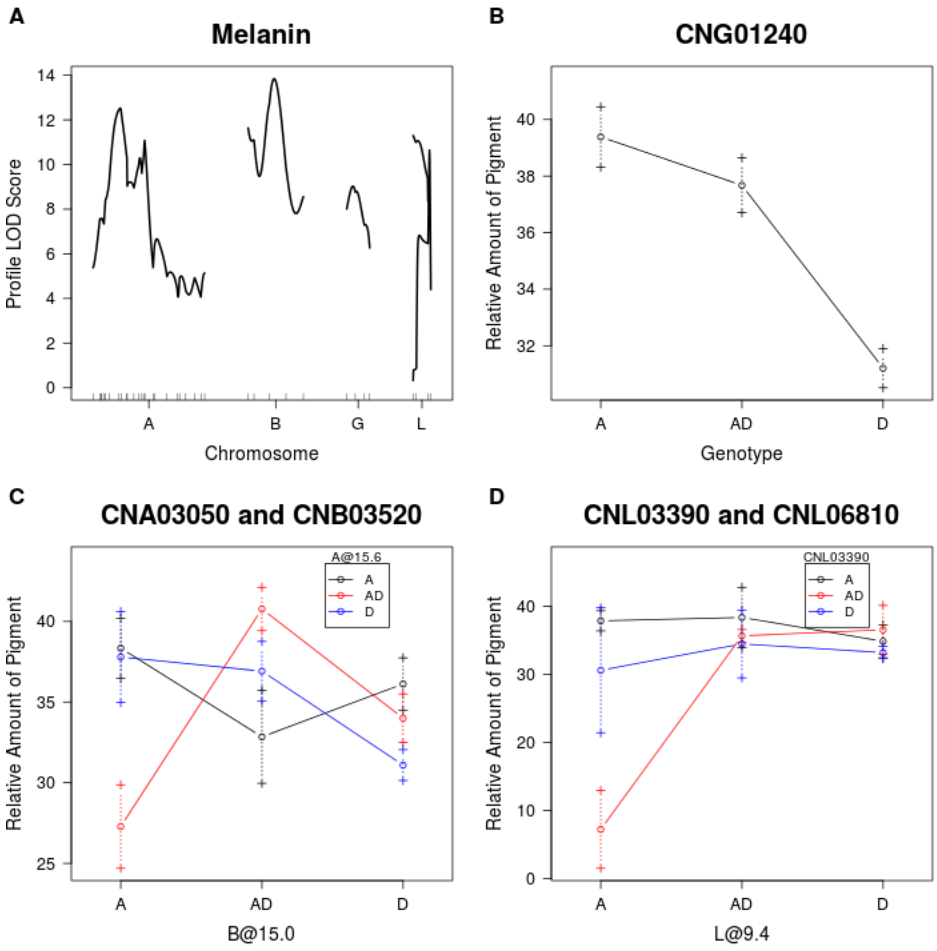

Figure 4.2: **A** QTLs identified for melanin production. **B - D** Average melanin production for given genotypes / genotypic combinations at markers closest to QTLs. Dashed lines represent standard error in the mean.

4.3.2 Cell Size

Cell diameter differed significantly between JEC20 and CDC15 when they were grown under inducing conditions (p=0.001) (see methods) (Figure 4.1). Six QTLs were identified, around markers CNA06130, CNA07310, CNA07990, RUM1, CNF00290, and CNI01350. The full model explains 51 % of phenotypic variance. Strains that are homozygous A or heterozygous at marker CNA06130 are larger than those which are homozygous D. At RUM1, strains that are heterozygous are larger than other genotypes. QTLs at markers CNA07310 and CNI01350 show an interaction effect where strains that are heterozygous at CNA07310 and heterozygous or homozygous A at marker CNI01350 are larger than strains of other genotypic combinations. QTLs at markers CNA07990 and CNF00290 also show an interaction effect. Strains which are heterozygous at both loci, heterozygous at CNF00290 and homozygous D at CNA07990, or heterzygous at CNA07990 and homozygous D at CNF00290 have the greatest cell sizes. Strains which are homozygous D at both markers have the smallest cell sizes (Figure 4.3).

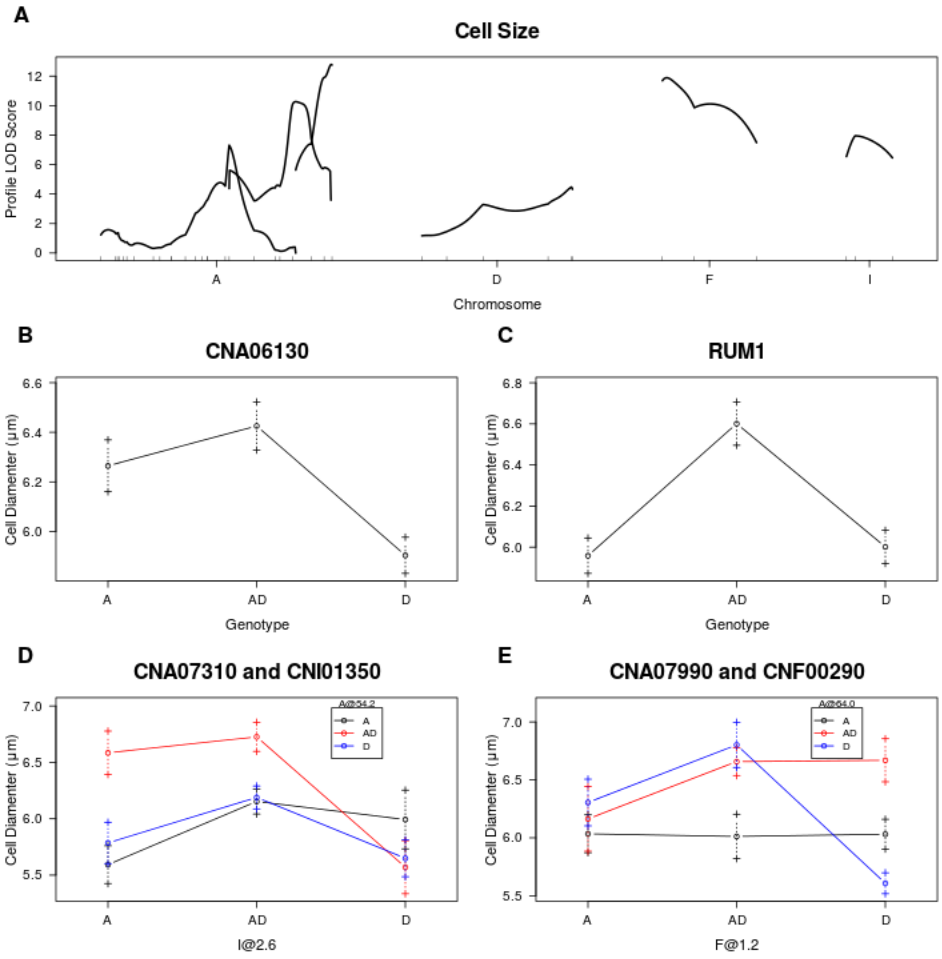

Figure 4.3: **A** QTLs identified for cell size. **B - F** Average cell diameter for given genotypes / genotypic combinations at markers closest to QTLs. Dashed lines represent standard error in the mean.

4.3.3 Cell Wall Thickness

Cell wall thickness was found to differ significantly between JEC20 and CDC15 ($p=4.8 \times 10^{-6}$) (Figure 4.1). It was also observed that for a given strain, larger cells produced thicker cell walls, although these two traits were not statistically correlated with each other when all the 230 hybrid progeny were analysed. Under non-inducing conditions, cell wall thickness has been reported as $\sim$0.05 µm (Feldmesser *et al.*, 2001). We observed a range of values from 0.17 a to 0.49 across all strains, indicating a 4 to 10 fold increase in cell wall thickness over non-inducing conditions. A single QTL was identified on chromosome A at marker CNA02700 that explains 8 % of the phenotypic variance. Strains that are heterozygous or homozygous D at this locus have thicker cell walls than those that are homozygous A (Figure 4.4).

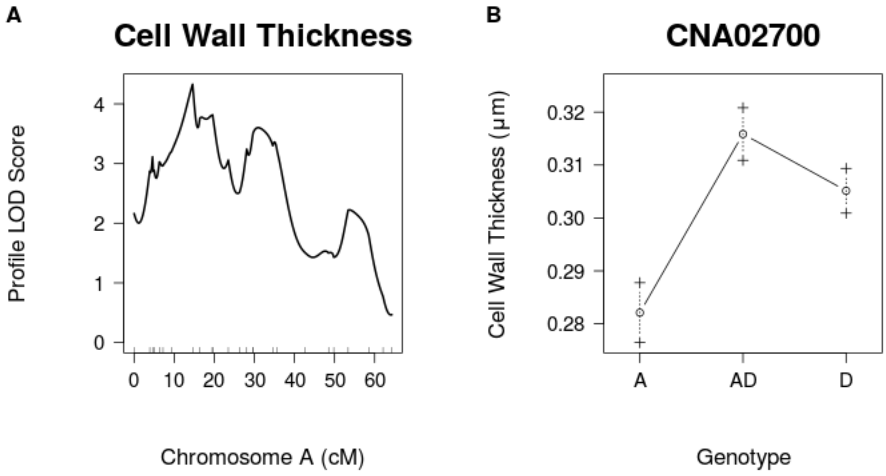

Figure 4.4: **A** LOD score profile for a QTL associated with cell wall thickness. **B** Average cell wall thickness for given genotypes at marker CNA02700. Dashed lines represent standard error in the mean.

4.3.4 Capsule Production

Under non-inducing conditions, no capsule was produced by either JEC20 or CDC15. Under inducing conditions there was still no capsule production after 2 d for the two parental strains, however after 7 d minimal capsule could be observed around CDC15 cells as a fuzzy halo. It was not possible to measure this small amount of capsule with the techniques applied here. In the progeny, capsule production was very heterogeneous. Many strains produced some cells that had large quantities of capsule after only 2 d, while other cells showed no capsule. When capsule area was used as a phenotypic trait, measured only for cells that produced at least some measurable amount of capsule and controlled for cell size, no QTLs were identified. When the frequency of capsule production, that is the proportion of cells which produced at least some measurable amount of capsule per strain, was analyzed as a quantitative trait, five QTLs were identified. These were around markers CNA00050, CNB00360, CND06160 CNH00030, and CNL04620, which together explain 43 % of the phenotypic variance. At marker CNA00050, strains which are homosygous A or heterozygous produce capsule at a higher frequency than those which are homozygous D. At markers CND06160 and CNH0030, strains which are heterozygous produce capsule more frequently than those which are homozygous for either parental allele. QTLs by markers CNB00360 and CNH0030 show an interaction effect where strains which are heterozygous at both markers, homozygous A at both markers, or homozygous A at one marker and heterozygous at the other marker produce capsule more frequently than strains which are homozygous D at both markers, or heterozygous at CNH00030 and homozygous D at CNB00360. (Figure 4.5).

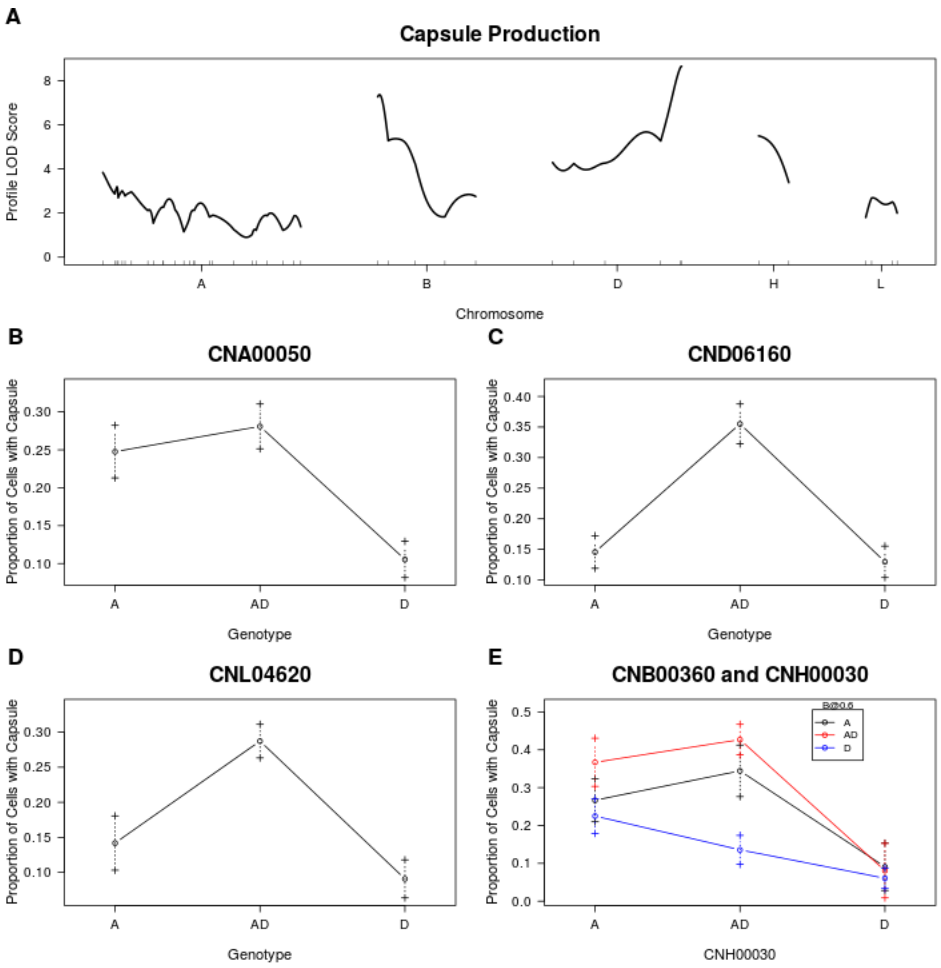

Figure 4.5: **A** QTLs identified for capsule production. **B - E** Average proportion of cells producing capsule for given genotypes / genotypic combinations at markers closest to QTLs. Dashed lines represent standard error in the mean.

