THE EFFECT OF ENVIRONMENTAL FACTORS ON HYBRID FITNESS IN *CRYPTOCOCCUS NEOFORMANS*

THE EFFECT OF ENVIRONMENTAL FACTORS ON HYBRID FITNESS IN *CRYPTOCOCCUS NEOFORMANS*

By

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Abstract

Recent population genetic studies have revealed that there are multiple natural hybridizations among divergent lineages in the human pathogenic fungus *Cryptococcus neoformans.* However, the biological and phenotypic effects of such hybridization are little understood. In this study, we constructed a laboratory cross between two genetically diverged strains; chosen because they differed in growth at different temperature and medium conditions and in their susceptibilities to the common antifungal drug Fluconazole. Our analyses indicated evidence of environment-specific outbreeding depression and intermediate phenotypes in the hybrid population of *C. neoformans.* A variable number of progeny displayed evidence of transgressive segregation. With increasing drug concentration, the relative fitness of transgressive hybrids also increased. The analyses showed that the progeny population had a greater phenotypic plasticity than the parental strains. Our study suggested that hybridization can play a significant role in the evolution of this important human pathogenic fungus.

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I am extremely thankful to my parents, my sister and brother in-law for their encouragement and support and unconditional love. By far the most important contribution is from my mother. She has shared all my success with me and supported me throughout all these years.

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Introduction

Cryptococcus neoformans has been the subject of intense study during the last decade because of its importance as a human pathogen. *C. neoformans* is an encapsulated basidiomycetous yeast and is capable of causing meningoencephalitis in both normal and immunocompromised patients. From the 1980's to early 1990's, due to a high number of immuno-compromised hosts, the incidence of human cryptococcosis was highly prevalent. However, since the mid 1990's the incidence has been decreasing in developed countries due to improved therapies for HIV and other immuno-suppressive conditions (Xu *et al.*, 2000c). It has been reported that globally, about 5 – 10 % of patients with AIDS are killed by *C. neoformans* (Casadevall & Perfect, 1998).

C. neoformans can be classified into five distinct serotypes (A, B, C, D, and AD) based on reactions to monoclonal antibodies. These serotypes represent divergent evolutionary lineages and three varieties have been proposed to reflect their historical divergence: *C. neoformans* var. *grubii*, representing serotype (A) strains. Almost 90% of clinical cases reported are caused by *C. neoformans* var. *grubii*. *C. neoformans* var. *neoformans* represents serotype (D) strains and *C. neoformans* var. *gattii* represents serotype (B) and (C) strains (Franzot *et al.*, 1999). At present, this three-variety classification system ignores the classification of serotype (AD) strains.

DNA sequence analysis has revealed that serotype (B) and (C) strains are phylogenetically more closely related to each other than to strains of serotype (A) or (D). Based on evidence from DNA sequencing, it was identified that serotype (AD) strains are hybrids of serotype (A) and (D) that have been separated by ~18.5 million years of evolution (Xu *et al.*, 2000b). Recent research suggests splitting *C. neoformans* into two separate species. Serotypes (A), (D), and (AD) belong to *Cryptococcus neoformans* (Sanfellice) Vuillemin, while serotypes (B) and (C) are included in the species *Cryptococcus bacillisporus* Kwon-Chung. This nomenclature refers to the original classification that was proposed for the teleomorphic species of *Filobasidiellla* (Kwon-Chung, 1976; Xu *et al.*, 2003).

All major serotypes differ in their ecology, morphology, epidemiology, physiology, molecular characteristics, pathogenicity, and geographical distribution. While serotypes (A), (D), and (AD) are found in temperate climates, serotypes (B) and (C) are mainly confined to tropical and subtropical areas associates with Eucalyptus trees. Clinical isolates of serotype (AD) are not very common in comparison to serotype (A). Serotype (A) is responsible for the majority of human infections and the majority of environmental isolates (Casadevall & Perfect, 1998; Franzot *et al.,* 1999), but serotype (AD) occurs in much higher frequency than other serotypes in a specific geographic area. For instance, 8 of 13 strains obtained from Belgium were serotype AD (Xu *et al.*, 2002) and 14% of isolates from San Francisco area were serotype AD, exceeding the combined number of isolates of serotypes (B), (C) and (D) from the same region (Brandt *et al.*, 1996).

The mating system in *C. neoformans* is heterothallic and is controlled by a large locus with two alleles; MAT-(a) and MAT-(α), which can easily mate with each other given suitable conditions. Under laboratory conditions, yeast cells with opposite mating types can fuse to form dikaryotic hyphae. In the basidia of dikaryotic hypha, nuclear fusion and meiosis occur to produce four chains of haploids basidiospores (Kwon-Chung, 1976).

Despite their significant divergence, many strains of serotypes (A) and (D) can mate in the laboratory (Yan *et al.*, 2002). Their meiotic progeny may contain alleles from both parental strains and are typically diploid or aneupliod (Lengeler, 2001). Most natural hybrid (AD) has one allele highly similar to the serotype (A) group and the other to the serotype (D) group. Experimental studies have shown that under laboratory conditions, two synthetic diploids from strains of serotype (A) and (D) had higher growth rates in vitro compared to their parental pairs (Toffaletti *et al.,* 2004). However, in a virulence test with rabbit model of crypotococcal meningitis, hybrid (AD) had virulence similar to serotype (A) parents but higher virulence than the serotype (D) parents (Toffaletti *et al.,* 2004). The phenotypic and genotypic consequences of hybridization between strains of serotype (A) and (D) remain largely unknown.

Hybridization

If populations come in contact before reproductive barriers are fully developed, they may form hybrids and exchange genes (Riesberg *et al.*, 2003). The role of hybridization in evolution has been debated for more than a century and it is thought that it may influence evolution in a variety of ways. There are two distinct viewpoints among biologists. At one extreme, hybridization is considered as an evolutionary force that creates opportunity for speciation and adaptive evolution. Proponents of this view argue that the increased genetic variation and new gene combinations resulting from hybridization promote the development and acquisition of novel adaptations. The contrasting position accords hybridization as an "evolutionary noise" that temporarily affects evolution but does not play an important role in it (Barton, 2001; Riesberg *et al.*, 2003). Some of the important consequences of hybridization include: the origin and transfer of genetic adaptations, the origin of new species, the breakdown of reproductive barriers, and the merger of species.

So far, most studies on hybridization have focused on plants and animals. Very little is known about the consequences of hybridization in fungi.

Hybridization is a process or product of the process in which an offspring is generated from mating between two genetically divergent parental strains (Schardl *et al.,* 2003). The outcome of hybridization can be a powerful tool for exploring the magnitude and genetic architecture of population differentiation. Hybrid progeny can be separated based on their phenotypic effects. For example, random genetic drift can lead to the fixation of alternate alleles in parents. Hybridization of these populations may result in hybrid vigor (heterosis). Alternately, hybrid progeny may express outbreeding depression and under-perform relative to their parents. Under natural conditions, loss of fitness may be due to a loss of local adaptation or breakdown of co-adapted genes at multiple loci to either parental environment (Galloway *et al.,* 2004).

Among hybrid progeny, there may be phenotypes that are extreme relative to either parent. The generation of these extreme phenotypes is referred to as transgressive segregation. Previous studies have shown that hybrid progeny can have extreme phenotypes relative to their parents. For example, plant hybrids often distribute differently from their parents and are typically found in more extreme habitats (Riesberg *et al.*, 2003). Literature searches indicate that most transgressive segregation in plants and animals results from the combination of alleles from both parents that have similar effects in the same direction on a phenotype (Riesberg *et al.*, 2003).

Several mechanisms have been proposed for observed transgressive phenotypes in segregating hybrid populations. These mechanisms include (i) elevation of mutation rate in hybrid; (ii) non-additive interaction between alleles at the same locus or overdominance;

(iii) non-additive interactions between alleles from different loci or epistasis; and (iv) unmasking rare recessive alleles that are normally heterozygous in the parental taxa (Riesberg *et al.*, 1999). The nature of these mechanisms responsible for transgressive segregation is critical to hybridization in adaptive evolution. For instance, overdominance could not be fixed in sexual diploid hybrids; therefore, it would be unlikely to contribute to long-term adaptive evolution in sexual diploids. Also the unmasking of rare recessive alleles that are normally heterozygous in the parental lines does not apply to parental populations with haploid genomes. On the other hand, extreme phenotypes generated by epistasis or the new combinations of alleles with opposing effects could become fixed in stabilized hybrid lineages through either selection or drift (Riesberg *et al.*, 1999).

All serotypes of *C. neoformans* reproduce primarily asexually. In laboratory conditions, it is possible to cross strains of opposite mating types and produce sexual progeny. Studies have demonstrated that strains of serotype (A) and (D) are genetically distinct but it is possible to obtain mating between isolates from the two serotypes (Kwon-Chung, 1975), thus making hybridization studies of *C. neoformans* under laboratory conditions possible. At present, less is known about how environmental factors such as antifungal drugs, nutrients and temperature influence the phenotypes of hybrid populations. Literatures demonstrate that hybrid (AD) serotype strains are more tolerant to Fluconazole when compared to strains of serotype (A) and (D) (Xu *et al.*, 2001). There has been no research on how the generation of extreme hybrids can be influenced by high temperature, high drug concentration and low nutrition. Lack of knowledge about the phenotypic and genotypic consequences of hybrids and the interaction of the hybrids with environmental factors makes such research valuable for understanding fungal hybridization and speciation.

Environmental factors affecting growth

One of the most important features of human pathogenic fungi is their ability to grow vigorously at 37°C. Most fungal species are not capable of growing at such elevated temperature. It has been suggested that pathogenicity of *C. neoformans* may have been evolved during the interactions with organisms in the soil (Steenbergen *et al.*, 2001). However, their ability to survive and grow rapidly at the human and other mammalian bodies' temperatures of 37 °C could not be the result of interacting with soil environments that have temperatures much lower than 37 °C.

Several proteins have been identified in *C. neoformans* as being essential for growth at high temperatures. These include CNA1, Ras1, the p21-activated kinase (PAK) kinase STE20, the phospholipid-binding protein Cts1, the vacuolar ATPase Vph1, the thiol peroxidase Tsa1, amongst others. The degeneration or deletion of genes encoding each of these proteins would cause either attenuation or loss of virulence in mammalian hosts (Karos *et al.*, 2000). This reveals that a high temperature environment is a necessary factor for the evolution and maintenance of pathogenicity in this fungus (Xu *et al.*, 2004).

C. neoformans show growth in most laboratory media. Requirements for the growth of this fungus are carbon, nitrogen, oxygen sources and trace elements. This fungus has been found in variety of places such as eucalyptus trees, birds droppings, and soils contaminated by avian excreta. Experimental evidence suggests that *C. neoformans* has the enzymes that allow it to live in harsh environments such as bird excreta (Casadevall & Perfect, 1998). Previous studies have revealed that the level of nutrition in media influences the ability of mating efficiency. Carbon and nitrogen source alteration can lead to an increase or decrease in mating rate (Dong & Courchesne, 1998).

The common antifungal drug Fluconazole has low toxicity, high oral bioavailability, broad spectrum efficacy, and ease of administration to the patient. Indeed, these features make it the preferred antifungal drug for treating many fungal infections. This antifungal drug can penetrate the patient's central nervous system and works against *C. neoformans*. It functions by disrupting the biosynthesis of ergosterol and blocking an alpha-14 demethylation step in the biosynthetic pathway (Xu *et al.*, 2001).

Drug resistance is the acquired ability of a microorganism to resist the effects of a chemotherapeutic agent to which it is normally susceptible. When a drug is used in excess, resistant mutations will be selected for. Microorganisms can become resistant to drugs immediately after the drug becomes available, even if it is used in moderation. For instance, penicillin was used extensively to treat *N. gonorrhoeae* but presently it is utterly useless against the pathogen (Madigan, 2006). Fluconazole-resistant fungal pathogens are becoming more common (Xu *et al.*, 2001). The evidence from previous studies indicates that *C. neoformans* are becoming more resistant to Fluconazole during the treatment period of infected patients. There is extensive surveillance effort over the use of Fluconazole for *Candida albicans,* another common fungal pathogen. However, there is very little surveillance of the use of Fluconazole to fight *C. neoformans* infections (Pfaller *et al.*, 2005). Testing for drug resistance in all types of pathogenic microorganisms is extremely important to find the way these microorganisms develop drug resistance.

Phenotypic plasticity

Phenotypic plasticity is the potential of an organism to alter its phenotype in response to changing environmental conditions, such as the previously mentioned temperature, media, and drug concentration. It is believed that phenotypic plasticity evolved to allow organisms a greater chance of survival in their ever-changing surroundings. Phenotypic plasticity may play an important role in organisms such as plants and animals in their adaptation and evolution by combining a physiological buffering to poor environmental conditions with an improved response to favorable conditions (Pigliucci, 2001). The understanding of how phenotypic diversity is generated by the coherent change of other integrated traits is a key challenge in evolutionary biology.

Plasticity is a property of the reaction norm of a genotype. Reaction norm describes the functional interrelationship of a range of environments to a range of phenotypes. The difference between measurements in different environment is called environmental sensitivity: the greater the difference, the greater the phenotypic plasticity. Not all the genotypes respond similarly to the same environmental changes. The variation in response among genotypes is termed genotype-environment interaction (Scheiner, 1993).

Previous studies have indicated significant plasticity in plants and that at least three genetic mechanisms contribute to plasticity. Overdominance occurs in an inverse relationship between plasticity and heterozygosity. Pleiotropy occurs as a function of differential expression of the same gene in different environment. Epistasis occurs when two distinct classes of genes control the two fundamental characteristics of a reaction norm: its plasticity and its across-environment average value. Evidence supporting the overdominance model is less prevalent than for the other two models. Models of plasticity evolution could account for both pleiotropic and epistatic effects (DeWitt & Scheiner, 2004)

The aim of this study is to investigate the phenotypic effects of hybridization in *C. neoformans.* We specifically address the following questions: (1) Is there any evidence for outbreeding depression or heterosis in this species with regards to vegetative fitness when comparing hybrid progeny with parents? (2) Will hybrid progeny show extreme phenotypes (i.e. transgressive segregation) relative to the parents? (3) Do environmental factors such as high temperature, high drug concentrations and limited nutrition influence the production of progeny with extreme phenotypes? (4) Will interactions between the environmental factors show significant influence on vegetative fitness of hybrids? And (5) if so, how much does each of these environmental factors contribute to the variation of hybrid progeny's fitness?

Material and methods

Experimental design and implementation

Stains, cross, and isolation of progeny

In this experiment, two parental strains (JEC20 and CDC15) and a total of 269 progeny from the cross of those parental strains were tested in environments that differ in temperatures, media and Fluconazole concentrations. The two parental strains, JEC20 and CDC15, were first used to construct the hybrid progeny population. These two stains were chosen due to their divergent genotypic and phenotypic traits. Strain JEC20 is a model laboratory strain. It is a standard tester for determining mating types in *C. neoformans*. The parental strains for the derivation of JEC20 are NIH433, a serotype D MATalpha environmental isolate from pigeon droppings in Denmark, and NIH12, a serotype D MATa clinical isolate from a patient with osteomyelitis in the United States (Hietman *et al.,* 1999). It belongs to *C. neoformans* var. *neoformans* and has the MATa allele at the mating type locus. It is susceptible to the antifungal drug Fluconazole at MIC=4 μg/mL, where MIC refers to lowest drug concentration that inhibits 80% of growth compared to the no drug control as (Xu *et al.*, 2001) based on standards set by the National Committee for Clinical Laboratory Standard (NCCLS). In contrast, strain CDC15 was isolated in 1992 from the United States clinical surveillance sources. It belongs to *C. neoformans* var. grubii, and has the (α) allele at the mating type locus. CDC15 was chosen because of its resistance to Fluconazole (MIC=64 μg/mL).

Crosses between JEC20 and CDC15 were conducted on V-8 juice agar (Xu *et al.*, 2000b). After incubation at 25°C for 2-4 weeks, hyphal filaments were observed outside of the mating mixture. For isolation of progeny, single basidiospores were obtained from various regions outside of mating mixture to maximize sampling of different mating and meiotic events (Xu *et al.*, 2000b). Agar containing hyphae and basidiospores was cut and washed gently in sterile distilled water. The spore suspension was vortexed, diluted, and plated onto YEPD agar (1% yeast extract, 2% dextrose, 2% Bacto Peptone, 1.5% agar). Individual germinated spores were transferred to new YEPD plates (Xu *et al.*, 2000b). A total of 269 basdiospores were stored permanently in a -70 °C freezer readily accessible for phenotypic analyses.

Fluconazole susceptibility testing

Vegetative fitness determined by colony size is quantitative and suitable for variety of statistical analyses. Because it's relatively inexpensive and simple, the use of colony size to measure vegetative fitness in fungi has had a long tradition, and has been extensively used in recent work (Pringle *et al.*, 2002). The procedure of estimating vegetative fitness based on colony size is described in Xu's paper (Xu *et al.*, 1998).

To determine the effect of environmental factors on hybrid vegetative fitness and the potential genotype-environment interactions, three environmental variables were examined: temperature, medium and Fluconazole concentrations. The two parental strains (JEC20 and CDC15) and all their 269 progeny were examined. We chose Fluconazole because it is one of the most widely used antifungal drug for long-term maintenance treatment of cryptococcal infections. In this study, two basic fungal media: the minimal medium SD (Difco-yeast nitrogen base supplemented with dextrose) and the common rich medium YEPD (yeast- extract, Bacto-peptone and dextrose) were used and supplemented with a series of Fluconazole concentrations as follows: 0 μg/mL, 0.5 μg/mL, 1 μg/mL, 2 μg/mL, 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL, 64 μg/mL, 100 μg/mL. Finally, the combination of medium and drug concentration was examined at 25 °C and 37 °C temperatures.

This experimental design thus includes 40 treatments (2 media x 2 temperatures x 10 drug concentrations) for 269 progeny and 2 parental strains. For each strain, a single colony was suspended in 200 μL of sterile water via vortexing and 1μL of liquid suspension was streaked on to SD and YEPD plates containing 0, 0.5, 1, 2, 4, 8, 16, 32, 64, 100 μg/mL of Fluconazole per mL respectively. The plates were incubated either at 37 °C or 25 °C for 48 hours. To maximize representation of measurement for each strain in each treatment, five independent colonies were measured separately in each treatment. The evidence of growth was examined under a microscope with 4x magnification (Xu *et al.*, 1998). The degree of drug-resistance of strains was measured according to the change of colony size after the treatment of Fluconazole on the isolates. A total of 54200 (40x271x5) data points were collected.

Data analysis

Vegetative fitness comparison between parental strains and hybrid progeny at different drug concentrations

The objective of this analysis is to examine whether there is evidence indicative of outbreeding depression and heterosis. The analysis is done by comparing the mean and standard deviations of the vegetative fitness between parents and progeny in each of the 40 testing environments. If the mean vegetative fitness of hybrids exceeded both parents, the result would be consistent with heterosis. In contrast, if the mean vegetative fitness of the progeny were lower than both parents, the result would be consistent with outbreeding depression. Finally if the mean vegetative fitness is between the mean vegetative fitness of the two parents, the result would be consistent with intermediate phenotype and additive gene function. A graphical overview of vegetative fitness distribution was used to show the relationship between progeny and the parental strains in each of the 40 conditions. To determine whether the Fluconazole concentration has any effect of producing different vegetative fitness on parents and hybrids, we performed two-sample t-tests on four different environments.

Estimates of transgressive phenotypes

To determine whether there is transgressive segregation and the fraction of hybrids with transgressive phenotypes in each of the 40 testing environments, we calculated the percentage of individuals with fitness exceeding that of the fitter parent (in this case CDC15) by two standard deviations. We also calculated the percentage of individuals with fitness lower than that of the smaller parent JEC20 by 2 standard deviations (De Vicente & Tanksley, 1993). To standardize the calculation, the vegetative fitness of the two parents in each of the four testing environments was scaled to 1 separately and the relative fitness of hybrids with transgressive phenotypes was adjusted accordingly as a simple ratio of the colony size of the hybrids with transgressive phenotypes over the parents. The standardization allowed the comparison of vegetative fitness differences among testing environments. To investigate the relationship between Fluconazole concentration and vegetative fitness of hybrids with transgressive phenotypes, regression analysis was preformed. Linear regression was used to find the relationship between a response variable vegetative fitness (Y) and a predictor variable, such as Fluconazole concentration (X) by extending the simple linear regression to modeled regression with the equation below. The equation shows maximum fitness reduced by applying the drug, where the reduction depends on drug concentration.

Equation 1 $$F = fmax - AExp[-k C]$$

or

Equation 2 $$\text{Log}[fmax - fitness] = \log[A] - k C$$

Where F represents vegetative fitness of transgressive hybrids, fmax is the maximum vegetative fitness of the transgressive hybrids and C represents the drug concentrations. The vegetative fitness and maximum fitness were log transformed for normalization. k represents linear regression coefficient that measures how drug concentration effects the fitness.

Genotype-environment interactions of hybrids

The factorial ANOVA method was employed to test the relative contributions of strains, medium, incubation temperature, drug concentration and their two-way, three-way, and four-way interactions to variation in vegetative fitness. Sums of squares were partitioned into sources attributable to four main effects and six pairwise two-way interactions, four three-way interactions, one four-way interaction, and the within-treatment random error. P values of <0.005 will indicate the interactions between genotype and environmental factors are significant and suggest that these factors are involved in determining hybrid vegetative fitness. This statistical tests followed the procedures in Sokal and Rohlf (1981), using the SPSS program.

Influence of environmental factors on vegetative fitness

To determine how much each of the four environmental factors and the interactions among these four factors contribute to hybrid vegetative fitness, the sums of squares were partitioned into sources attributable to the four main effects and eleven interaction effects. The percentage of each main effect was calculated by using the sum of squares of the variable divided by the total sums of squares.

Estimate of phenotypic plasticity of hybrids

Phenotypic plasticity is typically represented by reaction norm. Measurement of plasticity should indicate both the amount of change (slope) in the phenotype across environments and the pattern of that change. Genetic variation for plasticity occurs when genomes have different reaction norm functions. The genetic variance for plasticity can also be measured as an across-environment correlation. To estimate how genetic variation across environments contribute to the phenotypic plasticity of hybrids, the coefficient of variation (CV) was calculated by using standard deviation over the mean of hybrids in each concentration and environment. We compared the genetic variation for plasticity between the parental lines and hybrids by calculating the mean and the standard deviation for each progeny at the given fluconazole concentration. The progeny means were then used to calculate the average and standard deviation for that progeny across all the drug concentrations. The same method was used to obtain the coefficient of variation values for the parental lines. The graphical overviews of phenotypic plasticity were used to present the number of individuals that fall into different intervals of coefficient of variation and how they compare to the parental strains.

Phenotypic plasticity for temperature and medium were also estimated by comparing the correlation coefficients of progeny with their parents in the no fluconazole environment. Four sets of environmental conditions were tested: (i) at 25°C, the plasticity between SD and YEPD media; (ii) at 37°C, the plasticity between SD and YEPD media; (iii) on SD medium, the plasticity between temperatures 25°C and 37°C; and (iv) on YEPD, the plasticity between temperatures of 25°C and 37°C.

Results

Evidence for outbreeding depression and intermediate phenotype within hybrid progeny in T37/SD and T25/SD environments

When the temperature was set to 37 °C, and the low-nutrition (SD) medium was used, the hybrid progeny showed the mean vegetative fitness lower than both parental strains between fluconazole concentrations 0 and 2 μg/mL. Between 4 -100 μg/mL, hybrids showed intermediate mean vegetative fitness of the two parents (Figure 1). In the T37/SD environment, the parental CDC15 showed a significantly higher vegetative fitness than parent JEC20 in all drug concentrations (d.f. = 450, $p<0.001$). As the drug concentration increased, the difference between the two parents was more pronounced. In high drug concentrations, the means of hybrids were significantly lower than the parental strain CDC15, as confirmed by the statistical test (d.f. = 1569, $p<0.001$).

However, hybrid progeny and parent JEC20 showed no significant difference in any of the concentrations (Table 1), and JEC20 did not show any significant growth advantage over the hybrids. The overall results indicated that in the T37/SD environment, outbreeding depression occurred in certain drug concentrations in *C. neoformans.* As the concentration of Fluconazole increased from 0 to 2 μg/mL, vegetative fitness of hybrids was reduced. However none of the differences were statistically significant.

At 25 °C, hybrid progeny had lower mean vegetative fitness than both parental strains in all concentrations (Figure 2). Result from t-tests showed that the difference was statistically significant (d.f. = 450, $p<0.001$) between the two parents in all concentrations. This difference was more pronounced with increasing Fluconazole concentrations, with CDC15 having greater average fitness compared to JEC20, with advantages ranging between 13.4% to 66.3% for concentrations 0 to 100 µg/mL. The hybrids and CDC15 also showed significant differences (d.f. = 1569, $p<0.001$) in their mean vegetative fitness. Strain CDC15 had significant growth advantage (19% to 86.2% for 0-100 µg/mL concentrations) over hybrids in all the concentrations. When comparing the mean vegetative fitness of JEC20 to the hybrids, the differences were not statistically significant at any of the fluconazole concentrations (Table 1).

Hybrid progeny have intermediate phenotypes of the two parents in T25/YEPD and T37/YEPD environments

Hybrids in this cross showed intermediate vegetative fitness between the two parental strains in both T25/YEPD and T37/YEPD environments in all drug concentrations (Figure 3 and 4). In general, strains in both environments grew significantly faster at 37 °C than at 25 °C. At 37 and 25 °C, parent CDC15 showed a significantly higher vegetative fitness than JEC20 and the difference was statistically significant in all drug concentrations (d.f. = 450, $p<0.001$). The differences between the parents were more pronounced as the drug concentration increased.

At 37 °C, the hybrid progeny and the parent JEC20 showed significantly differences (d.f. = 1569, $p<0.001$) in all fluconazole concentrations, with hybrids having a greater growth advantage ranging from 11.1% to 51.3% for 0-100 µg/mL of Fluconazole concentrations. At 25 °C, JEC20 and hybrids also showed significant difference (d.f. = 1569, $p<0.001$) in all the concentrations with hybrids having greater growth advantage of 6.3% to 25.8% among the tested Fluconazole concentrations.

Table 1. Summary information of differences between JEC20 and hybrids in four testing environments. In T25/SD and T37/SD environments, hybrids and JEC20 strains showed no significant differences in any of the fluconazole concentrations. In contrast, in all YEPD environments, hybrids and JEC20 showed statistically significant differences in all the concentrations. The fluconazole concentration is in μg/mL.

Table 1: Summary of differences between JEC20 and Hybrids in various environments

	Testing Environments							
	T37/YEPD		T37/SD		T25/SD		T25/YEPD	
Drug Concentration	T value	p value	T value	p value	T value	p value	T value	p value
0	3.748	0.00018***	0.33	0.7408NS	1.352	0.176 NS	1.925	0.05435*
0.5	5.266	1.58E-07***	1.08	0.27851NS	1.728	0.084 NS	2.458	0.01405**
1	5.448	5.90E-08***	0.25	0.80488NS	1.295	0.195 NS	2.545	0.01103**
2	9.333	3.40E-20***	0.63	0.53127NS	0.986	0.324 NS	3.547	0.0004***
4	8.312	2.01E-16***	0.45	0.65455 NS	0.941	0.347 NS	4.187	0.0000***
8	7.918	4.54E-15***	3.12	0.00186 NS	0.437	0.662 NS	5.206	0.0000***
16	6.485	1.18E-10***	2.68	0.00742 NS	0.99	0.322 NS	6.243	0.0000***
32	4.218	0.000026***	2.08	0.03773 NS	0.079	0.936 NS	5.547	0.0000***
64	4.958	7.89E-07***	0.37	0.71403 NS	0.804	0.421 NS	5.112	0.0000***
100	5.172	2.61E-07***	0.53	0.59860 NS	2.25	0.024 NS	4.475	0.0000***

[ote: Only data from JEC20 and Hybrids were used in these analyses, *P<0.05; **P<0.01; **P<0.0005; NS, not significant.