4.3.5 Fluconazole Resistance

JEC20 has an MIC of 16 µg/ml on agar and an MIC of 4 µg/ml in broth for Fluconazole. CDC15 has an MIC on agar of 128 µg/ml and an MIC in broth of 32 µg/ml for Fluconazole. The progeny generally exhibited hybrid depression for Fluconazole resistance, however a number of strains had MICs of 128 µg/ml in broth (Figure 4.1). Three QTLs were identified for MIC on agar at markers CNA00290, CNE01630, and CNN02060. The QTL at CNA00290 has the largest effect by far, explaining 36 % of the phenotypic variance on its own. By contrast the model as a whole explains 46 % of the phenotypic variance. Progeny which are homozygous A or heterozygous at markers CNA00290 and CNE01630 have a higher MIC than those which are homozygous D at this marker. Strains that are homozygous A at CNN02060 have higher MICs than those which are heterozygous (Figure 4.6).

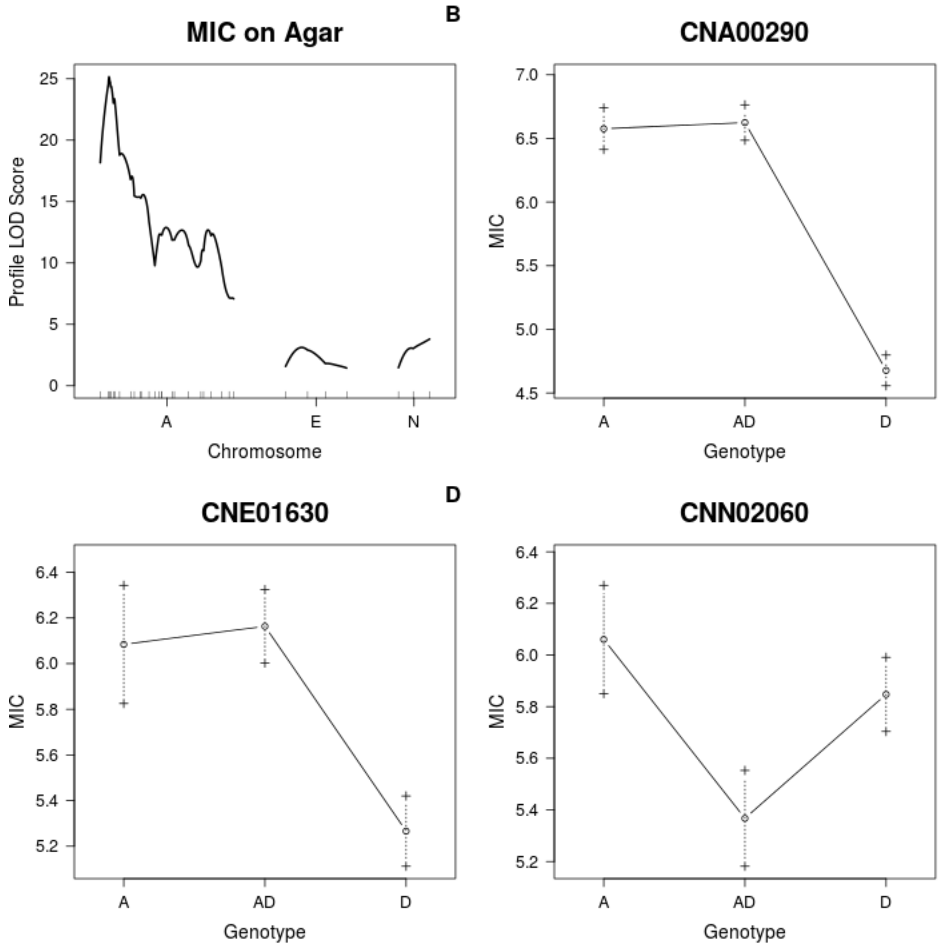

Figure 4.6: **A** QTLs identified for Fluconazole resistance on agar. **B - D** Average MIC when grown on agar for given genotypes / genotypic combinations at markers closest to QTLs. Dashed lines represent standard error in the mean.

The results from broth also showed three QTLs. Again there was one at CNA00290. The other two were different from those identified based on data from agar plates, located by markers CNA07470 and CNC06110. The full model explains 44 % of phenotypic variance. All markers show a similar trend, whereby strains that are homozygous A or heterozygous for a given marker have a higher MIC than those which are homozygous D (Figure 4.7).

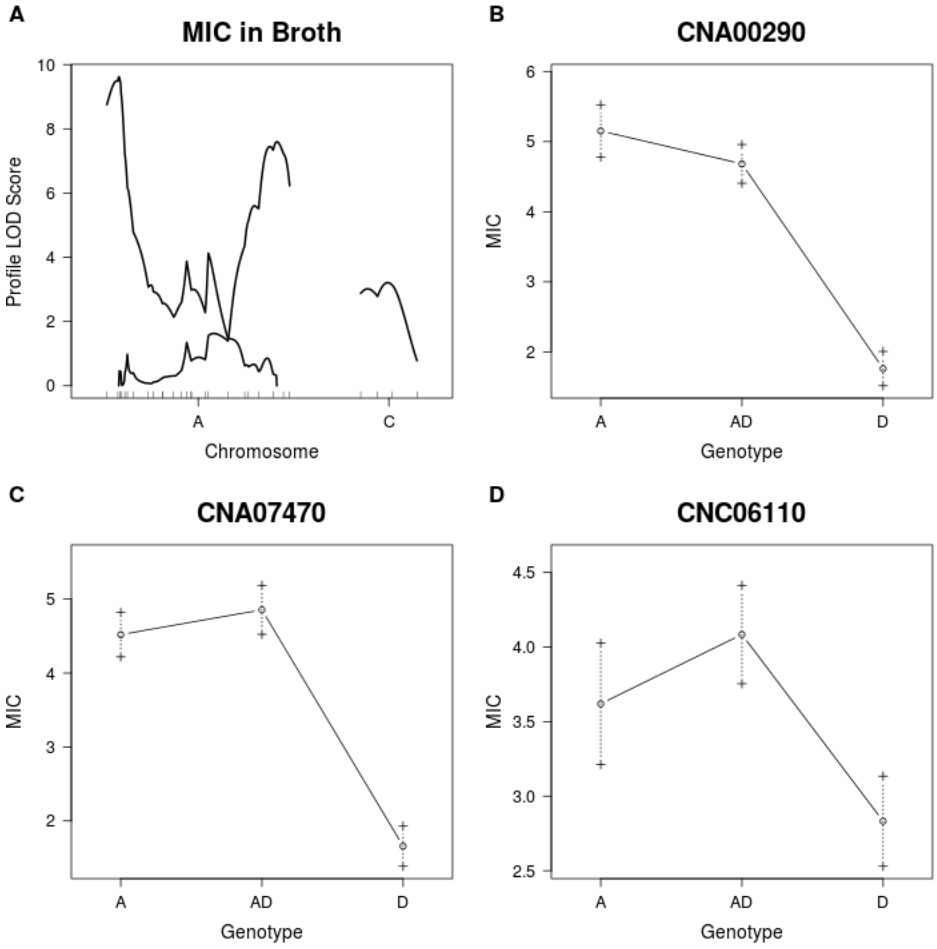

Figure 4.7: **A** QTLs identified for Fluconazole resistance in broth. **B - D** Average MIC when grown in broth for given genotypes / genotypic combinations at markers closest to QTLs. Dashed lines represent standard error in the mean.

The results from the MQM analysis agreed well with the results from the MIM evaluation of MIC. For growth on YEPD agar with Fluconazole, marker CNA00290 shows the highest LOD score at concentrations above 1.0 µg/ml with the rest of the markers contributing very little (Figure 4.8). At 1.0 µg/ml of Fluconazole, marker CNA05300 shows the highest LOD score, however, at 0.5 µg/ml CNA00290 once again shows the highest LOD score. For growth on RPMI with Fluconazole, marker CNA00290 has the highest LOD scores for concentrations of 0.5 µg/ml to 16.0 µg/ml with a peak LOD score at 2.0 µg/ml (Figure 4.9). No QTLs were observed at 0 µg/ml, 64.0 µg/ml and 128.0 µg/ml. In agreement with the MIM results in broth, at 32.0 µg/ml marker CNA07470 has the highest LOD score.

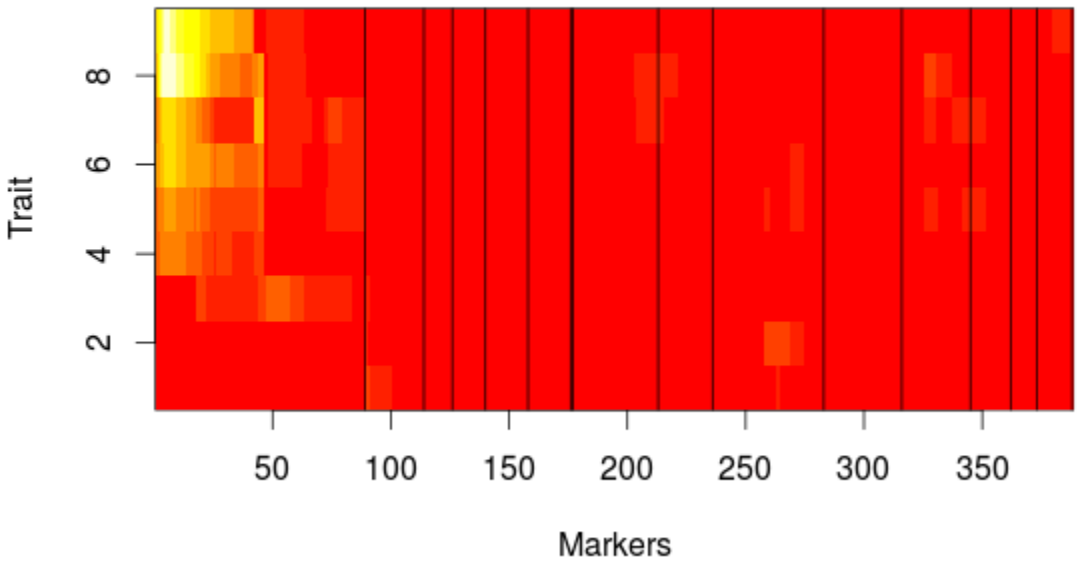

Figure 4.8: MQM results for growth with Fluconazole on agar. White indicates high LOD score. Red indicates low LOD score. X axis shows $\log_2\mu$M of Fluconazole. Markers represent pseudomarkers placed throughout the linkage map. Chromosomes are indicated by black vertical lines.

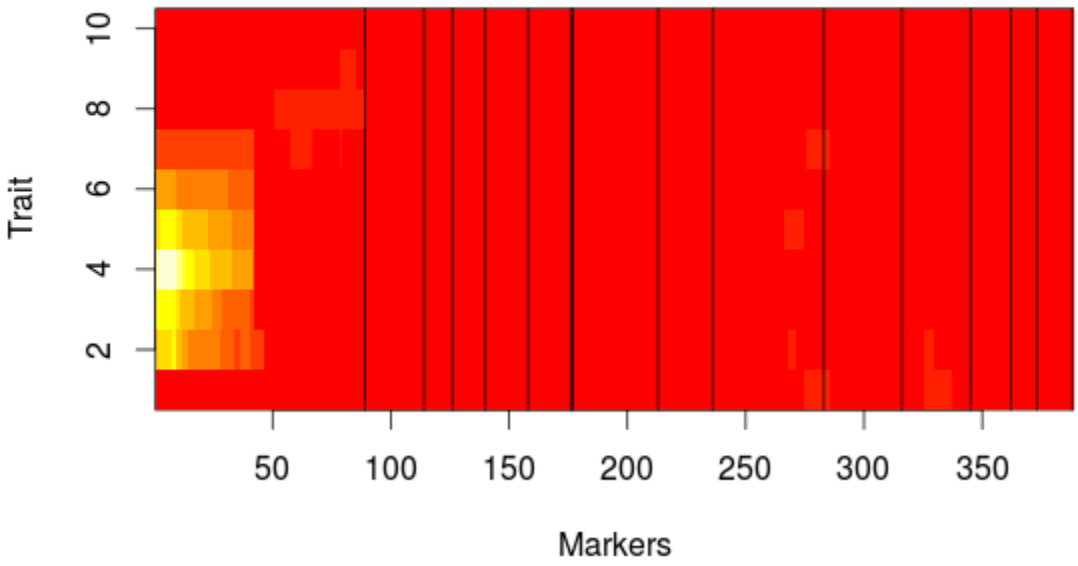

Figure 4.9: MQM results for growth with Fluconazole on agar. White indicates high LOD score. Red indicates low LOD score. X axis shows $\log_2\mu$M of Fluconazole. Markers represent pseudomarkers placed throughout the linkage map. Chromosomes are indicated by black vertical lines.