Figure 1. Fluconazole concentration-dependent outbreeding depression and intermediate phenotypes in *C. neoformans* at T37/SD environment. Bars in the graph represent the trends of distribution of vegetative fitness vs. Fluconazole concentration of parents and progeny. The upper and the lower error bars are the extent of the standard deviations. Fluconazole concentration is measured in μg/mL. Growth is expressed as the mean of the colony size.

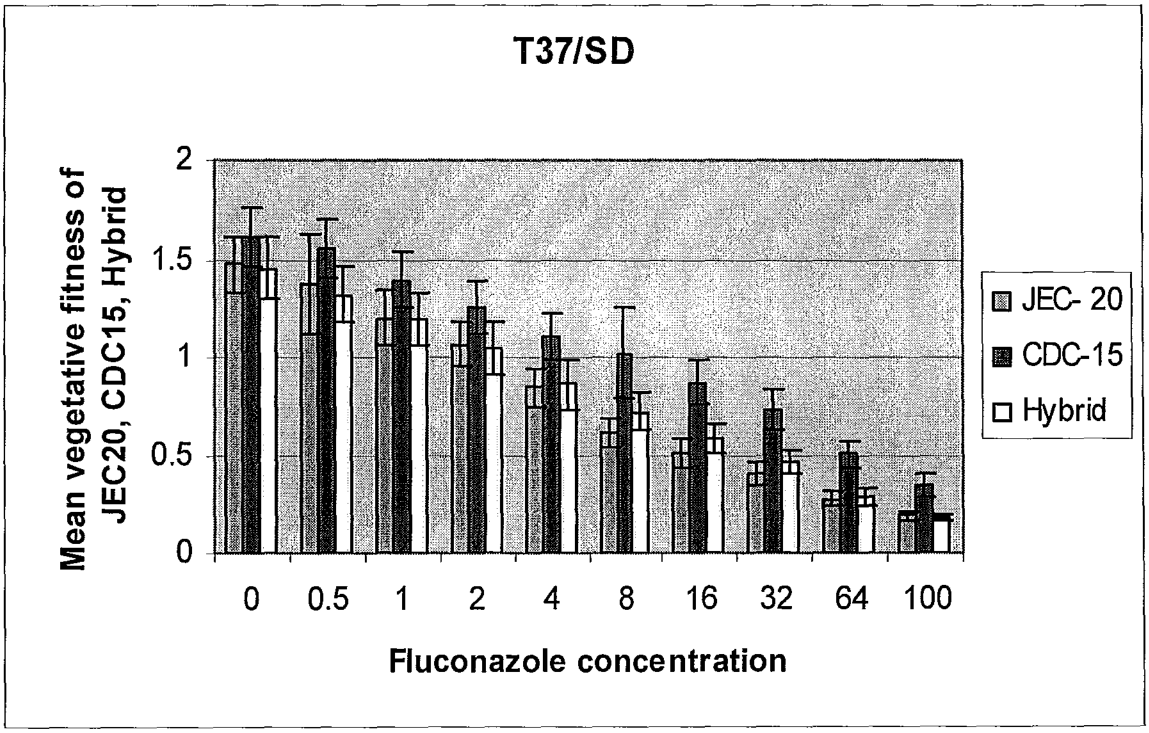

Figure 1. Relative vegetative fitness of JEC20, CDC15 and hybrid progeny at different Fluconazole concentrations in the T37/SD environment.

Figure 2. Fluconazole concentration-dependent outbreeding depression in *C. neoformans* at the T25/SD environment. Bars in the graph represent the trends of distribution of vegetative fitness vs. Fluconazole concentration of parents and progeny. The upper and the lower error bars are the extent of the standard deviations. Fluconazole concentration is measured in μg/mL. Growth is expressed as the mean of the colony size.

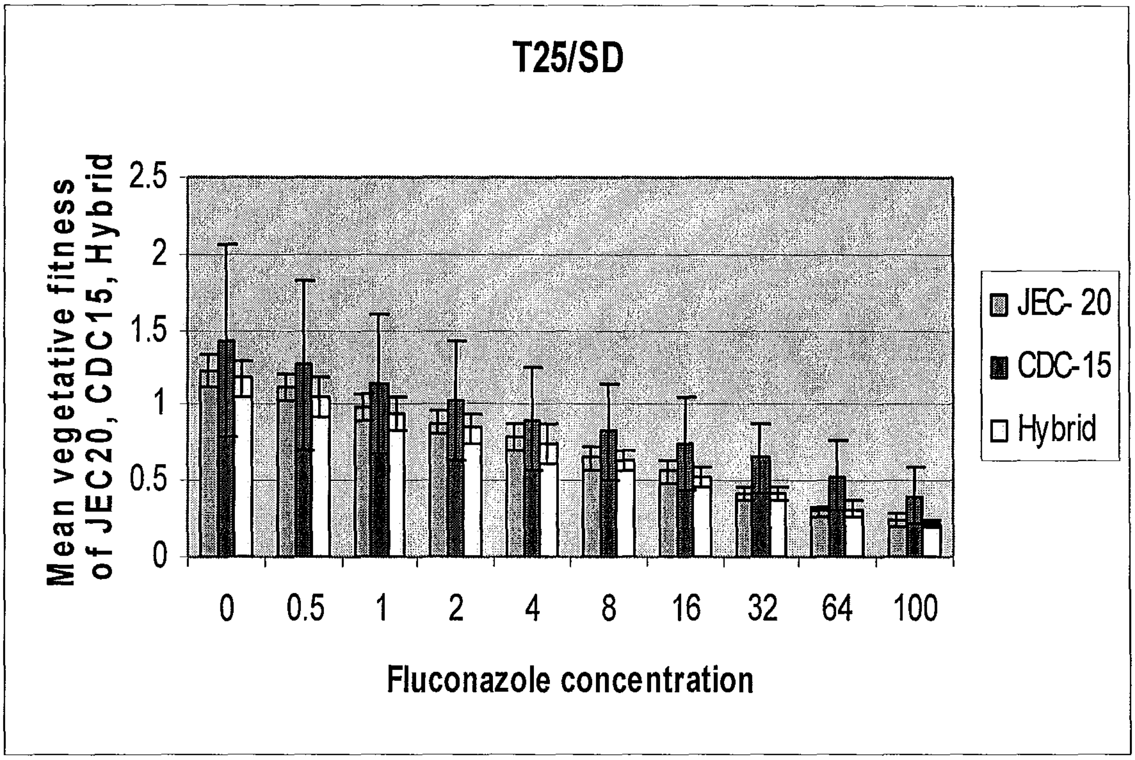

Figure 2. Relative vegetative fitness of JEC20, CDC15 and hybrid progeny at different Fluconazole concentrations in the T25/SD environment.

Figure 3. Fluconazole concentration-dependent intermediate phenotypes in *C. neoformans* at the T25/YEPD environment. Bars in the graph represent the trends of distribution of vegetative fitness vs. Fluconazole concentration of parents and progeny. The upper and the lower error bars are the extent of the standard deviations. Fluconazole concentration is measured in μg/mL. Growth is expressed as the mean of the colony size.

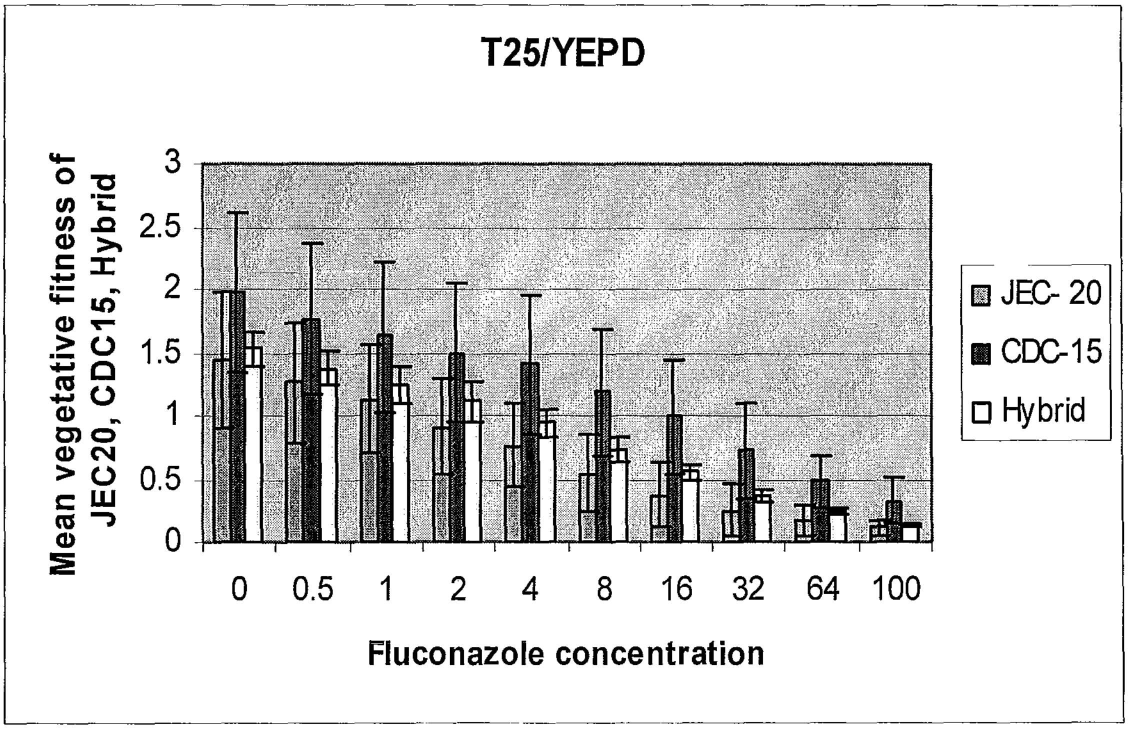

Figure 3. Relative vegetative fitness of JEC20, CDC15 and hybrid progeny at different Fluconazole concentrations in the T25/YEPD environment.

Figure 4. Fluconazole concentration-dependent intermediate phenotype in *C. neoformans* at the T37/YEPD environment. Bars in the graph represent the trends of distribution of vegetative fitness vs. Fluconazole concentration of parents and progeny. The upper and the lower error bars are the extent of the standard deviations. Fluconazole concentration is measured in μg/mL. Growth is expressed as the mean of the colony size.

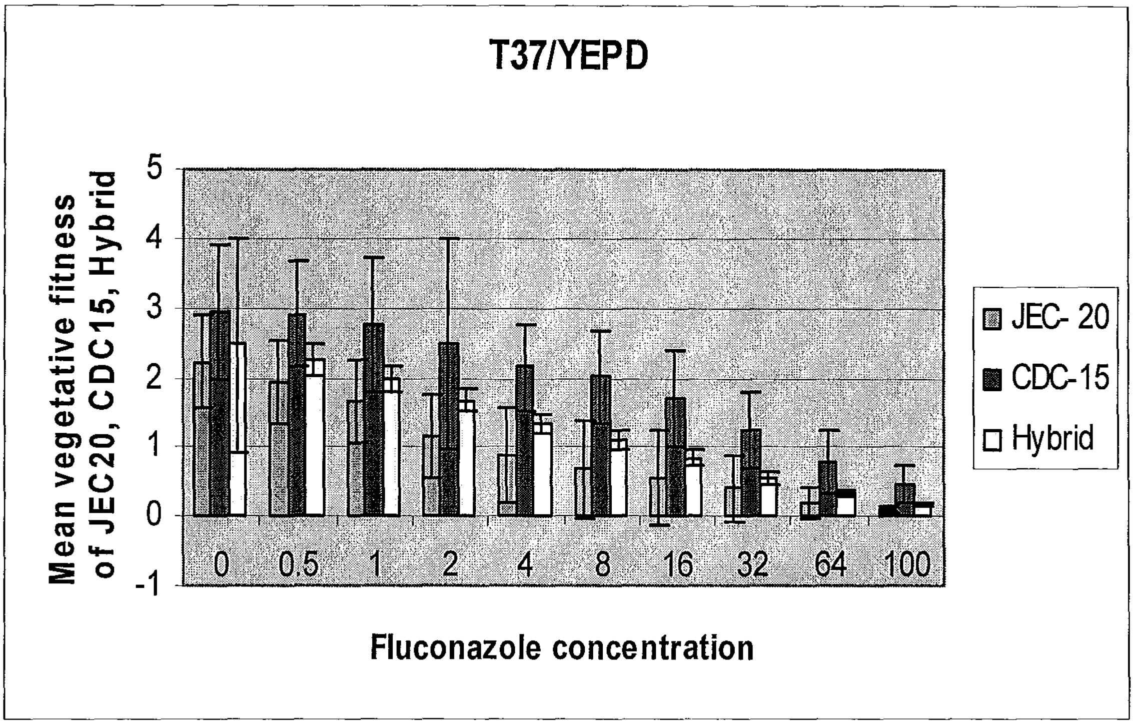

Figure 4. Relative vegetative fitness of JEC20, CDC15 and hybrid progeny at different Fluconazole concentrations in the T37/YEPD environment.

Evidence for transgressive segregation in hybrid progeny and environmental influence in production of extreme hybrids

To identify progeny with extreme phenotypes relative to parental strains, a comprehensive analysis was done in all 40 testing environments. The mean and standard deviation of the each progeny in each environment were compared to those of the two parents. Tables 2 to 5 indicate the percentage of individuals observed that exceeded or fell behind the means of both parents by at least 2 standard deviations in each of the 40 conditions. The goal of this analysis is to find progeny that are outside of the 95% range of parents and have significant higher or lower vegetative fitness than both parents.