4.4 Discussion

In this study we have identified 23 QTLs which contribute to the differential manifestation of several phenotypes between a laboratory strain of *C. deneoformans* (JEC20) and a clinical, drug resistant isolate of *C. neoformans* (CDC15). Among these, we have identified six QTLs involved in resistance to the azole drug Fluconazole. Interestingly, all of the six traits examined in this study possessed QTLs on chromosome A. Chromosome A is the largest chromosome, containing approximately 13 % of the genes in genome. However, given this and the total number of QTLs, the observed distribution of QTLs among the chromosomes is not unexpected (p=0.17, chi square distribution).

The primary goal of this work was to identify the regions of the genome where a difference in alleles contributes to major phenotypic differences between strains of two divergent lineages in the pathogenic *Cryptococcus* species complex. We note that interpreting the observed differences can be complicated by the total number of chromosomes among the hybrid progeny. In this study, we assumed that all hybrid progeny were diploid. However, a significant proportion of the progeny may be aneuploid and it has been demonstrated that disomy of specific chromosomes in *C. neoformans* can lead to Fluconazole resistance and increased virulence in mice (Sionov *et al.*, 2010, 2009). Additionally, it has been demonstrated in *Saccharomyces cerevisiae* that the beneficial effects of chromosomal duplication are a result of a few individual loci on the specific chromosome rather than a chromosome-wide response (Sunshine *et al.*, 2015). It is therefore possible that the QTLs identified here represent loci that result in higher levels of resistance or of a given virulence factor when present in two copies

versus one copy. In our analyses, all homozygous genotypes were coded as AA or DD. In actuality, a portion of the homozygous loci are likely A- or D- due to chromosome loss (Vogan *et al.*, 2013). As a result of this, if there is a benefit to having two copies of a given locus regardless of the specific allele, then the copy number effect or heterosis effect may be masked. This is because all heterozygous loci should be present as two copies whereas the pool of homozygous genotypes will be represented by individuals with either one or two copies of the locus.

Following this line of reasoning, we analyzed all of the QTLs for evidence of the effect of copy number on the traits. To do this, genotypes which were determined to be A or D were subdivided into AA, A-, or A? and DD, D-, or D? based on surrounding markers. For progeny that were A or D at the locus of interest, but heterozygous (H) at at least one other locus on the same chromosome, they were categorized as AA or DD. If at least one other locus on the same chromosome was the opposite genotype of the locus of interest, but no heterozygous loci were present on that chromosome, they were categorized as A- or D-. This assumes that we have genotyped a sufficient number of markers on each chromosome as to observe heterozygosity were it present. If the entire chromosome was homozygous for the given allele, the loci were categorized as A? or D?. See Vogan *et al.* (2013) for a detailed explanation as to why the copy number of fully homozygous chromosomes cannot be determined (Vogan *et al.*, 2013). If the number of alleles at a given locus was the sole reason for larger values of a given trait, than H, AA, and DD categories would be significantly different than A- or D-, but not differ significantly from each other. However, this was never observed in our dataset. For two cases (cell size at RUM1 and capsule production at CND06160) the results suggest that there may be a combined effect of copy number and heterosis.

Progeny which are H have significantly higher values than those which are A- or D-, indicative of heterosis. However, there are no differences between AA and DD categories and any other category (Supplementary Table 4.S2).

Melanin Production

One of the QTLs identified for melanin production is located near marker CNG01240. This marker lies within the LAC1 gene, which codes for laccase in *C. neoformans* (Williamson, 1994). Laccase is responsible for the key enzymatic step in the conversion of precursors, such as L-DOPA, into melanin and is necessary for virulence (Liu *et al.*, 1999; Salas *et al.*, 1996). There are numerous ($\frac{61}{265}$) amino acid substitutions between the sequences for laccase in the reference genomes of H99 (*C. neoformans*) and JEC21 (*C. deneoformans*), but relatively little within-species sequence divergence (Ito-Kuwa *et al.*, 2008). However, no studies have shown whether or not these amino acid changes affect melanin production differences between these two lineages. A detailed promoter analysis has been conducted on *C. deneoformans* and determined that multiple transcription factors control melanin expression (Zhu and Williamson, 2004), but a parallel experiment has not been conducted in *C. neoformans*. A comparative analysis using *in silico* methods is not viable as the ORF CNAG_07734 is present immediately upstream of LAC1 in *C. neoformans*, but absent in *C. deneoformans*. A follow-up experiment to replace the JEC20 allele with the CDC15 allele in JEC20 or vice versa at the LAC locus could help understand whether these amino acid differences contribute to melanin expression differences between the two strains.

Cell Size, Cell Wall Thickness, and Capsule Production

Measurements for cell size and cell wall thickness were conducted under nutrient limiting conditions. Previously, Feldmesser *et al.* observed cell wall thickening and an increase in cell size during the course of pulminary infection of a mouse (Feldmesser *et al.*, 2001) with *C. deneoformans*. After 48 h of infection, they reported the ratio of cell wall thickness to cell diameter as 0.061 ± 0.026 as compared to 0.480 ± 0.020 five minutes post infection. In agreement with this work, we found that after 48 h of growth in nutrient-poor media (inducing conditions) this ratio was 0.100 ± 0.032 for JEC20 and 0.140 ± 0.021 for CDC15. This suggests that starvation induces a similar physiological response as the host environment in *Cryptococcus*.

Interestingly, three of the five QTLs associated with cell size were located on chromosome A (Figure 4.3). Our result is consistent with previous observations about the importance of Chromosome A to cell size. For example, Sionov *et al.* (2010) reported that a clonal descendant of strain H99 but with two copies of Chromosome A exhibited increased cell size (Sionov *et al.*, 2010). Research on the model yeast *Saccharomyces cerevisiae* has revealed that factors controlling cell cycle can effect cell size in a ploidy dependant manner by lengthening the time a cell spends in the G1 phase of the cell cycle (Talia *et al.*, 2007). Thus, the genes underlying these three QTLs may code for cyclins, or cyclin dependent products, though none have been identified as such in the annotated genome of JEC21.

The QTL identified for cell wall thickness displays an unexpected pattern of association between genotype and cell wall thickness. Despite the fact that cells from CDC15 have thicker cell walls than JEC20 under inducing conditions, in the hybrid

progeny, strains which are homozygous D or heterozygous have thicker cell walls than homozygous A progeny(Figure 4.4). This suggests that the D allele at this locus promotes cell wall thickening in a hybrid background, but may have little or no effect in the JEC20 background. Alternatively, there may be a second interacting locus that is necessary for increased cell wall thickening in CDC15, but not in JEC20. However, we were unable to identify such a locus using the present data.

As neither JEC20 nor CDC15 showed any capsule production after 2 d in inducing media, it was expected that any QTLs would be associated with heterozygous advantage and/or aneuploidy. However, this was not the case for the QTL at marker CNA00050, one of the five QTLs identified associated with this trait. This QTL may therefore be responsible for the production of capsule observed in CDC15 after 7 d, but additional data is required to determine this.

A minor complication to the traits measured for cell size, cell wall and frequency of capsule production is that we assumed all the measured cells in a given progeny have exactly the same genotype. Previously, we demonstrated a loss of heterozygosity among separate colonies isolated from the same strain in this mapping population (Vogan *et al.*, 2013). For most of the traits analyzed here, we can safely assume that the phenotypes represent the major genotype of a given colony, but individual cell measurements mean that some cells may have different genotypes than others. If these different genotypes cause large shifts in the phenotype of that cell then a simple average may not represent the phenotype appropriately. However, the average coefficient of variation for all of the traits that required single cell measurements was 0.33 and only 5 % of measurements had coefficients of variation above 0.5, suggesting

that this is not a critical issue.

Fluconazole Resistance

Ongoing work in our lab has revealed that CDC15 contains a well known point mutation that confers resistance to Fluconazole (in preparation), namely Y145F in ERG11 (the gene that codes for the target of Fluconazole) (Sionov *et al.*, 2012). This gene lies adjacent to marker CNA00290, which is the location of the very strong QTL identified in all of the analyses for fluconazole resistance. Because of the strong effect of this mutation, it was unexpected to find that for the MQM analysis of MIC in liquid media, the QTL by marker CNA07470 was the only QTL identified at high drug concentrations. The efflux protein AFR1 has been shown to confer resistance to multiple azoles in *C. neoformans* (Posteraro *et al.*, 2003). Its genomic location is nearby to marker CNA07470, at locus CNA07090, suggesting that this may be the gene underlying this QTL. If so, then this suggests that at high concentrations of Fluconazole in RPMI broth, drug efflux contributes more to resistance than the point mutation in ERG11.

Previous work has determined that disomy at chromosome A resulted in higher MICs of laboratory strains, and may be a result of increased levels of the ERG11 protein (Sionov *et al.*, 2010). Additionally, Sionov *et al.* (2010) presented convincing evidence that disomy at chromosomes 4, 10, and 14 of H99 also contribute to resistance. These chromosomes correspond to Chromosomes L, J and H as annotated in JEC21 and used here. However, none of the other four QTLs identified for MIC on agar and in broth are located on these chromosomes, suggesting that there are additional loci contributing to Fluconazole resistance in *C. neoformans*.

Here we have identified 23 QTLs which are associated with increased expression of virulence factors and increased resistance to the antifungal drug Fluconazole. Our results represent an essential step from which to dissect the genetic bases of major phenotypic differences between two strains representing the two divergent lineages of the pathogenic *Cryptococcus* species complex. Future studies should be able to determine and/or confirm the identity of the genes underlying these QTLs, which may help elucidate the genetic mechanisms which allow this normally saprophitic organism to cause deadly disease in humans.

4.5 Supplement

Table 4.S1: Primers and marker names used for genotyping in this study.

ID	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Size D (bp)	Enzyme
CNA00050F	AGCAATTCCAAACCGACCCC	31347	1440	17738	1436	HaeIII
CNA00050R	TTACGCCCACACCAAGGCAT	29908		19173		
CNA00290F	AAACTCGCGGTTGCCAATCC	120407	1170	95921	1205	SacI
CNA00290R	TTGGAGGATGCAGGCGGTTT	121576		97125		
CNA00670F	TTGGGCCAGGAGTGAGTGAT	213956	1316	192305	1309	HaeIII
CNA00670R	AAGGCAGAAAGAGCCGCGTT	215271		193613		
CNA01100_2F	GCAAAGACTGACACCAGCGACC	322838	1060	298164	1061	HaeIII
CNA01100_2R	CTGCCTCTTTTCCAAGCACAGC	323897		299224		
CNA01490F	AGGCAGGCGCGTCAGCTTTTTG	438028	1303	401640	1320	HaeIII
CNA01490R	AAACAGGCGACCATTGCGGGAG	439330		402959		
CNA01890F	ATCACGCAACCCGTTGCCAT	543126	1227	508335	1230	SacI
CNA01890R	TGCCTTGGCGTTGGCTCTTT	544352		509564		
CNA02350F	CGAGGCTTTGAGAGGATGGGG	643257	1156	613955	1167	HindIII
CNA02350R	CCGCTCGAACATGTACTCACCC	644412		615121		
CNA02700F	TTGGATGTTGTCCATCCCGC	729033	1368	703078	1368	EcoRI
CNA02700R	TCAAGACCGGCATTGTCGCA	730400		704445		
CNA03050F	CCCCCATTGTCGCCAAACAT	819061	1183	795229	1178	HaeIII
CNA03050R	AAGCAATTCCCCACGGCGAT	820243		796406		
CNA03460F	CCACGGTTGGGCATGAACA	911508	1667	900629	1667	HaeIII
CNA03460R	TTGCCTGGCTATCCATTC	909842		902295		
CNA03720F	GCTGAGAGGTGCCCAGTTGAG	1008429	1177	999753	1170	HinfI
CNA03720R	ACAGCTTGATCGTGCCATCAGG	1009605		1000922		
CNA04100F	CCACCAGCGCAAAAACGAC	1109343	1726	1100766	1748	HindIII
CNA04100R	TGATGCGCCGCCTTTCTCTT	1111068		1102513		