Progeny with higher vegetative fitness compared to parental strains

There are several noteworthy observations regarding extreme hybrids from the four environmental conditions. First, at the T25/SD environment, hybrids had the highest number of extreme phenotypes in all the concentrations when compared with other three environments (Table 5). At the 0 μg/mL fluconazole concentration, 2.9% of the progeny had significantly greater vegetative fitness than the parents. In this environment, as drug concentration increased, the percentage of extreme hybrids increased, with the highest percentage at 4 μg/mL Fluconazole (7.4%). However, starting from the concentration of 8 μg/mL, the number of transgressive segregants decreased with increasing Fluconazole concentration.

Second, in the T37/SD environment, hybrids showed the highest percentage of extreme phenotypes (3.7%) at concentration 2 μg/mL. However at this concentration in the T37/YEPD environment, there was no transgressive segregants (Tables 3 & 4). Third, at the T37/SD environment with fluconazole concentrations 64 and 100 μg/mL, the number of transgressive segregants is similar to that in the T25/SD environment. Fourth, at the T25/YEPD environment, the 0.5 μg/mL drug concentration showed the highest percentage of transgressive segregants of 4.4% (Table 2). Fifth, except T37/YEPD, the three temperature-nutrient conditions showed the lowest number of transgressive segregants in the two highest drug concentrations, 64 and 100 μg/mL.

At the T37/SD environment with the drug concentration 100 μg/mL, we observed the greatest difference between any progeny and the parents, with the progeny 4.2 times larger than parental strains. Overall, the highest vegetative fitness of transgressive segregants was observed from concentrations 2 to 100 μg/mL in the T37/SD environment. Our results suggest that the environment with high temperature, high drug concentrations and limited nutrition showed the highest production of progeny with extreme vegetative fitness.

Progeny with lower vegetative fitness compared to parental strains

Results from the analysis of progeny with fitness lower than parental line JEC20 indicated that all the testing environments except T25/SD showed less fit progeny in low drug concentrations. At the 2 μg/mL concentration in the T37/ SD environment, 7 % progeny showed significantly lower vegetative fitness than parent JEC20, the less fit of the two parents (Table 4).

To standardize our comparison, the vegetative fitness of the parents in each of the four testing environments was scaled to 1 and the relative fitness of hybrids with transgressive phenotypes were adjusted accordingly as a simple ratio of the colony size of the hybrids with transgressive phenotypes over the parents. Table 6 and 7 indicate the colony size of the hybrids with transgressive phenotypes lager and smaller than both parents in the four testing environments. The results from Table 6 suggested that hybrids with transgressive phenotype showed different degrees of growth on different Fluconazole concentrations. As drug concentration increases, the fitness of hybrids with extreme phenotype increases. In general, we observed the lowest vegetative fitness of extremely small hybrids from concentration 0 to 1 μg/mL in the T37/SD environment. Relatively speaking, the smallest progeny was 14.7 times smaller than parental strain JEC20 in the no drug treatment in the T37/SD environment.

Regression analysis of transgressive hybrid with greater fitness than both parents

The goal of the regression analysis is to determine the parameters for a function that best fit a set of data. To find the best model to fit the set of data, we normalized the vegetative fitness data using natural logarithm (Thanks to the suggestion by Dr. Stone). The vegetative fitness of transgressive hybrids displayed an exponential relationship with fluconazole concentration in all four temperature-environment conditions. The following regression equations are the model equations derived from this study for four environments.

For the T25/SD environment: LN (fmax-f) = -0.0264*C - 0.0382

For the T37/SD environment: LN (fmax-f) = -0.03*C + 0.8125

For the T25/YEPD environment: LN (fmax-f) = -0.0454*C- 0.477

For the T37/YEPD environment: LN (fmax-f) = -0.0557*C + 0.3184

From the regression analysis we also obtain the R^2 for each of the four conditions, describing how much of the change in the dependent variable (log of colony size) is explained by changes in the independent variable (drug concentration). Our analysis indicated that the concentration of Fluconazole accounts for 74%, 78%, 87%, and 86% of the variation in vegetative fitness of the transgressive segregants in the T25/SD, T37/SD, T25/YEPD, and T37/YEPD environments receptively (Figures5 to 8).

Table 2. Summary information for the number of progeny with fitness higher and lower than both parents at different Fluconazole concentrations in the T25/YEPD environment. The number of progeny with higher fitness decreases with increasing Fluconazole concentration. Only concentrations 0-1 μg/mL of Fluconazole showed progeny with lower fitness than JEC20. Fluconazole concentration is measured in μg/mL.

Table 2: Summary information of the number of progeny with fitness higher and lower than both parents at different Fluconazole concentrations in T25/YEPD

	T25/YEPD				
Drug Concentration	Mean(±SD) of Hybrid	Mean(±SD) of CDC15	Number of Hybrid with fitness higher than the lager parent CDC15	Mean(±SD) of JEC20	Number of Hybrid with fitness lower than the smaller parent JEC20
0	1.53 ± 0.139	1.99 ± 0.639	6	1.44 ± 0.535	5
0.5	1.37 ± 0.136	1.78 ± 0.593	12	1.26 ± 0.476	5
1	1.25 ± 0.142	1.63 ± 0.589	9	1.13 ± 0.429	2
2	1.12 ± 0.157	1.50 ± 0.545	8	0.91 ± 0.374	0
4	0.95 ± 0.109	1.42 ± 0.538	7	0.76 ± 0.326	0
8	0.73 ± 0.088	1.19 ± 0.496	5	0.54 ± 0.299	0
16	0.55 ± 0.067	0.99 ± 0.451	8	0.37 ± 0.261	0
32	0.36 ± 0.047	0.72 ± 0.374	4	0.25 ± 0.211	0
64	0.24 ± 0.024	0.48 ± 0.209	4	0.16 ± 0.126	0
100	0.14 ± 0.012	0.33 ± 0.173	2	0.11 ± 0.060	0

Note: Data are presented as mean ± standard deviation of colony sizes in ocular micrometers.

Table 3. Summary information for the number of progeny with fitness higher and lower than both parents at different Fluconazole concentrations in the T37/YEPD environment. The number of progeny with higher fitness decreases with increasing Fluconazole concentration. Only concentrations 0-1 μg/mL of Fluconazole showed progeny with lower fitness than JEC20. Fluconazole concentration is measured in μg/mL.

Table 3: Summary information of the number of progeny with fitness higher and lower than both parents at different Fluconazole concentrations in T37/YEPD

	T37/YEPD				
Drug Concentration	Mean(±SD) of Hybrid	Mean(±SD) of CDC15	Number of Hybrid with fitness higher than the lager parent CDC15	Mean(±SD) of JEC20	Number of Hybrid with fitness lower than the smaller parent JEC20
0	2.46 ± 1.534	2.94 ± 0.969	4	2.21 ± 0.666	18
0.5	2.25 ± 0.223	2.91 ± 0.772	6	1.92 ± 0.592	9
1	1.97 ± 0.198	2.76 ± 0.967	3	1.64 ± 0.598	4
2	1.66 ± 0.161	2.48 ± 1.506	0	1.12 ± 0.585	0
4	1.35 ± 0.135	2.16 ± 0.606	6	0.86 ± 0.690	0
8	1.11 ± 0.120	2.02 ± 0.673	6	0.66 ± 0.715	0
16	0.85 ± 0.098	1.72 ± 0.672	2	0.54 ± 0.683	0
32	0.56 ± 0.094	1.24 ± 0.555	6	0.39 ± 0.478	0
64	0.31 ± 0.048	0.77 ± 0.448	4	0.19 ± 0.218	0
100	0.17 ± 0.015	0.46 ± 0.287	2	0.11 ± 0.047	0

Note: Data are presented as mean ± standard deviation of colony sizes in ocular micrometers.

Table 4. Summary information for the number of progeny with fitness higher and lower than both parents at different Fluconazole concentrations in the T37/SD environment. The number of progeny with higher fitness decreases with increasing Fluconazole concentration. Only concentrations 0, 0.5, 2 μg/mL of Fluconazole showed progeny with lower fitness than JEC20. Fluconazole concentration is measured in μg/mL.

Table 4: Summary information of number of progeny with fitness higher and lower than both parents at different Fluconazole concentrations in T37/SD

	T37/SD				
Drug Concentration	Mean(±SD) of Hybrid	Mean(±SD) of CDC15	Number of Hybrid with fitness higher than the lager parent CDC15	Mean(±SD) of JEC20	Number of Hybrid with fitness lower than the smaller parent JEC20
0	1.45 ± 0.156	1.62 ± 0.703	7	1.47 ± 0.598	2
0.5	1.32 ± 0.148	1.55 ± 0.678	7	1.37 ± 0.946	0
1	1.19 ± 0.133	1.39 ± 0.609	9	1.20 ± 0.499	9
2	1.04 ± 0.135	1.26 ± 0.53	10	1.06 ± 0.430	19
4	0.87 ± 0.129	1.10 ± 0.518	9	0.85 ± 0.429	0
8	0.72 ± 0.095	1.02 ± 0.824	3	0.61 ± 0.411	0
16	0.59 ±0.075	0.87 ± 0.464	5	0.50 ± 0.409	0
32	0.46 ± 0.063	0.73 ± 0.384	8	0.41 ± 0.344	0
64	0.28 ± 0.044	0.50 ± 0.310	4	0.27 ± 0.260	0
100	0.18 ± 0.018	0.34 ± 0.194	3	0.18 ± 0.159	0

Note: Data are presented as mean ± standard deviation of colony sizes in ocular micrometers.

Table 5. Summary information for the number of progeny with fitness higher and lower than both parents at different Fluconazole concentrations in the T25/SD environment. The number of progeny with higher fitness decreases with increasing Fluconazole concentration. This environment preformed the highest number of progeny in all the concentrations. In contrast, no progeny with lower fitness was observed in any concentration. Fluconazole concentration is measured in μg/mL.

Table 5: Summary information of number of progeny with fitness higher and lower than both parents at different Fluconazole concentrations in T25/SD

	T25/SD				
Drug Concentration	Mean(±SD) of Hybrid	Mean(±SD) of CDC15	Number of Hybrid with fitness higher than the lager parent CDC15	Mean(±SD) of JEC20	Number of Hybrid with fitness lower than the smaller parent JEC20
0	1.17 ± 0.116	1.39 ± 0.632	8	1.23 ± 0.616	0
0.5	1.04 ± 0.126	1.26 ± 0.564	13	1.11 ± 0.601	0
1	0.94 ± 0.105	1.13 ± 0.463	12	0.99 ± 0.515	0
2	0.84 ± 0.104	1.03 ± 0.399	14	0.87 ± 0.432	0
4	0.75 ± 0.125	0.90 ± 0.332	20	0.78 ± 0.361	0
8	0.63 ± 0.075	0.82 ± 0.312	18	0.64 ± 0.337	0
16	0.53 ± 0.065	0.75 ± 0.309	14	0.55 ± 0.323	0
32	0.41 ± 0.050	0.65 ± 0.233	9	0.42 ± 0.280	0
64	0.30 ± 0.049	0.53 ± 0.234	4	0.30 ± 0.223	0
100	0.21 ± 0.026	0.40 ± 0.192	3	0.24 ± 0.215	0

Note: Data are presented as mean ± standard deviation of colony sizes in ocular micrometers.

Table 6. Summary information of the colony size of the hybrids with transgressive phenotypes with fitness greater than CDC15 in the four testing environments. The colony size of transgressive hybrids increases with increasing Fluconazole concentrations. In T37/SD, concentrations 2-100 μg/mL showed the highest colony sizes compared to other environments. However, in T25/SD, concentrations 0-1 μg/mL showed the highest colony size compared to other environments. Fluconazole concentration is measured in μg/mL.

Table 6: Relative fitness of transgressive hybrids greater than CDC15 in various environments

Drug Concentration	Testing environments			
	T25/SD	T37/SD	T25/YEPD	T37/YEPD
0	2.301± 0.274	2.037± 0.098	1.868± 0.177	1.797± 0.061
0.5	2.234± 0.816	2.002± 0.084	1.979± 0.214	1.667± 0.081
1	2.176± 0.334	2.105± 0.141	2.040± 0.565	1.799± 0.098
2	2.175± 0.369	2.277± 0.977	1.875± 0.775	0.000± 0.000
4	2.083± 1.894	2.368± 0.425	1.942± 1.125	1.782± 0.198
8	1.985± 0.392	3.202± 0.382	2.029± 0.147	1.938± 0.143
16	2.047± 0.278	2.614± 0.360	2.030± 0.093	2.027± 0.000
32	2.274± 0.307	2.534± 0.437	2.048± 0.714	2.230± 0.125
64	2.867± 0.360	4.052± 1.722	2.434± 0.395	2.685± 0.493
100	2.986± 0.453	4.288± 1.486	2.456± 0.502	2.711± 0.415

Note: Vegetative fitness of the parents was scaled to 1 in each environment. Relative fitness of hybrids with transgressive phenotypes were adjusted as a simple ratio of the colony size of the hybrids with transgressive phenotypes over the parents.

Table 1.7. Summary information of the colony size of the hybrids with transgressive phenotypes with fitness smaller than the JEC20 parent in the four testing environments. The colony size of transgressive hybrids is smallest at the lowest drug concentration. At high concentrations there is no evidence of transgressive hybrids. The T25/SD environment has no transgressive hybrids in any concentrations. Fluconazole concentration is measured in μg/mL.