ID	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Size D (bp)	Enzyme
CNA04490F	TGCCCGACCTCCAAAAGCAGAC	1211780	1225	1203345	1222	HindIII
CNA04490R	CCAGATGCCATCACGATCCACG	1213004		1204566		
CNA04940F	GCTCAAAAAGACCAGCCGCTTC	1305670	1023	1307790	1026	PvuII
CNA04940R	GGACCTGCTAGCGTTGTGAGAG	1306692		1308815		
CNA05300F	TCGGACAGCAAGTGAGTCCAGG	1399036	1097	1406625	1096	HaeIII
CNA05300R	TAAGCCTGTTGAGCGAATCGGG	1400132		1407720		
CNA05600F	TGGGTTTGGCTTCAGGCAG	1484100	1068	1500087	1068	HaeIII
CNA05600R	CCGCTCTTCACATGCAGCAA	1485167		1501154		
CNA06030F	GGGTTACCAGCGGTGGCGATC	1596113	1099	1612358	1096	PstI
CNA06030R	GTGACGGCCAACCCTGAAGGAG	1597211		1613453		
CNA06130F	AGTTTCCTCCGATCCGGCGTC	1636495	1093	1656370	1102	HinfI
CNA06130R	CTGCCTCGATGACTTGGCTG	1637587		1657471		
CNA06310F	CGTATCCAGCGGCTGACCAC	1696056	1100	1715148	1105	PvuII
CNA06310R	AATGGTCGCTAGACTGGACGGG	1697155		1716252		
CNA06610F	GCCTCAATTCACGCCCAGACCC	1776337	1126	1803026	1123	SacI
CNA06610R	CTCCTTCGCTCCCTTCTTCGTC	1777462		1804148		
CNA06890F	CGTGCCCTTCCCCAAACAAT	1853447	1453	1879077	1455	BamHI
CNA06890R	TCATTGTGCGAGATGCCTGC	1851995		1880531		
CNA07310F	ACACCCCAAATTCCCCAACC	1973202	975	2000368	975	PstI
CNA07310R	ACATCCCAAACGAACCCAC	1974176		2001342		
CNA07470F	TCCGGCATGCATGATCCGAA	2018541	1345	2044802	1331	HinfI
CNA07470R	AATCGCCTGCAACTGCGCAA	2019885		2046132		
CNA07650F	AGAAGAGCATGGCAGCGGAA	2073146	1344	2098757	1352	HinfI
CNA07650R	ATGTCGTCGTCGTTTCGCCC	2074489		2100108		
CNA07990F	TCCAATGGACGAGGACGATG	2177600	1430	2200900	1424	HinfI
CNA07990R	TGACCGGTGTGGGTTGCAAT	2179029		2202323		
CNB00360F	AGTGCTCAGAGTCTGGGGCTGG	101410	1214	102897	1223	HincII
CNB00360R	GCCATTCGCAGGGGTGGAGG	102623		104119		
CNB01690F	CCTCCTCATCGCCTACGCC	492285	936	501104	936	HaeIII
CNB01690R	GGATCTCGTACACGCCGACG	493220		502039		
CNB03520F	TTTCGGGAGTTGGGAGCACA	1036775	1060	1051980	1061	HaeIII
CNB03520R	TTGGCTGCGGTACTGGCATTCTT	1037834		1053040		
CNB04550F	TGCCGAAATTAGTCGTAAGCG	1293628	962	1306077	962	PvuII
CNB04550R	TAGCACTTGCCATTGCCTGC	1292667		1305116		
CNB05710F	TTGGACAACGCAAGACCCCAG	1598321	1262	1601790	1263	HindIII
CNB05710R	TGTTGCAAGCAACGATGCC	1599582		1603052		
CNC00670F	TGTGCGGCTTTGGGATTGGT	195225	1110	191360	1108	HaeIII
CNC00670R	TTGCAGGTATGGCCGAATGG	196334		192467		
CNC02790F	GATGAAATGGCGAGGACGCA	866572	1469	799711	1470	EcoRI
CNC02790R	TTCCGCGTTGCAACACAACC	868040		801180		
CNC06110F	AAGGGCGTTGATCCGGCAAT	235559	1404	1795499	1403	HaeIII
CNC06110R	TTCCCCAGGTCGTTTGGGAT	236962		1796901		
CNC07180F	TGGAGGCGTTGGGCGAAATAGAG	15726	1316	2100876	1309	HaeIII
CNC07180R	TTCAGCCGTCGCCTTTACCACAA	17041		2102184		
CND00510F	CGGTGCCGCTTTATTTGTGGC	1650153	1325	150300	1327	HinfI
CND00510R	TCTAGCGCCAAAGCGTGCAAG	1651477		151626		
CND02060F	TCAGGTTCAAACCGCCAGCA	1261617	1223	553800	1222	HaeIII
CND02060R	AGTTTTGCCCCGCTTCGCTTGC	1262839		555021		

ID	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Size D (bp)	Enzyme
CND03630F	GCAAGCTCGAATACTTCCTGC	819135	888	1001545	888	HaeIII
CND03630R	CGCACCATCATCCGACCA	818248		1002432		
CND05120F	ACCCTCTCATCCCTCCCGCC	403556	1244	1398741	1251	AluI
CND05120R	GGGATTCGAGAAGGGCTCTTCG	402313		1399991		
RUM1	TGAAGATTTTGGATTCGAAGAAGGTGACG	258645	1345	59083	1340	EcoRV
RUM1	CAAGTGCAGAGCTGATCGGCATGGG	259989		57744		
CND06160F	TCAGCAAGCCTTGATAGCAGAGC	120306	1135	1701497	1140	PstI
CND06160R	CCACGTCCATAAGTCACATCTGGC	121440		1700358		
CNE00250F	TGGCGTCTCTTTGAACGCGATC	44253	1118	55046	1118	HaeIII
CNE00250R	ATGGCGGAATGTCCGGCTTT	45370		56163		
CNE01630F	CCGTAGACTGGTGGGCGTTGG	455696	1039	452609	1040	HaeIII
CNE01630R	CCTTCGGCTGCAGGCGTAGG	456734		453648		
CNE03010F	TTGCTGGCAACCACCAGCTT	822857	1111	855287	1117	HaeIII
CNE03010R	TTTCCGCACCGGTTGATGCT	823967		856403		
CNE05150F	CCACACTTCCAACCATCCTCG	1417863	1182	1445761	1185	HaeIII
CNE05150R	CATGACCTTCGGCTCCAGCAC	1416682		1444577		
CNF00290F	TCATGCCCCTTCGCCTTCAT	1307533	1457	96744	1455	HaeIII
CNF00290R	TTCTCCTTCTCCCCATCCCA	1308989		98198		
CNF02400F	TCAAAGCTTCCGCCCGTGTT	726889	1277	701994	1262	HindIII
CNF02400R	CAGCCGCCTCAAATCACGAA	728165		703255		
CNF04800F	GGTCTGCTCTATATGAGCTGCG	49162	1044	1397215	1058	HindIII
CNF04800R	GGAAACTGGTCCCCAACAATC	48119		1398272		
CNG00170F	TTTCTTCCGCCGCTTCTCAC	1348500	1245	42764	1232	
CNG00170R	ACAGCGCGTTGAGTTTCGGT	1349744		43995		HaeIII
CNG01240F	TCGTTTCGCTGGTCAGCAGC	1021799	1237	356266	1246	EcoRV
CNG01240R	CGGTACATGGGATGGGCGGT	1023035		355021		
CNG03250F	TTGCCAATAACGTGGCACGG	444807	1424	916601	1423	HindIII
CNG03250R	AAAGGGAGGCGGCTGATGATA	446230		918023		
CNG04610F	TGTTTCCCACAGGCCAAGGACT	59766	1466	1308769	1514	HinfI
CNG04610R	CGTGCGCGAATGCATCGATAT	61231		1310282		
CNH00030F	TGTCGATGTGCTTCTCGGCA	12634	1272	1186643	1274	PstI
CNH00030R	CTCCCTCCCATCCCAAAACAC	13905		1187916		
CNH02750F	TTGGATCGCTTGCTCGCGAA	818314	1428	349466	1411	EcoRI
CNH02750R	AGGCCCGAGCAAAGGAATGA	819741		350876		
CNH03360F	CGGGGCTATTTGGAGCGAAA	968260	1396	150788	1380	HaeIII
CNH03360R	ATGATGGGGCCTCTGGATTG	969655		152167		
CNI00070F	CCGCCTGCACACCTTTCTT	1142613	1214	20265	1200	XhoI
CNI00070R	TGTCTTCGGTTTGGATGGG	1143826		21464		
CNI01350F	GAGCGACATCGTCCCTATGTGA	790719	1250	402736	1232	PvuII
CNI01350R	ACTGGTAGCAATGGCGACATG	789470		403967		
CNI04370F	AGCGGCTACAGCAAAAGCGA	30620	1078	1166708	1084	HinfI
CNI04370R	AACATGTCCGCCTCACCCAA	31697		1167791		
CNJ00070F	ATGGCGGAAGAGGCGTATGA	1016363	1153	14214	1135	HinfI
CNJ00070R	CCTGTCCAGTGCGCATTTCG	1017515		15348		
CNJ01700F	TGGGACGCTGACACTCGGTATG	532587	1137	496402	1137	PstI
CNJ01700R	TTGACCTTCTGGACAAGCTGGC	531451		497538		
CNJ02920F	TGGGGGAGAAAGGACATTGG	175236	1555	904610	1568	HinfI
CNJ02920R	CAAATGCCGAGCTCCCTTC	176790		906177		

ID	Sequence	Location A (bp)	Size A (bp)	Location D (bp)	Size D (bp)	Enzyme
CNK00170F	AACATGGCATCTCCCCCCAA	1500435	1108	54380	1111	HinfI
CNK00170R	TCGTGCTGACCATGCGGTTT	1501542		55490		
CNK01700F	ACGCACTCTCACAGCTCCTTCG	1033365	1175	501568	1173	HaeIII
CNK01700R	GCAAAGCTCAGGCTCAAATCCAG	1034539		500396		
CNK03410F	TTTCGCCGCACCCCCTTTTT	1513810	1131	1002531	1125	HinfI
CNK03410R	CCTCGCCGCCCAATAATTCA	1514940		1003655		
CNL03990F	CCCAGGCAGCCGAGGATG	787025	1497	95393	1487	HaeIII
CNL03990R	GCTCGTCGTGACCAGAGGCG	785529		96879		
CNL04620F	TTCGTGGCGACAGGTTTTGGG	595892	1321	289852	1321	HindIII
CNL04620R	TTCAGCGATGGGTTGAGGCA	597212		291172		
CNL05760F	GGCTCGTGTCAGGCTCAATC	288054	1812	604149	1818	PvuII
CNL05760R	CCTCGCACACAGCACGCATA	286243		605966		
CNL06810F	TTAATGGACTGGGCAGATGCTCGTC	18019	868	894746	879	SacI
CNL06810R	ATGTCTTCTCCCGCCCTTTTTGCC	18886		895624		
CNM00180F	GCTCAAGAACCATACCTGCTCAT	65845	1607	39248	1633	HincII
CNM00180R	GGCGGCAGGTGACTTCAGTG	67451		40880		
CNM00630F	TGCCAATTGCAAGGGTGGCT	183577	1133	195041	1134	PstI
CNM00630R	TGCGTTGAACAACGCGACCT	184709		196174		
CNM02560F	ATGGACGCTCTCACATTACCTTGC	757906	1491	776201	1493	AccI
CNM02560R	ACGCTGCCCTCTCCCACAGTC	759396		777693		
CNN00060F	CCCAACCTCATCCCACCTC	40214	1223	18763	1228	XhoI
CNN00060R	ACAGAACCCATTGAGCCCGA	41436		19990		
CNN01360F	GACTGGGAGTCGGACATGC	415180	1119	405849	1119	EcoRV
CNN01360R	CACTTCAATCATCTGCACGCCA	416298		406967		
CNN02060F	TTGGAACAGGCCACTCGGAA	632500	1432	644695	1433	HaeIII
CNN02060R	ACCGCCAAGGATTCTTGCGA	633931		646127		

Table 4.S2: Average phenotype ± standard deviation for given genotypic class. The marker closest to the QTL of interest, as indicated by the position (pos) and Chromosome (Chr), is indicated.

Trait	Chr	Pos (cM)	Marker	AA	A-	A?	DD	D-	D?	H
Melanin	A	15.6	CNA03050	38.21 ± 6.66^{ab}	35.83 ± 7.19^{ab}	36.70 ± 7.27^{ab}	39.08 ± 4.03^{ab}	33.73 ± 5.55^{ab}	31.18 ± 10.14^{b}	36.16 ± 8.89^{a}
	B	15.0	CNB03520	36.56 ± 10.35^{ab}	31.27 ± 9.19^{b}	39.20 ± 5.91^{a}	30.55 ± 11.96^{ab}	32.72 ± 7.07^{ab}	33.03 ± 7.91^{b}	38.40 ± 7.14^{a}
	G	3.6	CNG01240	43.51^{ab}	39.82 ± 4.28^{a}	39.40 ± 5.69^{a}	26.39 ± 8.91^{ab}	33.45 ± 2.80^{ab}	30.88 ± 8.61^{b}	38.03 ± 6.40^{a}
	L	0.0	CNL03990	38.32 ± 5.56^{a}	34.22 ± 7.71^{a}	38.05 ± 5.25^{a}	32.84 ± 7.14^{a}	27.43^{a}	33.23 ± 7.39^{a}	35.03 ± 9.69^{a}
	L	9.4	CNL06810	4.98^{a}	32.14^{ab}	38.05 ± 5.25^{b}	34.38 ± 5.80^{ab}	34.87 ± 7.64^{b}	33.23 ± 7.39^{b}	35.29 ± 9.10^{b}
Cell Size	A	35.7	CNA06130	6.63 ± 0.78^{ac}	6.22 ± 0.86^{ab}	6.10 ± 0.54^{ab}	6.42 ± 0.88^{ab}	5.77 ± 0.67^{b}	5.87 ± 0.76^{bc}	6.44 ± 0.81^{a}
	A	54.2	CNA07310	6.39 ± 0.87^{ab}	5.85 ± 0.58^{b}	6.10 ± 0.54^{ab}	6.37 ± 0.83^{ab}	6.10 ± 0.94^{ab}	5.87 ± 0.76^{b}	6.48 ± 0.80^{a}
	A	64.0	CNA07990	6.59 ± 1.00^{ab}	5.91 ± 0.68^{b}	6.10 ± 0.54^{ab}	5.85 ± 0.77^{b}	5.99 ± 0.87^{b}	5.87 ± 0.76^{b}	6.60 ± 0.72^{a}
	D	41.6	RUM1	5.56 ± 0.68^{ab}	5.96 ± 0.75^{b}	5.94 ± 0.67^{b}	6.52 ± 0.82^{ab}	5.85 ± 0.70^{b}	5.99 ± 0.67^{b}	6.57 ± 0.88^{a}
	F	1.2	CNF00290	6.52 ± 0.33^{ab}	6.25 ± 0.77^{ab}	6.08 ± 0.59^{ab}	6.08 ± 0.76^{ab}	5.94 ± 0.69^{b}	5.83 ± 0.78^{b}	6.56 ± 0.81^{a}
	I	2.6	CNI01350	5.91 ± 0.62^{ab}	5.42 ± 0.55^{b}	6.15 ± 0.69^{ab}	5.79 ± 1.28^{ab}	6.03 ± 0.39^{ab}	5.72 ± 0.62^{b}	6.32 ± 0.82^{a}
Cell Wall Thickness	A	14.6	CNA02700	0.05 ± 0.00^{abc}	0.05 ± 0.01^{b}	0.05 ± 0.01^{abc}	0.04 ± 0.01^{ab}	0.05 ± 0.01^{ac}	0.06 ± 0.01^{c}	0.05 ± 0.01^{ac}
Capsule	A	0.0	CNA00050	0.27 ± 0.28^{ab}	0.19 ± 0.21^{ab}	0.22 ± 0.22^{ab}	0.12 ± 0.19^{ab}	0.07 ± 0.14^{b}	0.09 ± 0.16^{b}	0.22 ± 0.22^{a}
	B	0.6	CNB00360	0.07 ± 0.09^{ab}	0.19 ± 0.23^{ab}	0.21 ± 0.23^{ab}	0.07 ± 0.17^{b}	0.09 ± 0.12^{b}	0.10 ± 0.16^{b}	0.28 ± 0.22^{a}
	D	41.9	CND06160	0.16 ± 0.14^{ab}	0.05 ± 0.08^{b}	0.16 ± 0.19^{b}	0.14 ± 0.13^{ab}	0.12 ± 0.19^{b}	0.10 ± 0.16^{b}	0.29 ± 0.23^{a}
	H	0.0	CNH00030	0.24 ± 0.21^{a}	0.20 ± 0.24^{abc}	0.20 ± 0.22^{ab}	0.05 ± 0.12^{bc}	0.07 ± 0.12^{abc}	0.06 ± 0.12^{c}	0.23 ± 0.22^{a}
	L	2.2	CNL04620	0.00^{ab}	0.08 ± 0.14^{ab}	0.14 ± 0.18^{ab}	0.01 ± 0.02^{ab}	0.04 ± 0.04^{ab}	0.08 ± 0.13^{b}	0.23 ± 0.22^{a}
MIC on agar	A	4.2	CNA00290	6.33 ± 1.15^{abc}	6.74 ± 0.67^{a}	6.31 ± 1.20^{ac}	5.11 ± 1.17^{cd}	4.93 ± 1.21^{bd}	4.40 ± 1.76^{bd}	6.62 ± 0.95^{a}
	E	7.8	CNE01630	5.75 ± 1.50^{ab}	6.31 ± 1.14^{ab}	6.12 ± 1.32^{ab}	6.07 ± 1.77^{ab}	5.00 ± 1.15^{ab}	5.13 ± 1.77^{b}	6.15 ± 1.27^{a}
	N	15.0	CNN02060	5.67 ± 1.41^{ab}	5.46 ± 1.66^{ab}	6.43 ± 1.10^{b}	5.50 ± 2.12^{ab}	5.40 ± 1.78^{ab}	5.89 ± 1.36^{ab}	5.35 ± 1.81^{a}
MIC in Broth	A	4.2	CNA00290	6.67 ± 0.58^{a}	4.61 ± 1.24^{a}	5.69 ± 1.14^{a}	3.00 ± 2.55^{abc}	2.56 ± 3.00^{b}	1.00 ± 2.34^{c}	4.63 ± 2.17^{a}
	A	59.8	CNA07470	4.45 ± 1.97^{ab}	4.00 ± 2.65^{a}	5.69 ± 1.14^{a}	3.70 ± 2.54^{ab}	2.04 ± 2.42^{bc}	1.00 ± 2.34^{c}	4.79 ± 2.23^{a}
	C	9.4	CNC06110	1.60 ± 1.95^{a}	1.80 ± 1.92^{a}	4.08 ± 2.44^{a}	3.33 ± 2.66^{a}	4.00 ± 4.12^{a}	2.73 ± 2.88^{a}	4.10 ± 2.65^{a}

Superscript letters refer to significant differences between groups based on Tukey's HSD at $p<0.05$

Chapter 5

Conclusions

In this body of work I provided evidence for three separate genetic features that have important implications for the evolution of *Cryptococcus*. In Chapter two, evidence was presented which showed that during a hybrid cross mitotic recombination occurs and possible models which could explain this were outlined. In Chapter 3, multiple candidate Bateson – Dobzhansky – Muller incompatibilities were identified. In Chapter 4, QTLs implicit in virulence related phenotypes and drug resistance to Fluconazole were ascertained. Previous work in our group identified multiple fixed rearrangements between *C. deneoformans* and *C. neoformans*, together with the evidence of BDM incompatibilities presented in Chapter 3, these indicate that there has been little historic gene flow between the two groups. This was part of the justification for splitting *C. neoformans* into *C. neoformans* and *C. deneoformans* as explained in the introduction (Hagen *et al.*, 2015). However, these species may not remain separate for long. Hybridization has the ability to erode species boundaries. Mixing of alleles from the two species can potentially lead to the extinction of the alleles from one of the parental species, particularly if there is a selective advantage for one or

the other. It is not clear how often hybridization is occurring in the wild, but it is clear that both species now have largely overlapping distributions. The MATa locus is extremely rare in natural populations. It appears to be slightly more prevalent in *C. deneoformans* than in *C. neoformans*, where it has only been located in isolated populations in southern Africa (Litvintseva *et al.*, 2003). Despite this, many hybrids possess a *C. neoformans* type MATa allele, denoted A**a**D$\boldsymbol{\alpha}$ (Litvintseva *et al.*, 2007). Their results suggest that this hybridization occurred once, and then the hybrids spread throughout the globe through clonal reproduction. The hybrids are generally not as efficient at mating as non-hybrids, particularly if they are self-fertile. So the A**a**D$\boldsymbol{\alpha}$ strains, may not introduce *C. neoformans* type alleles into *C. deneoformans* populations through mating. However, in Chapter 2 it was demonstrated that hybrid strains can lose heterozygosity rapidly. Thus, it is possible that hybrid strains with both MAT types could become homozygous MATa and as a result be more efficient at mating. Since the MATa allele is rare, this means that if a MATa hybrid strain which can mate well encounters another strain of *Cryptococcus* it will likely be MAT$\boldsymbol{\alpha}$, and so the potential for mating is very high.

There is compelling evidence that this has already occurred once in the past. Kavanaugh *et al.* identified a 14-gene fragment that's nearly homogenous between *C. neoformans* and *C. deneoformans*. They suggest that the region was integrated into a *C. deneoformans* strain, likely through hybridization, and then became fixed in many populations (Kavanaugh *et al.*, 2006). The ancestral range of *C. neoformans* is likely Africa, while most *C. deneoformans* isolates come from Europe, which hints that this historic hybridization event may have occurred around the Mediterranean. In agreement with this, *C. deneoformans* strains from Spain and Italy were found to

have the fragment, while two strains from Denmark did not. The authors suggest that since the fragment is widespread, it may confer a selective advantage, however comparative studies need to be done to conclude this.

Although intraspecific crosses in *Cryptococcus* have only a transient diploid stage, there are other structures which are occasionally produced that have multiple copies of the genome. In these structures, the mitotic recombination described in Chapter 2 could play a significant role. At 37 °C it has been observed that *Cryptococcus* can exist as stable diploids. If moved to 24 °C, these strains will begin forming filaments and produce viable haploid progeny (Sia *et al.*, 2000). It is therefore possible, that if a patient becomes infected with a diploid strain, mitotic recombination could lead to an array of mixed phenotypes, rather than a homogenous clonal population. It is also known that *Cryptococcus* is commonly found in pigeon guano, where strains would also endure temperatures of 37 °C (Emmons, 1960). So the potential for mitotic recombination may be high even in non-clinical situations.

One of the major questions that are still unanswered surrounding *Cryptococcus* is: why does *C. neoformans* appear to cause infection more readily than *C. deneoformans*? Both species have the same canonical virulence factors, melanin production, polysaccharide capsule, and growth at high temperature. Chapter 4 provides evidence that this is due to the quantitative nature of these virulence factors. Therefore, strains that produce more melanin and capsule, or that grow better at high temperature should be expected to be more virulent. Preliminary results suggest that the differences noted between CDC15 and JEC20 are representative largely of just those strains, and may not be indicative of the differences between *C. neoformans*

and *C. deneoformans* as a whole. For example, there are some clinical isolates of *C. deneoformans* which produce as much melanin as CDC15, and some strains of *C. neoformans* that produce less melanin than CDC15. In agreement with this statement, other research has found that most environmental isolates of *C. neoformans* are not able to cause infection in a mouse model (Litvintseva and Mitchell, 2009). This implies that *C. neoformans* acquire the necessary adaptations to cause infection more readily than *C. deneoformans*. If this is the case then it is expected to be due to the presence of compensatory alleles. For example, if there is a metabolic cost to producing more melanin, than a strain which has an allele at another locus that can compensate for this cost would grow better than one which does not. Thus, while experiments under *in vitro* conditions may show that two strains produce the same amount of melanin, it would not fully capture how much melanin the strains could produce *in vivo*.

A second part to the question posed in the previous paragraph is: did selection for virulence traits drive the divergence between *C. neoformans* and *C. deneoformans*? A prevailing theory as to what led to the evolution of virulence traits in *Cryptococcus* points to predation by amboebae as they engulf prey in a similar manner as macrophages and are found cohabiting with *Cryptococcus* (Steenbergen *et al.*, 2001). However, this selective pressure would be expected to impact *C. neoformans* in a more or less equal fashion as *C. deneoformans*, since amoeba are relatively ubiquitous in the environment. It is therefore possible that *C. neoformans* has adapted to animal hosts specifically. To address this, future research that identifies the genes responsible for the differential expression of virulence between the species should also determine if these genes are under positive selection. If so, it may significantly impact our view

on how we see *cryptococcus* as an opportunistic pathogen.

Bibliography

Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J. E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C. A., Buggs, R., Butlin, R. K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S. H., Hermansen, J. S., Hewitt, G., Hudson, A. G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Möst, M., Mullen, S., Nichols, R., Nolte, A. W., Parisod, C., Pfennig, K., Rice, A. M., Ritchie, M. G., Seifert, B., Smadja, C. M., Stelkens, R., Szymura, J. M., Väinölä, R., Wolf, J. B. W., and Zinner, D. (2013). Hybridization and speciation. *Journal of Evolutionary Biology*, **26**(2), 229–246.

Abramoff, M. D., Magalhães, P. J., and Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics international*, **11**(7), 36–42.

Alcázar, R., García, A. V., Parker, J. E., and Reymond, M. (2009). Incremental steps toward incompatibility revealed by *Arabidopsis* epistatic interactions modulating salicylic acid pathway activation. *Proceedings of the National Academy of Sciences*, **106**(1), 334–339.

Arnold, M. L., Hamlin, J. A., Brothers, A. N., and Ballerini, E. S. (2012). Natural hybridization as a catalyst of rapid evolutionary change. In R. S. Singh, J. Xu, and

R. J. Kulathinal, editors, *Rapidly Evolving Genes and Genetic Systems*, pages 256 – 266. Oxford University Press.

Barchiesi, F., Cogliati, M., Esposto, M., Spreghini, E., Schimizzi, A., Wickes, B., Scalise, G., and Viviani, M. (2005). Comparative analysis of pathogenicity of *Cryptococcus neoformans* serotypes A, D and AD in murine cryptococcosis. *Journal of Infection*, **51**(1), 10–16.

Barton, N. H. (2001). The role of hybridization in evolution. *Molecular Ecology*, **10**(3), 551–568.

Bauer, M., Thomas, A., and Larsen, R. (2012). *Cryptococcus neoformans*: The model organism for yeast antifungal drug susceptibility testing. *Mycopathologia*, **173**(5), 435–443.

Beale, M. A., Sabiiti, W., Robertson, E. J., Fuentes-Cabrejo, K. M., O'Hanlon, S. J., Jarvis, J. N., Loyse, A., Meintjes, G., Harrison, T. S., May, R. C., Fisher, M. C., and Bicanic, T. (2015). Genotypic diversity is associated with clinical outcome and phenotype in cryptococcal meningitis across Southern Africa. *PLoS Negl Trop Dis*, **9**(6), e0003847.

Benham, R. W. (1956). The genus *Cryptococcus*. *Bacteriological Reviews*, **20**(3), 189–201.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, **57**(1), 289–300.

Boekhout, T., Theelen, B., Diaz, M., Fell, J. W., Hop, W. C. J., Abeln, E. C. A., Dromer, F., and Meyer, W. (2001). Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. *Microbiology*, **147**(4), 891 –907.

Bovers, M., Hagen, F., Kuramae, E., and Boekhout, T. (2008). Six monophyletic lineages identified within *Cryptococcus neoformans* and *Cryptococcus gattii* by multilocus sequence typing. *Fungal Genetics and Biology*, **45**(4), 400–421.