Table 7: Relative fitness of transgressive hybrids smaller than JEC20 parents in various environments

Drug Concentration	Testing environments			
	T25/SD	T37/SD	T25/YEPD	T37/YEPD
0	0.000± 0.000	0.067± 0.000	0.069± 0.000	0.234± 0.106
0.5	0.000± 0.000	0.000± 0.000	0.091± 0.029	0.182± 0.096
1	0.000± 0.000	0.072± 0.000	0.088± 0.000	0.080± 0.046
2	0.000± 0.000	0.083± 0.000	0.000± 0.000	0.000± 0.000
4	0.000± 0.000	0.000± 0.000	0.000± 0.000	0.000± 0.000
8	0.000± 0.000	0.000± 0.000	0.000± 0.000	0.000± 0.000
16	0.000± 0.000	0.000± 0.000	0.000± 0.000	0.000± 0.000
32	0.000± 0.000	0.000± 0.000	0.000± 0.000	0.000± 0.000
64	0.000± 0.000	0.000± 0.000	0.000± 0.000	0.000± 0.000
100	0.000± 0.000	0.000± 0.000	0.000± 0.000	0.000± 0.000

Note: Vegetative fitness of the parents was scaled to 1 in each environment. Relative fitness of hybrids with transgressive phenotypes was adjusted as a simple ratio of the colony size of the hybrids with transgressive phenotypes over the parents.

Figure 5. Vegetative fitness of transgressive hybrids of *C. neoformans* displayed an exponential relationship vs. the concentration of Fluconazole in the T37/SD environment. Regression equation: LN (fmax-f) = -0.03*C + 0.8125. The R^2 = 78.6%indicates that concentration of Fluconazole accounts for 78.6% of the variation in colony size among transgressive hybrids.

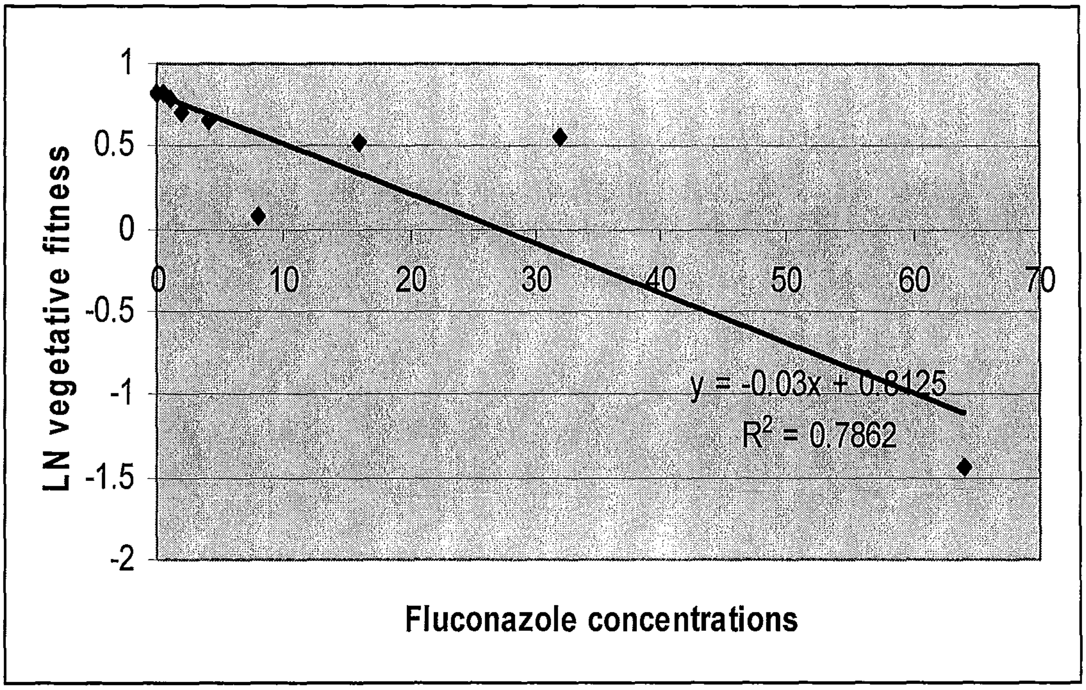

Figure 5. Linear regression of relative vegetative fitness (mm) of hybrids with transgressive phenotypes at 10 drug concentration (μg/ml) in T37/SD testing environment.

Figure 6. Vegetative fitness of transgressive hybrids of *C. neoformans* displayed an exponential relationship vs. the concentration of Fluconazole in the T25/YEPD environment. Regression equation: LN (fmax-f) = -0.0454*C- 0.477. The $R^2 = 87.4\%$ indicates that the concentration of Fluconazole accounts for 87.4% of the variation in colony size of transgressive hybrids.

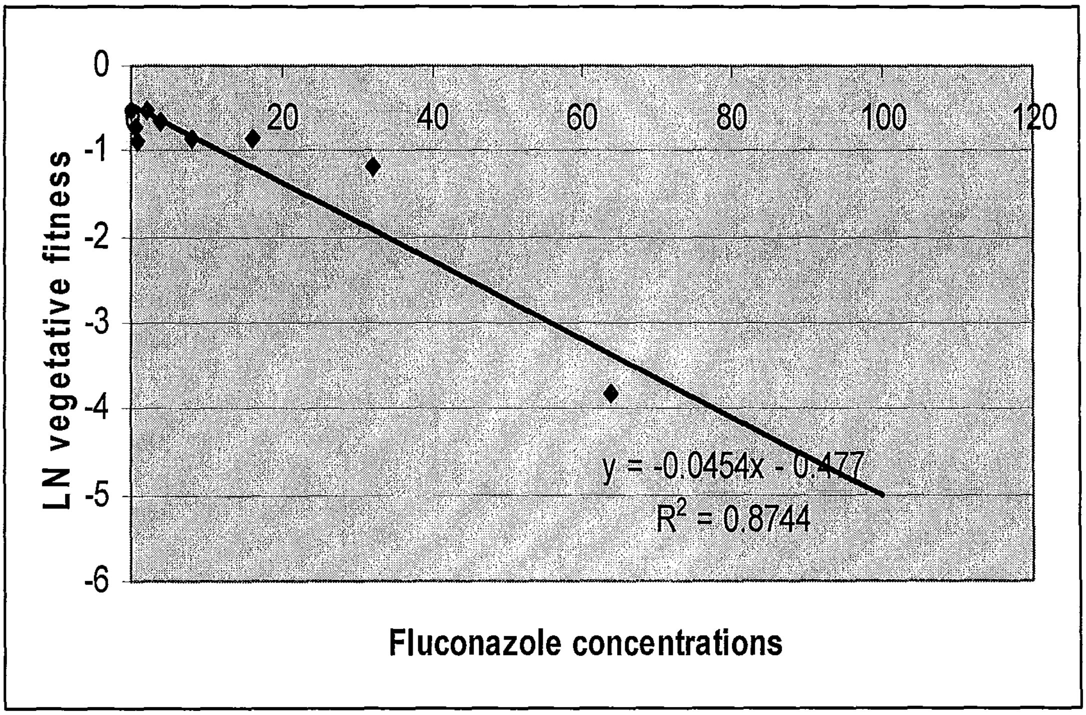

Figure 6. Linear regression of relative vegetative fitness (mm) of hybrids with transgressive phenotypes at 10 drug concentration (μg/ml) in T25/YEPD testing environment.

Figure 7. Vegetative fitness of transgressive hybrids of *C. neoformans* displayed an exponential relationship vs. the concentration of Fluconazole in the T37/YEPD environment. Regression equation: LN (fmax-f) = -0.0557*C + 0.3184. The R^2 = 86.5% indicates that the concentration of Fluconazole accounts for 86.5% of the variation in colony size of transgressive hybrids.

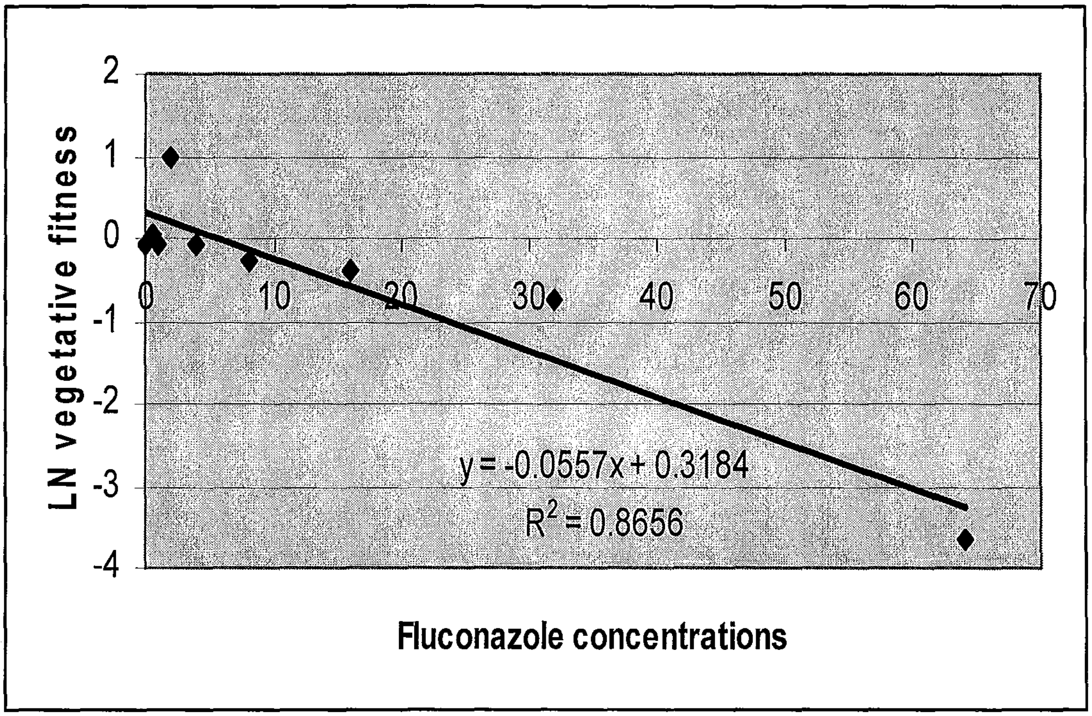

Figure 7. Linear regression of relative vegetative fitness (mm) of hybrids with transgressive phenotypes at 10 drug concentration (µg/ml) in T37/YEPD testing environment.

Figure 8. Vegetative fitness of transgressive hybrids of *C. neoformans* displayed an exponential relationship vs. the concentration of Fluconazole in the T25/SD environment. Regression equation: LN (fmax-f) = -0.0264*C - 0.0382. The R^2 = 74.3% indicates that the concentration of Fluconazole accounts for 74.3% of the variation in colony size of transgressive hybrids.

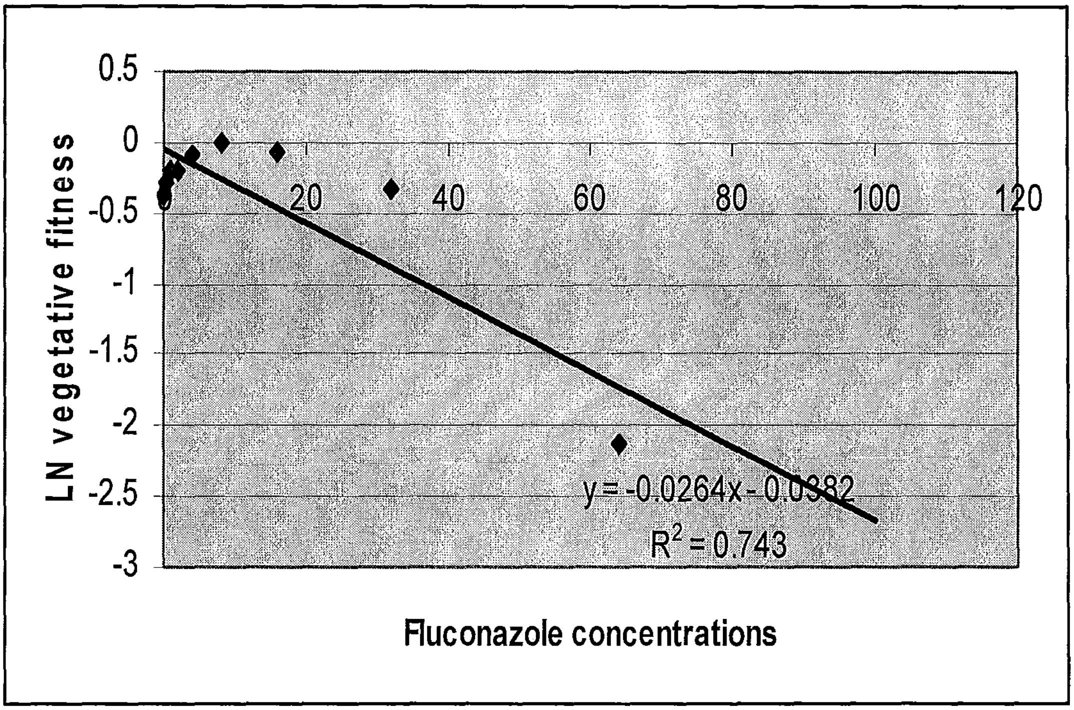

Figure 8. Linear regression of relative vegetative fitness (mm) of hybrids with transgressive phenotypes at 10 drug concentration (μg/ml) in T25/SD testing environment.

Genotype-environment interactions and their effects on vegetative fitness of hybrids

C. neoformans can be easily grown in the lab in a variety of temperature and media conditions. However, medium, temperature and drug concentrations may interact and influence the fitness of hybrid genotypes in different directions. To test this hypothesis, we first tested the main effect of these factors individually. Table 8 shows summarized results of the factorial ANOVA test. The data were first fitted into various models and the best model suggested that natural logarithm transformation allow the data to fit into a normal distribution. Because of the large data sets, no computer program can analyze all the 269 progeny at once. We ran the test for 5 progeny at the time and averaged all the 54 sets to get the total values. Table 8 shows degrees of freedom of only 5 progeny, however, mean squares; F-ratio and p values were adjusted based on the entire population of hybrids.

Statistical significances of genotype-environment interactions of vegetative fitness were determined by a four-way ANOVA. Based on this analysis we conclude that the combinations of genotype-environmental factors significantly affect vegetative fitness. For single-factor analysis, there was a significant contribution by each of the four factors to vegetative fitness. For bi-factorial analyses, statistically significant interactions affecting vegetative fitness were observed in all six pairwise interactions between progeny and medium, medium and temperature, progeny and temperature, temperature and drug, progeny and drug and drug and medium respectively (Table 8). In addition, there were significant three-way interactions among progeny, drug and temperature; medium, progeny and drug; medium, progeny and temperature; and medium, drug and temperature respectively. Similarly, significant four-way interaction among progeny, drug, temperature and medium was also found (Table 8). The analysis indicated that genotype, temperature, medium and drug concentration as well as various genotype-environment interactions account for 98.5% of the variation in vegetative fitness among the hybrids analyzed here.

Representative reaction norm graphs of hybrids in the four environments are shown in Figures 9 and 10. If there were no genotype-environment interactions, the vegetative fitness lines should be parallel and the crossing of reaction norms should not occur. However, as shown in Figures 9 and 10, crossing of reaction norm lines occurred in the nutrient-poor medium at intermediate drug concentrations and in the nutrient-rich medium at high drug concentrations in both the T25 and T37 environments. This result is consistent with the presence of genotype-environment interactions of vegetative fitness among hybrids.

Environmental factors contribute to the variation of hybrids

On the basis of these observations, we hypothesized that genotype-environment interactions contribute significantly to phenotypic variations of the hybrids. To estimate their relative contributions to the total fitness variation, we calculated the percentage contribution of each environmental factor by using sum of squares of the variable, divided by the total sums of squares. For single factor analysis, the results show that most phenotypic variations are explained by variation in drug concentration (64%) followed by progeny genotype (8%), medium (2.5%), and lastly temperature (2%). The combined contribution of genotype-environment interactions is 18.04% (Table 9).

Correlation of variation (CV) among hybrid progeny

The histograms of the correlation of variation (CV) distribution of hybrids and parents in T25/YEPD and 37/YPED environments showed that most hybrid progeny had greater phenotypic plasticity than the parental strains with regard to fluconazole concentrations (Figures 11 and 12). Specifically, in the T25/YEPD environment, 95.1% of progeny showed greater plasticity than parent CDC15. In the T37/YEPD environment, 78.8% of hybrid progeny displayed greater phenotypic plasticity compared to JEC20. However, in the other two environments T25/SD and T37/SD, the mean phenotypic plasticity of fluconazole concentration was similar to those of the two parents (Figure 13 and 14). In the T25/SD environment, about 39.4% of the hybrids had greater plasticity than JEC20 and in the T37/SD environment only 50% of hybrids had greater plasticity compared to JEC20 (Table 9).

Hybrid progeny had a greater range of phenotypic plasticity than parental strains with respect to medium and temperature conditions

Figures 15-18 showed the quantitative distribution of phenotypic plasticity arranged by each of the four treatment variables: 25 °C, 37 °C, YEPD, and SD. For each of the two temperatures 25 °C and 37 °C, we compared the vegetative fitness variation between the two growth media YEPD and SD (Figures 15 and 16). Similarly, for each of the two medium conditions YEPD and SD, we compared the vegetative fitness variation between the two growth temperatures (Figures 17 and 18). The phenotypic plasticity here for each strain (i.e. the two parents and the 269 progeny) is expressed as a correlation coefficient between the environmental variable (25 °C vs. 37 °C, or YEPD vs. SD) and their vegetative fitness. The correlation coefficients of the progeny are then grouped into 10 categories

(X-axis) and the respective number of progeny in each category is presented on the Y-axis. The correlation coefficients for the parental strains are indicated in each of the four graphs.

As can be seen from these graphs, at both the 25 °C and 37 °C temperature environments, the two parental strains showed low fitness variation (low phenotypic plasticity) between the rich YEPD medium and the minimal SD medium. In contrast, there was a significant variation in phenotypic plasticity among the progeny (Figures 15 and 16). Of the two temperatures, the 25 °C environment revealed a greater spread of phenotypic plasticity among the progeny than the 37 °C environment.

However, a different pattern was seen in Figures 17 and 18. Both parental strains showed significant phenotypic plasticity between the two temperatures with a much higher growth at 37 °C than at 25 °C on either YEPD or SD media. While the majority of the progeny had similar phenotypic plasticity as the parental strains, a significant number showed a pattern different from the two parental strains. Furthermore, the SD medium revealed a larger number of progeny than the YEPD medium that showed different phenotypic plasticity as the parents (Figures 17 and 18).

Table 8. Four-way factorial analysis of variance comparing gene-environment interactions among all progeny derived from the cross of JEC20 and CDC15. All gene-environment interactions were statistically significant, and together were able to explain 98.5% of the variation among progenies. This means that the four factors, genotype, temperature, medium, and drug concentration can almost fully explain progeny growth rates.

d.f. stands for degrees of freedom, SS stands for sum of squares, MS stands for mean squares, and F is the F statistic.

Table 8: Four-way ANOVA for genotype-environment interactions of LN vegetative fitness in experimental population of C. *neoformans*

Source	d.f.	SS	MS	F
Medium(M)	1	23.984	23.984	1591.474***
Progeny(P)	4	77.210	19.302	1336.930***
Drug(D)	9	611.269	67.919	4204.369***
Temperature(T)	1	20.005	20.005	1063.670***
M X P	4	28.055	7.014	489.091***
M X D	9	14.858	1.651	104.206***
D X P	36	40.606	1.128	74.429***
M X T	1	12.180	12.180	818.332***
P X T	4	11.284	2.821	195.997***
D X T	9	17.228	1.914	111.067***
M X P X T	4	16.602	4.151	266.802***
M X P X D	36	22.222	0.617	40.757***
M X D X T	9	5.062	0.562	37.946***
P X D X T	36	33.596	0.933	59.429***
M X P X D X T	36	19.733	0.548	33.561***
Error	800	14.275	0.018	

Note: Only data from 5 hybrids were used in these analyses. ***P<0.001

Table 9: Percentage of genotype- environment factors contribution to vegetative fitness.

Source	Contribution Percentage
Medium (M)	2.51%
Progeny(P)	8.09%
Drug (D)	64.08%
Temperature (T)	2.10%
M X P	2.94%
M X D	1.56%
D X P	4.26%
M X T	1.28%
P X T	1.18%
D X T	1.81%
M X P X T	1.74%
M X P X D	2.33%
M X D X T	0.53%
P X D X T	3.52%
M X P X D X T	2.07%

Note: Data is derived from Table 8.

Figure 9. Relative vegetative fitness of 269 progeny displayed genotype-environment interactions at T25/T37 on YEPD medium environment in 10 drug concentrations. The interactions are more pronounced at higher drug concentrations. For normal distribution, the natural logarithm was applied to the vegetative fitness of the progeny. This natural logarithm was used to estimate the marginal mean via the SPSS program. Fluconazole concentration is measured in μg/mL.

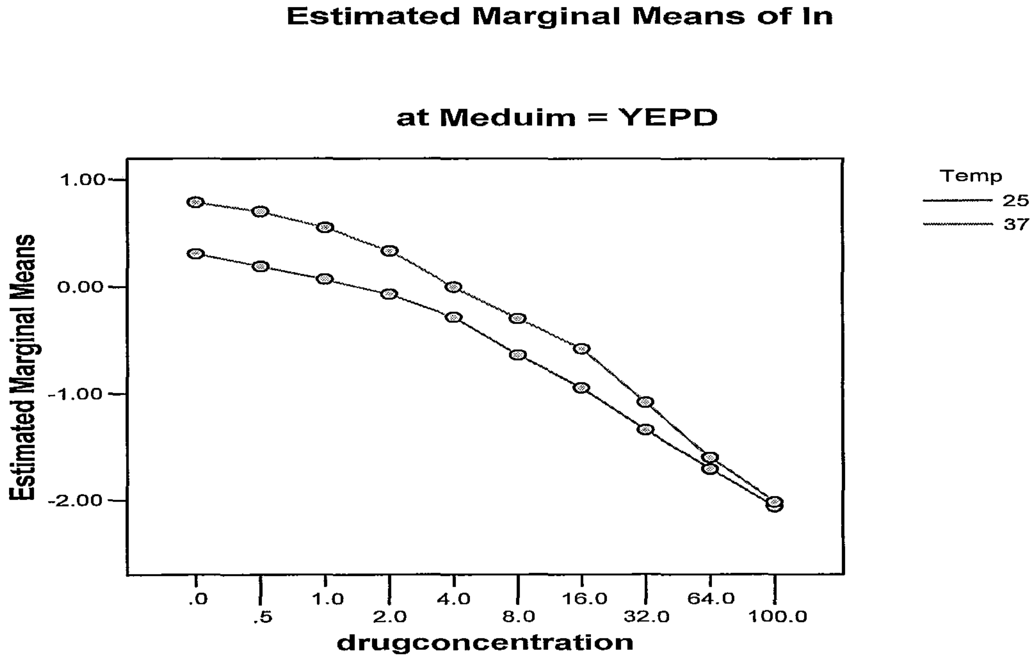

Figure 9. Relative vegetative fitness of 269 progeny grown at T25/ 37 on YEPD medium environment.

Figure 10. Relative vegetative fitness of 269 progeny displayed genotype-environment interactions at T25/T37 on SD medium environment in 10 drug concentrations. The interactions are more pronounced at higher drug concentrations. For normal distribution, the natural logarithm was applied to the vegetative fitness of the progeny. This natural logarithm was used to estimate the marginal mean via the SPSS program. Fluconazole concentration is measured in μg/mL.

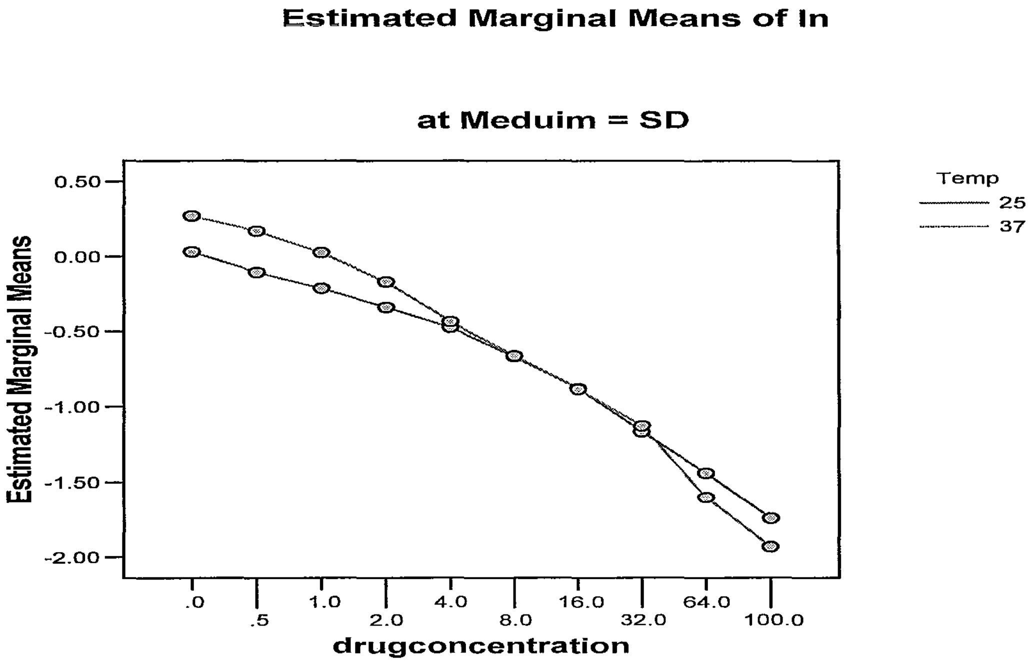

Figure 10. Relative vegetative fitness of 269 progeny grown at T25/ 37 on SD medium environment.

Figure 11. Coefficient of variation for 269 hybrids displays similar plasticity compared to parental strains in the T37/SD environment. The higher the CV, the higher the plasticity. These CV's are averaged over all concentrations and the distribution is skewed slightly to the left.

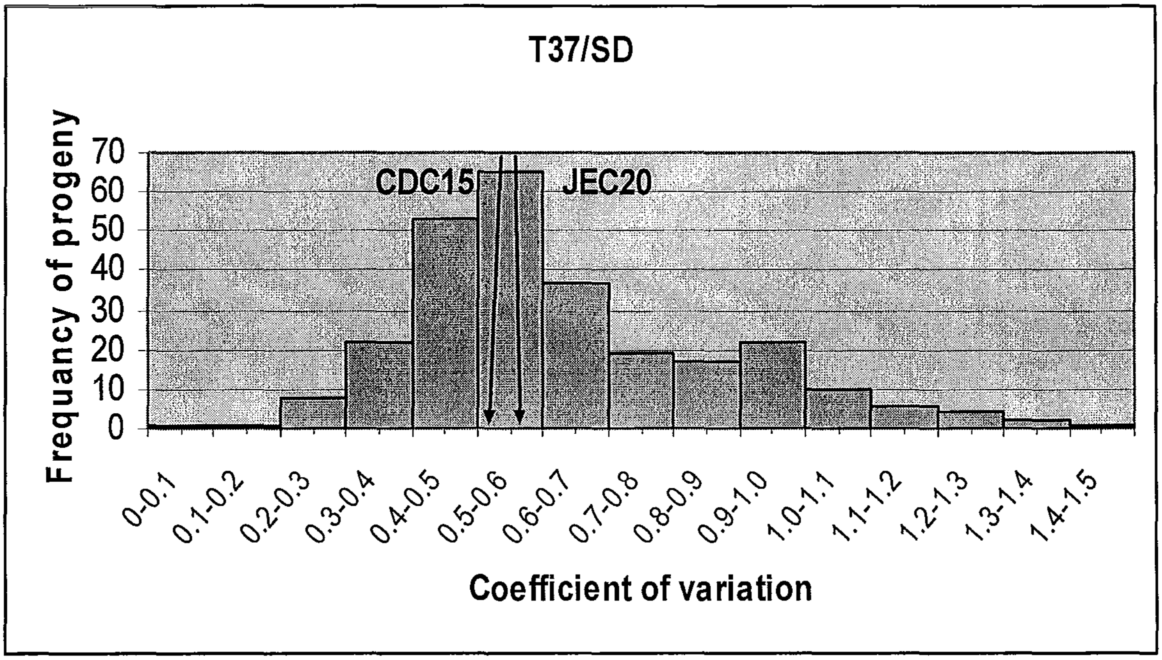

Figure 11. Distribution of coefficient of variation for hybrids in T37/SD environment.