Brandt, M. E., Hutwagner, L. C., Klug, L. A., Baughman, W. S., Rimland, D., Graviss, E. A., Hamill, R. J., Thomas, C., Pappas, P. G., Reingold, A. L., and Pinner, R. W. (1996a). Molecular subtype distribution of *Cryptococcus neoformans* in four areas of the United States. Cryptococcal Disease Active Surveillance Group. *Journal of Clinical Microbiology*, **34**(4), 912–917.

Brandt, M. E., Pfaller, M. A., Hajjeh, R. A., Graviss, E. A., Rees, J., Spitzer, E. D., Pinner, R. W., and Mayer, L. W. (1996b). Molecular subtypes and antifungal susceptibilities of serial *Cryptococcus neoformans* isolates in human immunodeficiency virus-associated cryptococcosis. *The Journal of Infectious Diseases*, **174**(4), 812–820. ArticleType: research-article / Full publication date: Oct., 1996 / Copyright © 1996 Oxford University Press.

Brandt, M. E., Pfaller, M. A., Hajjeh, R. A., Hamill, R. J., Pappas, P. G., Reingold, A. L., Rimland, D., and Warnock, D. W. (2001). Trends in antifungal drug susceptibility of *Cryptococcus neoformans* isolates in the United States: 1992 to 1994 and 1996 to 1998. *Antimicrobial Agents and Chemotherapy*, **45**(11), 3065–3069.

Brasier, C. M. (2001). Rapid evolution of introduced plant pathogens via interspecific

hybridization is leading to rapid evolution of Dutch elm disease and other fungal plant pathogens. *BioScience*, **51**(2), 123–133.

Brasier, C. M., Cooke, D. E. L., and Duncan, J. M. (1999). Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proceedings of the National Academy of Sciences*, **96**(10), 5878–5883.

Broman, K. W., Wu, H., Sen, Ś., and Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics*, **19**(7), 889–890.

Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G., and White, T. C. (2012). Hidden killers: Human fungal infections. *Science Translational Medicine*, **4**(165), 165rv13.

Cabot, E. L., Davis, A. W., Johnson, N. A., and Wu, C. I. (1994). Genetics of reproductive isolation in the *Drosophila simulans* clade: Complex epistasis underlying hybrid male sterility. *Genetics*, **137**(1), 175–189.

Campbell, L. T., Simonin, A. R., Chen, C., Ferdous, J., Padula, M. P., Harry, E., Hofer, M., Campbell, I. L., and Carter, D. A. (2015). *Cryptococcus* strains with different pathogenic potential have diverse protein secretomes. *Eukaryotic Cell*, pages EC.00052–15.

Casadevall, A. and Perfect, J. R. (1998). *Cryptococcus neoformans*. ASM Press, Washington DC US.

Casadevall, A., Steenbergen, J. N., and Nosanchuk, J. D. (2003). Ready made virulence and dual use virulence factors in pathogenic environmental fungi the *Cryptococcus neoformans* paradigm. *Current Opinion in Microbiology*, **6**(4), 332–337.

Chang, Y. C., Wickes, B. L., Miller, G. F., Penoyer, L. A., and Kwon-Chung, K. J. (2000). *Cryptococcus neoformans* STE$\boldsymbol{\alpha}$12 regulates virulence but is not essential for mating. *The Journal of Experimental Medicine*, **191**(5), 871–882.

Chen, J., Varma, A., Diaz, M. R., Litvintseva, A. P., Wollenberg, K. K., and Kwon-Chung, K. J. (2008). *Cryptococcus neoformans* strains and infection in apparently immunocompetent patients, China. *Emerging Infectious Diseases*, **14**(5), 755–762.

Chiavarino, A. M., Rosato, M., Manzanero, S., Jiménez, G., González-Sánchez, M., and Puertas, M. J. (2000). Chromosome nondisjunction and instabilities in tapetal cells are affected by B chromosomes in maize. *Genetics*, **155**(2), 889–897.

Chou, J.-Y. and Leu, J.-Y. (2010). Speciation through cytonuclear incompatibility: Insights from yeast and implications for higher eukaryotes. *BioEssays*, **32**(5), 401–411.

Chou, J.-Y., Hung, Y.-S., Lin, K.-H., Lee, H.-Y., and Leu, J.-Y. (2010). Multiple molecular mechanisms cause reproductive isolation between three yeast species. *PLoS Biol*, **8**(7), e1000432.

Christians, J. K., Cheema, M. S., Vergara, I. A., Watt, C. A., Pinto, L. J., Chen, N., and Moore, M. M. (2011). Quantitative trait locus (QTL) mapping reveals a role for unstudied genes in *Aspergillus* virulence. *PLoS ONE*, **6**(4), e19325.

Cruz, M. C., Sia, R. A. L., Olson, M., Cox, G. M., and Heitman, J. (2000). Comparison of the roles of calcineurin in physiology and virulence in serotype D and serotype A strains of *Cryptococcus neoformans*. *Infection and Immunity*, **68**(2), 982–985.

Dannaoui, E., Abdul, M., Arpin, M., Michel-Nguyen, A., Piens, M. A., Favel, A., Lortholary, O., Dromer, F., and Group, t. F. C. S. (2006). Results obtained with various antifungal susceptibility testing methods do not predict early clinical outcome in patients with cryptococcosis. *Antimicrobial Agents and Chemotherapy*, **50**(7), 2464–2470.

Davis, A. W., Noonburg, E. G., and Wu, C. I. (1994). Evidence for complex genic interactions between conspecific chromosomes underlying hybrid female sterility in the *Drosophila simulans* clade. *Genetics*, **137**(1), 191–199.

Delneri, D., Colson, I., Grammenoudi, S., Roberts, I. N., Louis, E. J., and Oliver, S. G. (2003). Engineering evolution to study speciation in yeasts. *Nature*, **422**(6927), 68–72.

Dobzhansky, T. (1936). Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics*, **21**(2), 113–135.

Dromer, F., Mathoulin, S., Dupont, B., Letenneur, L., and Ronin, O. (1996). Individual and environmental factors associated with infection due to *Cryptococcus neoformans* serotype D. *Clinical Infectious Diseases*, **23**(1), 91–96.

Dromer, F., Mathoulin-Pélissier, S., Launay, O., Lortholary, O., and the French Cryptococcosis Study Group (2007). Determinants of disease presentation and outcome during cryptococcosis: The CryptoA/D Study. *PLoS Med*, **4**(2), e21.

Ellis, D., Marriott, D., Hajjeh, R. A., Warnock, D., Meyer, W., and Barton, R. (2000). Epidemiology: Surveillance of fungal infections. *Medical Mycology*, **38**(sup1), 173–182.

Emmons, C. W. (1960). Prevalence of *Cryptococcus neoformans* in pigeon habitats. *Public Health Reports*, **75**(4), 362–364.

Evans, E. E. (1949). An Immunologic comparison of twelve strains of *Cryptococcus neoformans* (*Torula histolytica*). *Experimental Biology and Medicine*, **71**(4), 644–646.

Fang, S., Yukilevich, R., Chen, Y., Turissini, D. A., Zeng, K., Boussy, I. A., and Wu, C.-I. (2012). Incompatibility and competitive exclusion of genomic segments between sibling *Drosophila* species. *PLoS Genet*, **8**(6), e1002795.

Farrer, R. A., Weinert, L. A., Bielby, J., Garner, T. W. J., Balloux, F., Clare, F., Bosch, J., Cunningham, A. A., Weldon, C., Preez, L. H. d., Anderson, L., Pond, S. L. K., Shahar-Golan, R., Henk, D. A., and Fisher, M. C. (2011). Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proceedings of the National Academy of Sciences*, **108**(46), 18732–18736.

Feldmesser, M., Kress, Y., and Casadevall, A. (2001). Dynamic changes in the morphology of *Cryptococcus neoformans* during murine pulmonary infection. *Microbiology*, **147**(8), 2355–2365.

Fell, J. W., Boekhout, T., Fonseca, Á., Scorzetti, G., and Statzell-Tallman, A. (2000). Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *International Journal of Systematic and Evolutionary Microbiology*, **50**, 1351–1371.

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., and Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, **484**(7393), 186–194.

Fishman, L., Kelly, A. J., Morgan, E., and Willis, J. H. (2001). A Genetic map in the *Mimulus guttatus* species complex reveals transmission ratio distortion due to heterospecific interactions. *Genetics*, **159**(4), 1701–1716.

Franzot, S. P., Mukherjee, J., Cherniak, R., Chen, L.-C., Hamdan, J. S., and Casadevall, A. (1998). Microevolution of a standard strain of *Cryptococcus neoformans* resulting in differences in virulence and other phenotypes. *Infect. Immun.*, **66**(1), 89–97.

Franzot, S. P., Salkin, I. F., and Casadevall, A. (1999). *Cryptococcus neoformans* var. *grubii*: Separate varietal status for *Cryptococcus neoformans* serotype A isolates. *Journal of Clinical Microbiology*, **37**(3), 838–840.

Fredslund, J., Schauser, L., Madsen, L., Sandal, N., and Stougaard, J. (2005). PriFi: Using a multiple alignment of related sequences to find primers for amplification of homologs. *Nucleic Acids Res*, **1**(33), W516–20.

Giraud, T., Refrégier, G., Le Gac, M., de Vienne, D. M., and Hood, M. E. (2008). Speciation in fungi. *Fungal Genetics and Biology*, **45**(6), 791–802.

Gupta, G. and Fries, B. C. (2010). Variability of phenotypic traits in *Cryptococcus* varieties and species and the resulting implications for pathogenesis. *Future microbiology*, **5**(5), 775–787.

Hagen, F., Khayhan, K., Theelen, B., Kolecka, A., Polacheck, I., Sionov, E., Falk, R., Parnmen, S., Lumbsch, H. T., and Boekhout, T. (2015). Recognition of seven species in the *Cryptococcus gattii/Cryptococcus neoformans* species complex. *Fungal Genetics and Biology*, **78**, 16–48.

Hamoir, G. (1992). The discovery of meiosis by E. Van Beneden, a breakthrough in the morphological phase of heredity. *The International journal of developmental biology*, **36**(1), 9–15.

Heitman, J. (2011). *Cryptococcus: From human pathogen to model yeast*. American Society for Microbiology, Washington DC/US.

Heitman, J., Allen, B., Alspaugh, J. A., and Kwon-Chung, K. J. (1999). On the origins of congenic MAT$\boldsymbol{\alpha}$ and MATa strains of the pathogenic yeast *Cryptococcus neoformans*. *Fungal Genetics and Biology*, **28**(1), 1–5.

Herskowitz, I. (1988). Life cycle of the budding yeast *Saccharomyces cerevisiae*. *Microbiological Reviews*, **52**(4), 536.

Hertwig, O. (1876). Beitrage zur Kenntniss der Bildung, Befruchtung und Theilung des thlerischen Eles. *Morpholog. Jahrbuch*, **1**, 347–434.

Hopfer, R. L. and Blank, F. (1975). Caffeic acid-containing medium for identification of *Cryptococcus neoformans*. *Journal of Clinical Microbiology*, **2**(2), 115–120.

Hu, G., Wang, J., Choi, J., Jung, W. H., Liu, I., Litvintseva, A. P., Bicanic, T., Aurora, R., Mitchell, T. G., Perfect, J. R., and Kronstad, J. W. (2011). Variation in chromosome copy number influences the virulence of *Cryptococcus neoformans* and occurs in isolates from AIDS patients. *BMC Genomics*, **12**(1), 526.

Idnurm, A. (2010). A tetrad analysis of the basidiomycete fungus *Cryptococcus neoformans*. *Genetics*, **185**(1), 153–163.

Idnurm, A., Bahn, Y.-S., Nielsen, K., Lin, X., Fraser, J. A., and Heitman, J. (2005). Deciphering the model pathogenic fungus *Cryptococcus neoformans*. *Nat Rev Micro*, **3**(10), 753–764.

Ikeda, R., Shinoda, T., Fukazawa, Y., and Kaufman, L. (1982). Antigenic characterization of *Cryptococcus neoformans* serotypes and its application to serotyping of clinical isolates. *J. Clin. Microbiol.*, **16**(1), 22–29.

Ito-Kuwa, S., Nakamura, K., Valderrama, B., Aoki, S., Vidotto, V., and Osafune, T. (2008). Diversity of laccase among *Cryptococcus neoformans* serotypes. *Microbiology and Immunology*, **52**(10), 492–498.

Jasnos, L. and Korona, R. (2007). Epistatic buffering of fitness loss in yeast double deletion strains. *Nature Genetics*, **39**(4), 550–554.

Jorgensen, P., Nishikawa, J. L., Breitkreutz, B.-J., and Tyers, M. (2002). Systematic identification of pathways that couple cell growth and division in yeast. *Science*, **297**(5580), 395–400.

Kao, K. C., Schwartz, K., and Sherlock, G. (2010). A genome-wide analysis reveals no nuclear Dobzhansky-Muller pairs of determinants of speciation between *S. cerevisiae* and *S. paradoxus*, but suggests more complex incompatibilities. *PLoS Genet*, **6**(7), e1001038.

Kavanaugh, L. A., Fraser, J. A., and Dietrich, F. S. (2006). Recent evolution of the

human pathogen *Cryptococcus neoformans* by intervarietal transfer of a 14-gene fragment. *Molecular Biology and Evolution*, **23**(10), 1879 –1890.