Figure 12. Coefficient of variation for 269 hybrids displays similar plasticity compared to parental strains in T25/SD. The higher the CV, the higher the plasticity. These CV's are averaged over all concentrations and the distribution is skewed slightly to the left.

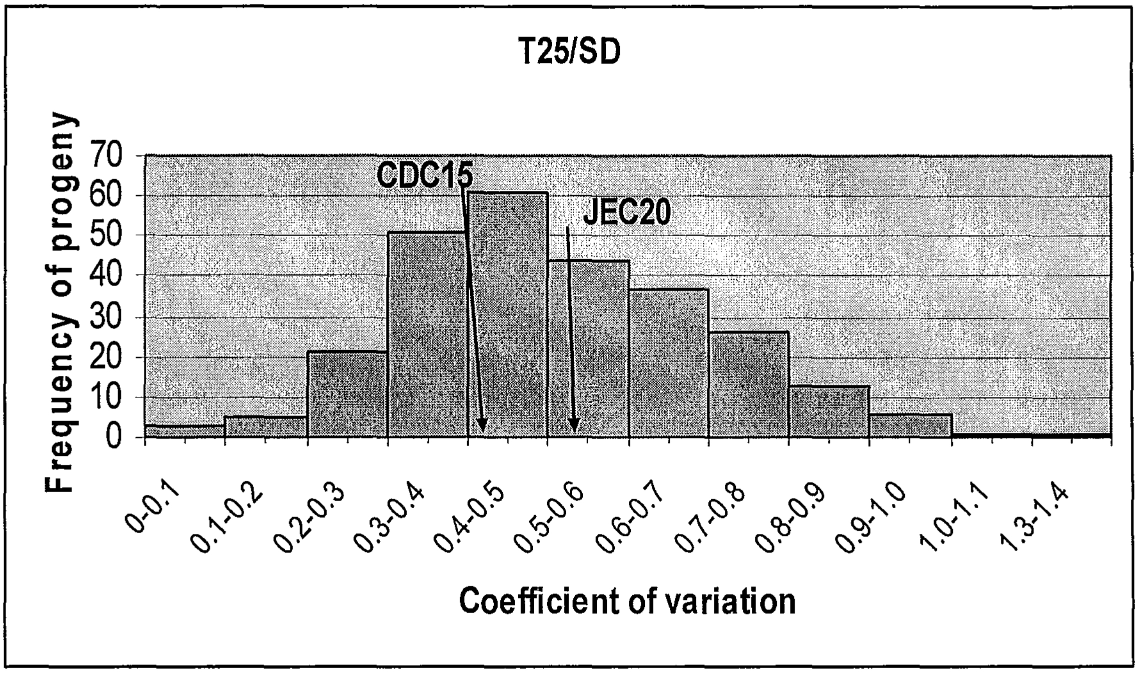

Figure 12. Distribution of coefficient of variation for hybrids in T25/SD environment.

Figure 13. Coefficient of variation of 269 hybrids displays much greater plasticity compared to parental strains in T25/YEPD. The higher the CV, the higher the plasticity. These CV's are averaged over all concentrations and the distribution is skewed slightly to the left.

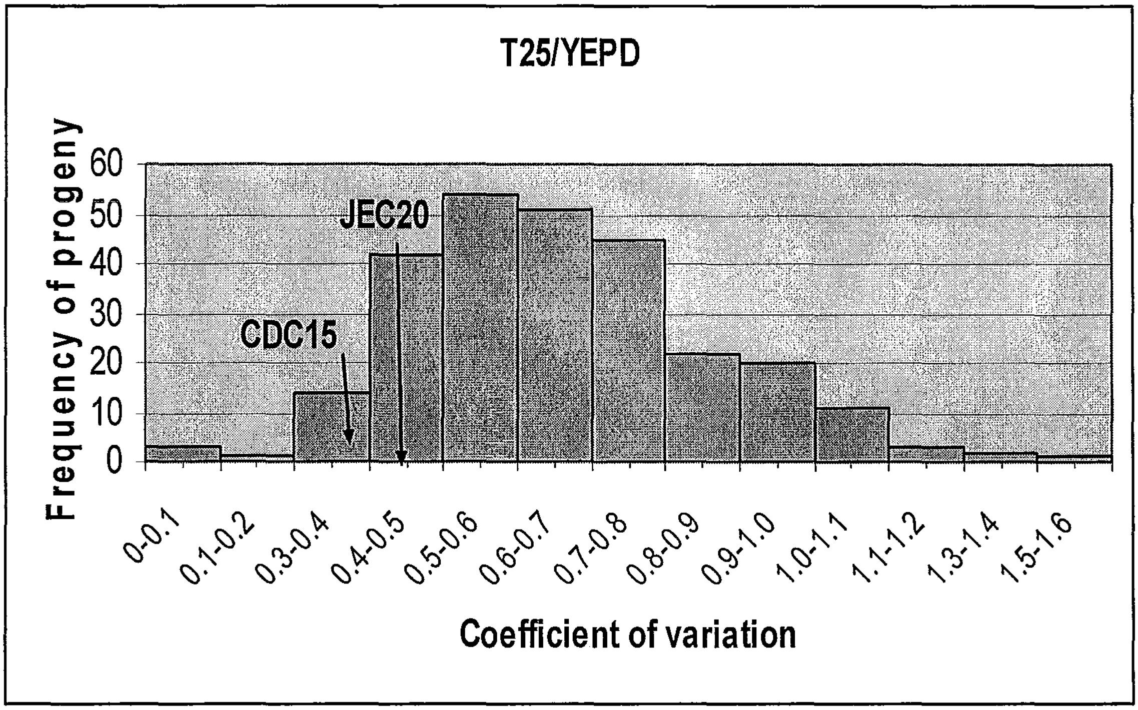

Figure 13. Distribution of coefficient of variation for hybrids in T25/YEPD environment.

Figure 14. Coefficient of variation of 269 hybrids displays much greater plasticity compared to parental strains in T37/YEPD. The higher the CV, the higher the plasticity. These CV's are averaged over all concentrations and the distribution is skewed slightly to the left.

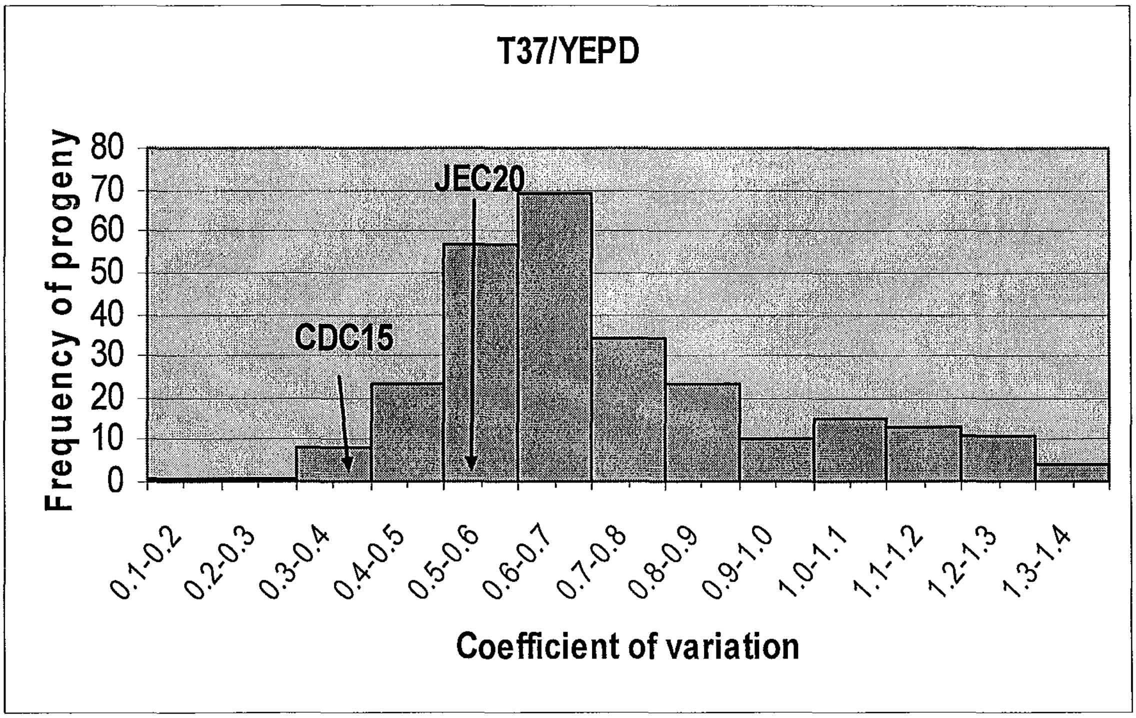

Figure 14. Distribution of coefficient of variation for hybrids in T37/YEPD environment.

Table 9. Summary information for percentage of progeny with lower and higher coefficient of variation than both parents in different environmental conditions. The T37/YEPD environment showed highest phenotypic plasticity of progeny compared to parental strain CDC15. Only 2.6% of progeny displayed phenotypic plasticity lower than CDC15. The coefficient of variation in the T37/ YEPD environment was greater than those of the other three environments.

Table 10: Summary information for percentage of progeny with lower and higher phenotypic plasticity than both parents in different environmental conditions

nvironmental condition	CV of CDC15	% of Hybrid with plasticity higher than CDC15	% of Hybrid with plasticity lower than CDC15	CV of JEC20	% of Hybrid with plasticity lower than JEC20	% of Hybrid with plasticity lower than JEC20
T37YEPD	0.383	97.00%	2.60%	0.546	78.80%	20.80%
T25/YEPD	0.382	95.10%	4.40%	0.446	85.80%	13.70%
T25/SD	0.414	66.50%	33.00%	0.547	39.40%	60.20%
T37/SD	0.502	67.20%	32.30%	0.566	50.10%	49.40%

Note: Data are presented as coefficient of variation (SD/Mean) values.

Figure 15. Distribution of correlation coefficient of progeny at T37 in both YEPD and SD medium. The correlation coefficient was calculated based on going from YEPD to SD, hence a greater negative number indicates greater plastic growth. The chart clearly shows that the progeny have significantly greater plasticity than parental strains, with 72% of the progeny having a correlation coefficient lower than -0.8.

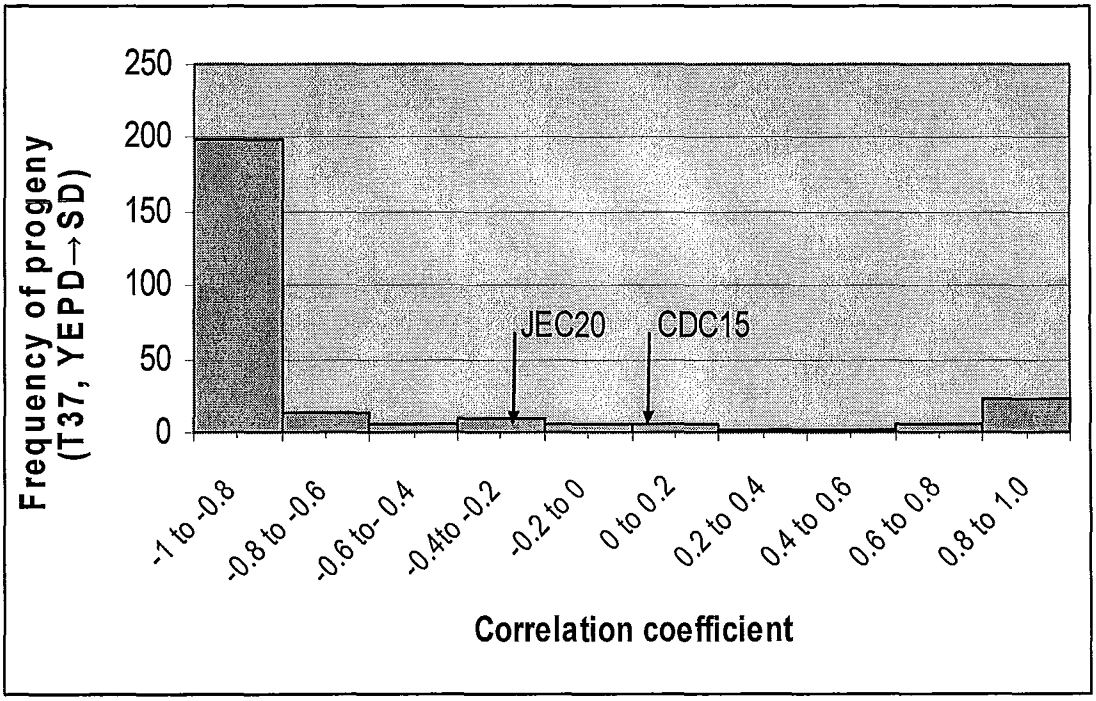

Figure 15. Distribution of correlation coefficient of progeny at T37 in both YEPD and SD medium.

Figure 16. Distribution of correlation coefficient of progeny at T25 in both YEPD and SD medium. The correlation coefficient was calculated based on going from YEPD to SD, hence a greater negative number indicates greater plastic growth. The chart clearly shows that the progeny have significantly greater plasticity than parental strains, with 58% of the progeny having a correlation coefficient of lower than -0.8.