Kerrigan, R. W., Imbernon, M., Callac, P., Billette, C., and Olivier, J.-M. (1994). The heterothallic life cycle of *Agaricus bisporus* var. *burnettii* and the inheritance of its tetrasporic trait. *Experimental Mycology*, **18**(3), 193–210.

Kidd, S. E., Hagen, F., Tscharke, R. L., Huynh, M., Bartlett, K. H., Fyfe, M., MacDougall, L., Boekhout, T., Kwon-Chung, K. J., and Meyer, W. (2004). A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). *Proceedings of the National Academy of Sciences of the United States of America*, **101**(49), 17258–17263.

Kwon-Chung, K. J. (1975). A new genus, *Filobasidiella*, the perfect state of *Cryptococcus neoformans*. *Mycologia*, **67**(6), 1197–1200.

Kwon-Chung, K. J. (1976a). Morphogenesis of *Filobasidiella neoformans*, the sexual state of *Cryptococcus neoformans*. *Mycologia*, **68**(4), 821–833. ArticleType: research-article / Full publication date: Jul. - Aug., 1976 / Copyright © 1976 Mycological Society of America.

Kwon-Chung, K. J. (1976b). A new species of *Filobasidiella*, the sexual state of *Cryptococcus neoformans* B and C serotypes. *Mycologia*, **68**(4), 942–946.

Kwon-Chung, K. J. (1980). Nuclear genotypes of spore chains in *Filobasidiella neoformans* (*Cryptococcus neoformans*). *Mycologia*, **72**(2), 418–422. ArticleType: research-article / Full publication date: Mar. - Apr., 1980 / Copyright © 1980 Mycological Society of America.

Kwon-Chung, K. J. and Rhodes, J. C. (1986). Encapsulation and melanin formation as indicators of virulence in *Cryptococcus neoformans*. *Infection and Immunity*, **51**(1), 218–223.

Kwon-Chung, K. J. and Varma, A. (2006). Do major species concepts support one, two or more species within *Cryptococcus neoformans*? *FEMS Yeast Research*, **6**(4), 574–587.

Kwon-Chung, K. J., Bennett, J. E., and Theodore, T. S. (1978). *Cryptococcus bacillisporus* sp. nov.: Serotype B-C of *Cryptococcus neoformans*. *International Journal of Systematic Bacteriology*, **28**(4), 616–620.

Kwon-Chung, K. J., Bennett, J. E., and Rhodes, J. C. (1982). Taxonomic studies on *Filobasidiella* species and their anamorphs. *Antonie van Leeuwenhoek*, **48**(1), 25–38.

Kwon-Chung, K. J., Edman, J. C., and Wickes, B. L. (1992). Genetic association of mating types and virulence in *Cryptococcus neoformans*. *Infection and Immunity*, **60**(2), 602–605.

Kwon-Chung, K. J., Boekhout, T., Fell, J. W., and Diaz, M. (2002). (1557) Proposal to conserve the name *Cryptococcus gattii* against *C. hondurianus* and *C. bacillisporus* (Basidiomycota, Hymenomycetes, Tremellomycetidae). *Taxon*, **51**(4), 804–806.

Lee, H.-Y., Chou, J.-Y., Cheong, L., Chang, N.-H., Yang, S.-Y., and Leu, J.-Y. (2008). Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. *Cell*, **135**(6), 1065–1073.

Lengeler, K. B., Cox, G. M., and Heitman, J. (2001). Serotype AD strains of *Cryptococcus neoformans* are diploid or aneuploid and are heterozygous at the mating-type locus. *Infect. Immun.*, **69**(1), 115–122.

Li, J., Pattaradilokrat, S., Zhu, F., Jiang, H., Liu, S., Hong, L., Fu, Y., Koo, L., Xu, W., Pan, W., Carlton, J. M., Kaneko, O., Carter, R., Wootton, J. C., and Su, X.-z. (2011). Linkage maps from multiple genetic crosses and loci linked to growth-related virulent phenotype in *Plasmodium yoelii*. *Proceedings of the National Academy of Sciences*, **108**(31), E374–E382.

Lin, X. (2006). The biology of the *Cryptococcus neoformans* species complex. *Annual Review of Microbiology*, **60**, 69–105.

Lin, X., Hull, C. M., and Heitman, J. (2005). Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. *Nature*, **434**(7036), 1017–1021.

Lin, X., Huang, J. C., Mitchell, T. G., and Heitman, J. (2006). Virulence attributes and hyphal growth of *C. neoformans* are quantitative traits and the MAT$\boldsymbol{\alpha}$ allele enhances filamentation. *PLoS Genet*, **2**(11), e187.

Lin, X., Nielsen, K., Patel, S., and Heitman, J. (2008). Impact of mating type, serotype, and ploidy on virulence of *Cryptococcus neoformans*. *Infect. Immun.*, pages IAI.00168–08.

Lin, X., Patel, S., Litvintseva, A. P., Floyd, A., Mitchell, T. G., and Heitman, J. (2009). Diploids in the *Cryptococcus neoformans* serotype A population homozygous for the $\boldsymbol{\alpha}$ mating type originate via unisexual mating. *PLoS Pathog*, **5**(1), e1000283.

Lind, M., Dalman, K., Stenlid, J., Karlsson, B., and Olson, . (2007). Identification of quantitative trait loci affecting virulence in the basidiomycete *Heterobasidion annosum s.l. Current Genetics*, **52**(1), 35–44.

Litvintseva, A. P. and Mitchell, T. G. (2009). Most environmental isolates of *Cryptococcus neoformans* var. *grubii* (serotype A) are not lethal for mice. *Infect. Immun.*, **77**(8), 3188–3195.

Litvintseva, A. P. and Mitchell, T. G. (2012). Population genetic analyses reveal the African origin and strain variation of *Cryptococcus neoformans* var. *grubii*. *PLoS Pathog*, **8**(2), e1002495.

Litvintseva, A. P., Marra, R. E., Nielsen, K., Heitman, J., Vilgalys, R., and Mitchell, T. G. (2003). Evidence of sexual recombination among *Cryptococcus neoformans* serotype A isolates in Sub-Saharan Africa. *Eukaryotic Cell*, **2**(6), 1162–1168.

Litvintseva, A. P., Kestenbaum, L., Vilgalys, R., and Mitchell, T. G. (2005). Comparative analysis of environmental and clinical populations of *Cryptococcus neoformans*. *J. Clin. Microbiol.*, **43**(2), 556–564.

Litvintseva, A. P., Lin, X., Templeton, I., Heitman, J., and Mitchell, T. G. (2007). Many globally isolated AD hybrid strains of *Cryptococcus neoformans* originated in Africa. *PLoS Pathog*, **3**(8), e114.

Liu, L., Tewari, R. P., and Williamson, P. R. (1999). Laccase protects *Cryptococcus neoformans* from antifungal activity of alveolar macrophages. *Infection and Immunity*, **67**(11), 6034 –6039.

Liu, O. W., Chun, C. D., Chow, E. D., Chen, C., Madhani, H. D., and Noble, S. M. (2008). Systematic genetic analysis of virulence in the human fungal pathogen *Cryptococcus neoformans*. *Cell*, **135**(1), 174–188.

Loftus, B. J., Fung, E., Roncaglia, P., Rowley, D., Amedeo, P., Bruno, D., Vamathevan, J., Miranda, M., Anderson, I. J., Fraser, J. A., Allen, J. E., Bosdet, I. E., Brent, M. R., Chiu, R., Doering, T. L., Donlin, M. J., D'Souza, C. A., Fox, D. S., Grinberg, V., Fu, J., Fukushima, M., Haas, B. J., Huang, J. C., Janbon, G., Jones, S. J. M., Koo, H. L., Krzywinski, M. I., Kwon-Chung, J. K., Lengeler, K. B., Maiti, R., Marra, M. A., Marra, R. E., Mathewson, C. A., Mitchell, T. G., Pertea, M., Riggs, F. R., Salzberg, S. L., Schein, J. E., Shvartsbeyn, A., Shin, H., Shumway, M., Specht, C. A., Suh, B. B., Tenney, A., Utterback, T. R., Wickes, B. L., Wortman, J. R., Wye, N. H., Kronstad, J. W., Lodge, J. K., Heitman, J., Davis, R. W., Fraser, C. M., and Hyman, R. W. (2005). The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. *Science*, **307**(5713), 1321 –1324.

Maheshwari, S. and Barbash, D. A. (2011). The genetics of hybrid incompatibilities. *Annual Review of Genetics*, **45**(1), 331–355.

Marra, R. E., Huang, J. C., Fung, E., Nielsen, K., Heitman, J., Vilgalys, R., and Mitchell, T. G. (2004). A genetic linkage map of *Cryptococcus neoformans* variety *neoformans* serotype D (*Filobasidiella neoformans*). *Genetics*, **167**(2), 619–631.

McDaniel, S. F., Willis, J. H., and Shaw, A. J. (2007). A linkage map reveals a complex basis for segregation distortion in an interpopulation cross in the moss *Ceratodon purpureus*. *Genetics*, **176**(4), 2489–2500.

Meyer, W., Marszewska, K., Amirmostofian, M., Igreja, R. P., Hardtke, C., Methling,

K., Viviani, M. A., Chindamporn, A., Sukroongreung, S., John, M. A., Ellis, D. H., and Sorrell, T. C. (1999). Molecular typing of global isolates of *Cryptococcus neoformans* var. neoformans by polymerase chain reaction fingerprinting and randomly amplified polymorphic DNA–a pilot study to standardize techniques on which to base a detailed epidemiological survey. *ELECTROPHORESIS*, **20**(8), 1790–1799.

Mitchell, T. and Perfect, J. (1995). Cryptococcosis in the era of AIDS–100 years after the discovery of *Cryptococcus neoformans*. *Clin. Microbiol. Rev.*, **8**(4), 515–548.

Morales, L. and Dujon, B. (2012). Evolutionary role of interspecies hybridization and genetic exchanges in yeasts. *Microbiology and Molecular Biology Reviews*, **76**(4), 721–739.

Morrison, L. J., Tait, A., McLellan, S., Sweeney, L., Turner, C. M. R., and MacLeod, A. (2009). A major genetic locus in *Trypanosoma brucei* is a determinant of host pathology. *PLoS Negl Trop Dis*, **3**(12), e557.

Morrow, C. A., Lee, I. R., Chow, E. W. L., Ormerod, K. L., Goldinger, A., Byrnes, E. J., Nielsen, K., Heitman, J., Schirra, H. J., and Fraser, J. A. (2012). A unique chromosomal rearrangement in the *Cryptococcus neoformans* var. *grubii* type strain enhances key phenotypes associated with virulence. *mBio*, **3**(2), e00310–11.

Movahed, E., Munusamy, K., Tan, G. M. Y., Looi, C. Y., Tay, S. T., and Wong, W. F. (2015). Genome-wide transcription study of *Cryptococcus neoformans* H99 clinical strain versus environmental strains. *PLoS ONE*, **10**(9), e0137457.

Moyle, L. C. and Nakazato, T. (2009). Complex epistasis for Dobzhansky–Muller hybrid incompatibility in *Solanum*. *Genetics*, **181**(1), 347–351.

Muller, H. (1942). Isolating mechanisms, evolution and temperature. *Biol. Symp*, **6**(811), 71–125.

Network, F. C. (2002). European Confederation of Medical Mycology (ECMM) prospective survey of cryptococcosis: Report from Italy. *Medical Mycology*, **40**(5), 507–517.

Newcombe, G., Stirling, B., McDonald, S., and Bradshaw Jr, H. D. (2000). *Melampsora* × *columbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*. *Mycological Research*, **104**(3), 261–274.

Nielsen, K., Cox, G. M., Wang, P., Toffaletti, D. L., Perfect, J. R., and Heitman, J. (2003). Sexual cycle of *Cryptococcus neoformans* var. *grubii* and virulence of congenic a and $\boldsymbol{\alpha}$ isolates. *Infection and Immunity*, **71**(9), 4831–4841.

Nishikawa, M. M., Lazera, M. S., Barbosa, G. G., Trilles, L., Balassiano, B. R., Macedo, R. C. L., Bezerra, C. C. F., Perez, M. A., Cardarelli, P., and Wanke, B. (2003). Serotyping of 467 *Cryptococcus neoformans* isolates from clinical and environmental sources in Brazil: Analysis of host and regional patterns. *J. Clin. Microbiol.*, **41**(1), 73–77.

Noor, M. A. F., Grams, K. L., Bertucci, L. A., and Reiland, J. (2001). Chromosomal inversions and the reproductive isolation of species. *Proceedings of the National Academy of Sciences*, **98**(21), 12084–12088.

Odom, A., Muir, S., Lim, E., Toffaletti, D. L., Perfect, J., and Heitman, J. (1997). Calcineurin is required for virulence of *Cryptococcus neoformans*. *The EMBO Journal*, **16**(10), 2576–2589.

O'Donnell, K. and Cigelnik, E. (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution*, **7**(1), 103–116.

Olson, Å. and Stenlid, J. (2002). Pathogenic fungal species hybrids infecting plants. *Microbes and Infection*, **4**(13), 1353–1359.

Orr, H. A. (1996). Dobzhansky, Bateson, and the genetics of speciation. *Genetics*, **144**(4), 1331–1335.

Orr, H. A. (2005). The genetic basis of reproductive isolation: Insights from *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 6522–6526. ArticleType: research-article / Issue Title: Systematics and the Origin of Species: On Ernst Mayr's 100th Anniversary / Full publication date: May 3, 2005 / Copyright © 2005 National Academy of Sciences.

Orr, H. A. and Irving, S. (2001). Complex epistasis and the genetic basis of hybrid sterility in the *Drosophila pseudoobscura* Bogota-USA hybridization. *Genetics*, **158**(3), 1089–1100.

Park, B. J., Wannemuehler, K. A., Marston, B. J., Govender, N., Pappas, P. G., and Chiller, T. M. (2009). Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS*, **23**(4), 525–530.

Park, R. F., Burdon, J. J., and Jahoor, A. (1999). Evidence for somatic hybridization in nature in *Puccinia recondita f. sp. tritici*, the leaf rust pathogen of wheat. *Mycological Research*, **103**(6), 715–723.

Poss, K. D., Nechiporuk, A., Stringer, K. F., Lee, C., and Keating, M. T. (2004). Germ cell aneuploidy in zebrafish with mutations in the mitotic checkpoint gene mps1. *Genes & Development*, **18**(13), 1527–1532.

Posteraro, B., Sanguinetti, M., Sanglard, D., La Sorda, M., Boccia, S., Romano, L., Morace, G., and Fadda, G. (2003). Identification and characterization of a *Cryptococcus neoformans* ATP binding cassette (ABC) transporter-encoding gene, CnAFR1, involved in the resistance to fluconazole. *Molecular Microbiology*, **47**(2), 357–371.

Presgraves, D. C. (2010). The molecular evolutionary basis of species formation. *Nature Reviews Genetics*, **11**, 175–180.

Raper, C. A. and Raper, J. R. (1966). Mutations modifying sexual morphogenesis in *Schizophyllum*. *Genetics*, **54**(5), 1151–1168.

Raper, C. A., Raper, J. R., and Miller, R. E. (1972). Genetic analysis of the life cycle of *Agaricus bisporus*. *Mycologia*, **64**(5), 1088–1117. ArticleType: research-article / Full publication date: Sep. - Oct., 1972 / Copyright © 1972 Mycological Society of America.

Rieseberg, L. H., Baird, S. J. E., and Gardner, K. A. (2000). Hybridization, introgression, and linkage evolution. *Plant Molecular Biology*, **42**(1), 205–224.

Rogers, S. M., Isabel, N., and Bernatchez, L. (2007). Linkage maps of the dwarf and normal Lake Whitefish (*Coregonus clupeaformis*) species complex and their hybrids reveal the genetic architecture of population divergence. *Genetics*, **175**(1), 375–398.

Saeij, J. P. J., Boyle, J. P., Coller, S., Taylor, S., Sibley, L. D., Brooke-Powell, E. T., Ajioka, J. W., and Boothroyd, J. C. (2006). Polymorphic secreted kinases are key virulence factors in toxoplasmosis. *Science (New York, N.Y.)*, **314**(5806), 1780–1783.

Salas, S. D., Bennett, J. E., Kwon-Chung, K. J., Perfect, J. R., and Williamson, P. R. (1996). Effect of the laccase gene CNLAC1, on virulence of *Cryptococcus neoformans*. *The Journal of Experimental Medicine*, **184**(2), 377–386.

Sasaki, T. and Ohshima, Y. (1987). Induction and characterization of artificial diploids from the haploid yeast *Torulaspora delbrueckii*. *Applied and Environmental Microbiology*, **53**(7), 1504–1511.

Scannell, D. R., Zill, O. A., Rokas, A., Payen, C., Dunham, M. J., Eisen, M. B., Rine, J., Johnston, M., and Hittinger, C. T. (2011). The awesome power of yeast evolutionary genetics: New genome sequences and strain resources for the *Saccharomyces sensu stricto* genus. *G3: Genes, Genomes, Genetics*, **1**(1), 11–25.

Schardl, C. L. and Craven, K. D. (2003). Interspecific hybridization in plant-associated fungi and oomycetes: A review. *Molecular Ecology*, **12**(11), 2861–2873.

Sebastiani, F., Barberio, C., Casalone, E., Cavalieri, D., and Polsinelli, M. (2002). Crosses between *Saccharomyces cerevisiae* and *Saccharomyces bayanus* generate fertile hybrids. *Research in Microbiology*, **153**(1), 53–58.

Shahid, M., Han, S., Yoell, H., and Xu, J. (2008). Fitness distribution and transgressive segregation across 40 environments in a hybrid progeny population of the human-pathogenic yeast *Cryptococcus neoformans*. *Genome*, **51**(4), 272–281.

Sia, R. A., Lengeler, K. B., and Heitman, J. (2000). Diploid strains of the pathogenic basidiomycete *Cryptococcus neoformans* are thermally dimorphic. *Fungal Genetics and Biology*, **29**(3), 153–163.

Sionov, E., Chang, Y. C., Garraffo, H. M., and Kwon-Chung, K. J. (2009). Heteroresistance to fluconazole in *Cryptococcus neoformans* is intrinsic and associated with virulence. *Antimicrobial Agents and Chemotherapy*, **53**(7), 2804–2815.

Sionov, E., Lee, H., Chang, Y. C., and Kwon-Chung, K. J. (2010). *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog*, **6**(4), e1000848.

Sionov, E., Chang, Y. C., Garraffo, H. M., Dolan, M. A., Ghannoum, M. A., and Kwon-Chung, K. J. (2012). Identification of a *Cryptococcus neoformans* cytochrome P450 lanosterol 14$\boldsymbol{\alpha}$-demethylase (Erg11) residue critical for differential susceptibility between fluconazole/voriconazole and itraconazole/posaconazole. *Antimicrobial Agents and Chemotherapy*, **56**(3), 1162–1169.

Sora, S., Lucchini, G., and Magni, G. E. (1982). Meiotic diploid progeny and meiotic nondisjunction in *Saccharomyces Cerevisiae*. *Genetics*, **101**(1), 17–33.

Steenbergen, J. N., Shuman, H. A., and Casadevall, A. (2001). *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proceedings of the National Academy of Sciences*, **98**(26), 15245 –15250.

Stephens, M. and Scheet, P. (2005). Accounting for decay of linkage disequilibrium

in haplotype inference and missing-data imputation. *The American Journal of Human Genetics*, **76**(3), 449–462.

Stephens, M., Smith, N. J., and Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *The American Journal of Human Genetics*, **68**(4), 978–989.

Strausberg, R. L. and Perlman, P. S. (1978). The effect of zygotic bud position on the transmission of mitochondrial genes in *Saccharomyces cerevisiae*. *Molecular and General Genetics MGG*, **163**(2), 131–144.

Summerbell, R. C., Castle, A. J., Horgen, P. A., and Anderson, J. B. (1989). Inheritance of restriction fragment length polymorphisms in *Agaricus brunnescens*. *Genetics*, **123**(2), 293–300.

Sun, S. and Xu, J. (2007). Genetic analyses of a hybrid cross between serotypes A and D strains of the human pathogenic fungus *Cryptococcus neoformans*. *Genetics*, **177**(3), 1475 –1486.

Sun, S. and Xu, J. (2009). Chromosomal rearrangements between serotype A and D strains in *Cryptococcus neoformans*. *PLoS ONE*, **4**(5), e5524.

Sunshine, A. B., Payen, C., Ong, G. T., Liachko, I., Tan, K. M., and Dunham, M. J. (2015). The fitness consequences of aneuploidy are driven by condition-dependent gene effects. *PLoS Biol*, **13**(5), e1002155.

Talia, S. D., Skotheim, J. M., Bean, J. M., Siggia, E. D., and Cross, F. R. (2007). The effects of molecular noise and size control on variability in the budding yeast cell cycle. *Nature*, **448**(7156), 947–951.

Tan, Y.-D., Wan, C., Zhu, Y., Lu, C., Xiang, Z., and Deng, H.-W. (2001). An amplified fragment length polymorphism map of the silkworm. *Genetics*, **157**(3), 1277–1284.

Thompson, G. R., Wiederhold, N. P., Fothergill, A. W., Vallor, A. C., Wickes, B. L., and Patterson, T. F. (2008). Antifungal susceptibility among different serotypes of *Cryptococcus gattii* and *Cryptococcus neoformans*. *Antimicrob. Agents Chemother.*, pages AAC.01216–08.

Torres, E. M., Sokolsky, T., Tucker, C. M., Chan, L. Y., Boselli, M., Dunham, M. J., and Amon, A. (2007). Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science*, **317**(5840), 916–924.

Turelli, M. and Orr, H. A. (2000). Dominance, epistasis and the genetics of postzygotic isolation. *Genetics*, **154**(4), 1663–1679.

Vogan, A. A. and Xu, J. (2014). Evidence for genetic incompatibilities associated with post-zygotic reproductive isolation in the human fungal pathogen *Cryptococcus neoformans*. *Genome*, **57**(6), 335–344.

Vogan, A. A., Khankhet, J., and Xu, J. (2013). Evidence for mitotic recombination within the basidia of a hybrid cross of *Cryptococcus neoformans*. *PLoS ONE*, **8**(5), e62790.

Wang, P., Nichols, C. B., Lengeler, K. B., Cardenas, M. E., Cox, G. M., Perfect, J. R., and Heitman, J. (2002). Mating-type-specific and nonspecific PAK kinases play shared and divergent roles in *Cryptococcus neoformans*. *Eukaryotic Cell*, **1**(2), 257–272.

Wang, Y., Aisen, P., and Casadevall, A. (1995). *Cryptococcus neoformans* melanin and virulence: Mechanism of action. *Infection and Immunity*, **63**(8), 3131–3136.

Warnecke, L., Turner, J. M., Bollinger, T. K., Lorch, J. M., Misra, V., Cryan, P. M., Wibbelt, G., Blehert, D. S., and Willis, C. K. R. (2012). Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Sciences*, **109**(18), 6999–7003.

Wickes, B. L. (2002). The role of mating type and morphology in *Cryptococcus neoformans* pathogenesis. *International Journal of Medical Microbiology*, **292**(5-6), 313–329.

Wickes, B. L., Mayorga, M. E., Edman, U., and Edman, J. C. (1996). Dimorphism and haploid fruiting in *Cryptococcus neoformans*: Association with the alpha-mating type. *Proceedings of the National Academy of Sciences*, **93**(14), 7327–7331.

Willett, C. S. (2011). Complex deleterious interactions associated with malic enzyme may contribute to reproductive isolation in the copepod *Tigriopus californicus*. *PLoS ONE*, **6**(6), e21177.

Williamson, P. R. (1994). Biochemical and molecular characterization of the diphenol oxidase of *Cryptococcus neoformans*: Identification as a laccase. *Journal of Bacteriology*, **176**(3), 656–664.

Wilson, D. E., Bennett, J. E., and Bailey, J. W. (1968). Serologic grouping of *Cryptococcus neoformans*. *Experimental Biology and Medicine*, **127**(3), 820–823.

Wu, C. (2001). The genic view of the process of speciation. *Journal of Evolutionary Biology*, **14**(6), 851–865.

Xu, J. (2012). Rapid evolution in experimental populations of major life forms. In R. S. Singh, J. Xu, and R. J. Kulathinal, editors, *Rapidly Evolving Genes and Genetic Systems*, pages 40 – 55. Oxford University Press.

Xu, J. and Mitchell, T. G. (2003). Comparative gene genealogical analyses of strains of serotype AD identify recombination in populations of serotypes A and D in the human pathogenic yeast *Cryptococcus neoformans*. *Microbiology*, **149**(8), 2147–2154.

Xu, J., Vilgalys, R., and Mitchell, T. G. (2000a). Multiple gene genealogies reveal recent dispersion and hybridization in the human pathogenic fungus *Cryptococcus neoformans*. *Molecular Ecology*, **9**(10), 1471–1481.

Xu, J., Ali, R. Y., Gregory, D. A., Amick, D., Lambert, S. E., Yoell, H. J., Vilgalys, R. J., and Mitchell, T. G. (2000b). Uniparental mitochondrial transmission in sexual crosses in *Cryptococcus neoformans*. *Current Microbiology*, **40**(4), 269–273.

Xu, J., Luo, G., Vilgalys, R. J., Brandt, M. E., and Mitchell, T. G. (2002). Multiple origins of hybrid strains of *Cryptococcus neoformans* with serotype AD. *Microbiology*, **148**(1), 203 –212.

Xu, J., Yan, Z., and Guo, H. (2009). Divergence, hybridization, and recombination in the mitochondrial genome of the human pathogenic yeast *Cryptococcus gattii*. *Molecular Ecology*, **18**(12), 2628–2642.

Yan, Z. and Xu, J. (2003). Mitochondria are inherited from the MATa parent in crosses of the basidiomycete fungus *Cryptococcus neoformans*. *Genetics*, **163**(4), 1315 –1325.

Yue, C., Cavallo, L. M., Alspaugh, J. A., Wang, P., Cox, G. M., Perfect, J. R., and Heitman, J. (1999). The STE12$\boldsymbol{\alpha}$ homolog is required for haploid filamentation but largely dispensable for mating and virulence in *Cryptococcus neoformans*. *Genetics*, **153**(4), 1601–1615.

Zhu, X. and Williamson, P. R. (2004). Role of laccase in the biology and virulence of *Cryptococcus neoformans*. *FEMS Yeast Research*, **5**(1), 1–10.