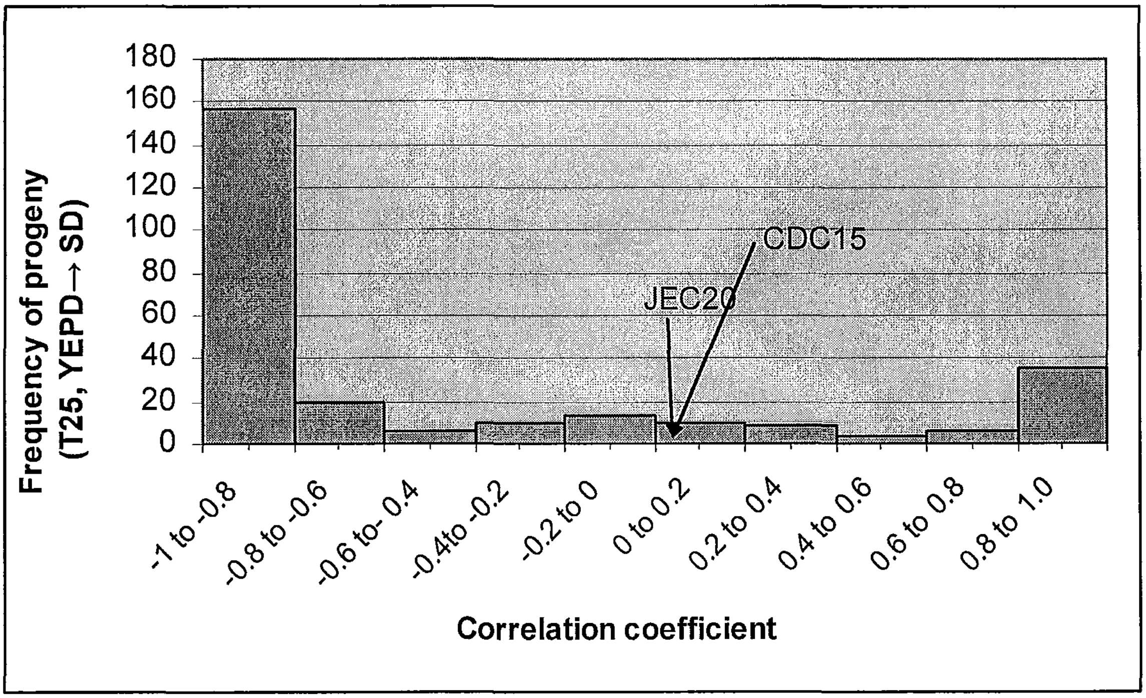

Figure 16. Distribution of correlation coefficient of progeny at T25 in both YEPD and SD medium.

Figure 17. Correlation coefficient of progeny and parents are high and similar in magnitude. The correlation coefficient was calculated using SD and based on going from 25 °C to 37 °C, hence a greater positive number indicates greater plastic growth. The chart clearly shows that the progeny have almost identical plasticity to parental strains.

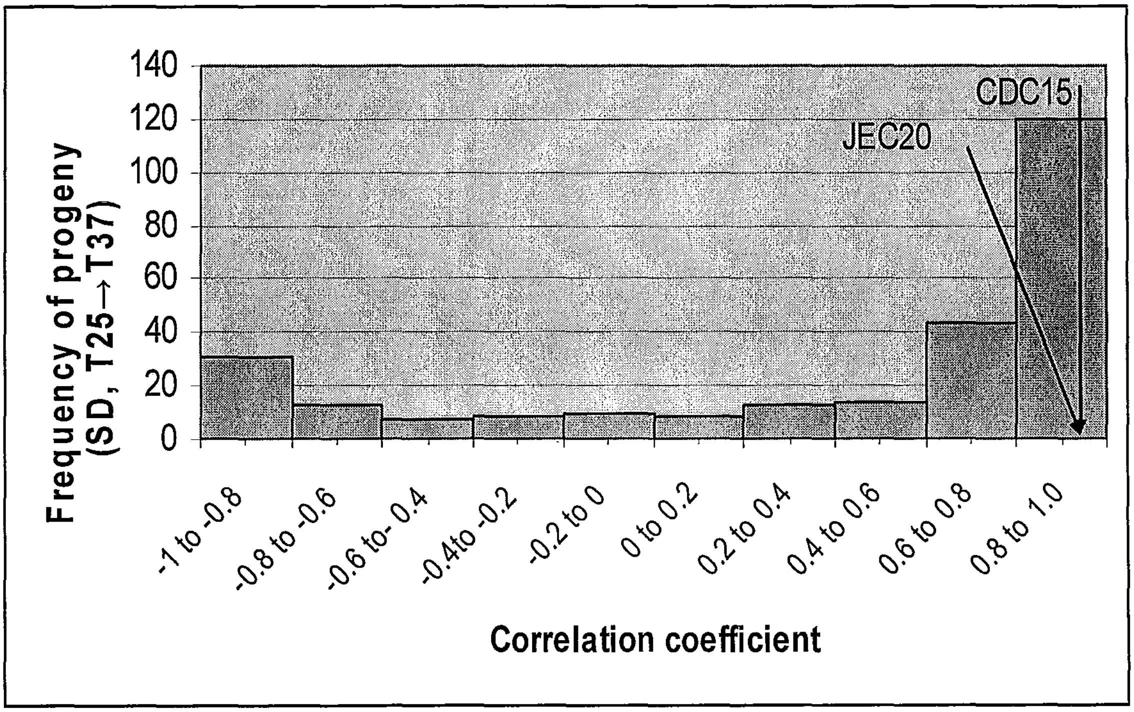

Figure 17. Distribution of correlation coefficient of progeny in SD medium at both T25 and T37.

Figure 18. Correlation coefficient of progeny and parents are high and similar in magnitude. The correlation coefficient was calculated using YEPD and based on going from 25 °C to 37 °C, hence a greater positive number indicates greater plastic growth. The chart clearly shows that the progeny have almost identical plasticity to parental strains.

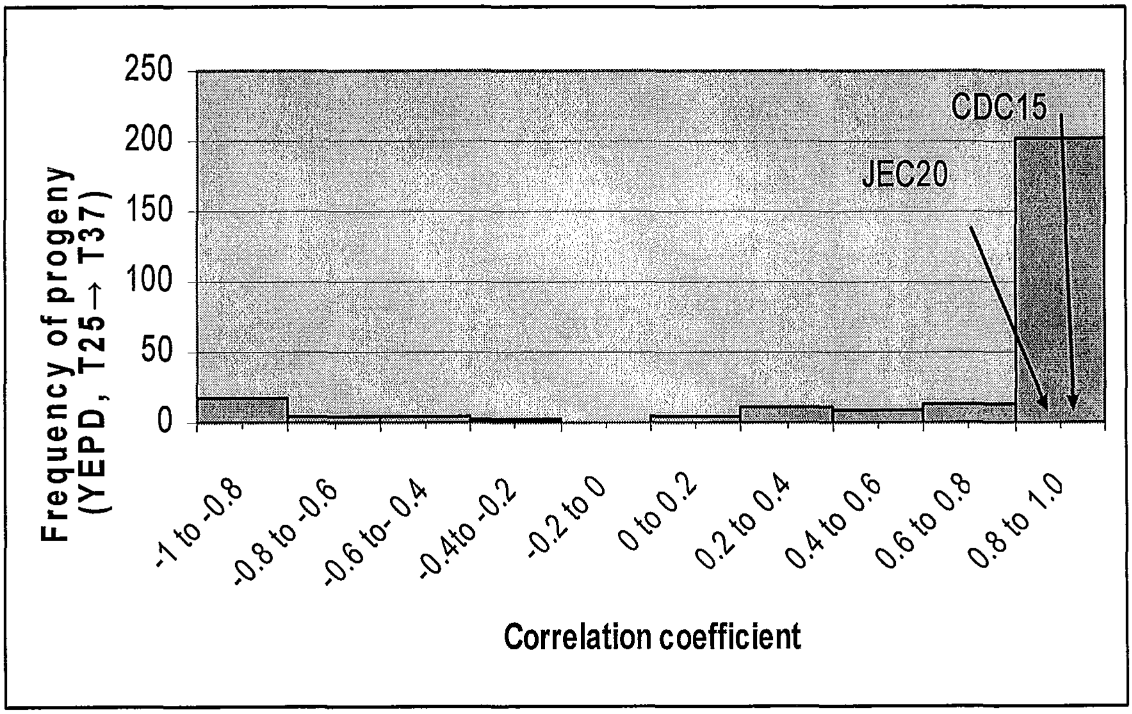

Figure 18. Distribution of correlation coefficient of progeny in YEPD medium at both T25 and T37.

Discussion

Outbreeding depression

The first evidence of outbreeding depression in fungi was reported in 2004 in our laboratory (Han, 2004). In that study, only one media (YMA) and one temperature (37 °C) was examined. The current experimental protocols are identical to what was presented there. Our current results in four media x temperature conditions confirmed and extended previous observations of outbreeding depression in *C. neoformans*. The results showed that the observation of outbreeding depression is environmental condition-specific. Among the forty tested environments (2 temperatures x two media x 10 drug concentrations), only 14 showed unambiguous evidence of outbreeding depression at the population level: in all 10 drug concentrations on SD at 25 °C and 4 drug concentrations on YEPD at 37 °C.

Two mechanisms have been proposed to explain outbreeding depression in animals and plants. Relative decline in vegetative fitness among outcrossed progeny can be attributed to a break-up of co-adapted gene complexes in the parental lines. In this case ourbreeding depression arises from (under-dominance between alleles or interaction between loci "complementary epistatis", Waser *et al.*, 2000) disrupted allelic co-adaptation within or across gene loci. Alternatively, disruption of locally adaptive genes is another mechanism explaining the genetic basis of outbreeding depression (Lynch, 1990; Lynch & Walsh, 1998). If hybrid individuals express genes or mixed genome that are not adapted to parental environments or to local conditions, they will suffer fitness decline in these environments. These two mechanisms could explain outbreeding depression in fungi as well.

Based on some estimates, strains of serotypes (A) and (D) have diverged from each other for about 18.5 million years. Such a long history of divergence could have allowed the accumulation of different co-adapted gene complexes. Indeed, low spore viability was observed in a sexual cross between strains of serotypes (A) and (D) (Lengeler *et al.,* 2001), suggesting some form of genomic incompatibility between the two serotypes. The hypotheses of locally adapted genes and co-adapted gene complexes are not mutually exclusive. They both may operate at once and may share some genetic features, such as allelic co-dapatation that might underlie adaptation to local environment. Further genetic analysis using high-density linkage maps may reveal the relative contributions of these two mechanisms as well as the potential gene loci to the outbreeding depression in *C. neoformans*.

Outbreeding depression in crosses between strains of serotypes (A) and (D) in *C. neoformans* was genetically and environmentally dependent. When progeny were exposed to no drug environments, they showed significant outbreeding depression. As drug concentration increased, outbreeding depression decreased.

My results revealed outbreeding depression in the low-nutrition environment at both temperatures. This result is consistent with previous observations in plants that outbred and inbred progeny typically have relatively lower fitness compared to their parents in nutrient-deprived environments (Dudash, 1990). Indeed, a recent investigation in *C. neoformans* identified that the nutrient-poor environment allow a greater expression of deleterious mutations in *C. neoformans* (Xu, 2004).

Interestingly, we observed a sudden relative fitness change in the T37/SD environment with the mean progeny fitness at drug concentrations 0-2 μg/mL significantly lower than both parents (outbreeding depression) but an intermediate fitness in between the two parents at drug concentrations between 4 μg/mL to 100 μg/mL. This sudden change might be related to the mechanisms of drug resistance in *C. neoformans*. At lower concentrations of Fluconazole, genes related to resistance to Fluconazole might not be activated. At intermediate and high concentrations of Fluconazole, these genes will be expressed. Since one parent JEC20 is sensitive to Fluconazole and the other parent CDC15 is resistant, it is expected that the expression of these resistance genes in the progeny would result in higher fitness than the Fluconazole-sensitive parent JEC20. At present, the number of genes and the mechanisms of these genes underlying Fluconazole resistance in strain CDC15 are unknown.

Intermediate Phenotype

In my study, the progeny mean vegetative fitness showed an intermediate value between the two parental strains in most environments. For example, in both T25/YEPD and T37/YEPD environments, the mean progeny fitness is in-between the two parental strains. This result suggests that most genes affecting vegetative fitness and Fluconazole resistance might be partially dominant/recessive or completely additive. Though the two parental strains are haploid, a preliminary survey suggests that most progeny of serotype (A) and (D) crosses contained alleles from both parents (Han, 2004), consistent with the hypothesis that most progeny were either diploid or aneuploid (Lengeler *et al.*, 2001).

A closer examination indicate that most progeny have a fitness value closer to one or the other parent, suggesting some form of partial dominance. It's interesting to note that in sexual crosses of *C. neoformans,* the mitochondria are usually inherited from the MAT-(a) parent (Yan & Xu, 2003). The MAT-(a) parent in this study was the JEC20 strain and all the progeny inherited mitochondria from JEC20 strain. In both T25/37YEPD and T37/SD environments, the mean fitness of progeny was more similar to that of parent JEC20. Thus, this result indicates either the mitochondria or the nuclear-mitochondrial gene combinations could have contributed to the phenotypes observed here.

Transgressive segregation

In this cross, a small fraction of hybrids showed transgressive segregation in almost all 40 tested environments. Overall, there are more progeny with fitness greater than both parents than progeny with fitness lower than both parents. In the T25/SD environment progeny showed the highest number of extreme phenotypes in all the drug concentrations when compared with other environments. In general, the100 μg/mL Fluconazole concentration environment had fewer progeny with extreme phenotypes than those with lower drug concentrations. However, in the T37/SD environment, the 100 μg/mL Fluconazole environment had the fittest transgressive hybrids.

As was discussed in the introduction, many different mechanisms have been proposed to account for transgressive segregation during hybridization. Each mechanism may contribute to transgressive segregation in specific systems, but some seemed unlikely to explain the observations here in *C. neofomans*. For example, mutation rates are known to be elevated in hybrid populations perhaps due to the activation of previously quiescent transposable elements (Egels, 1983), but due to the short time frame of our experiments (about 2 weeks) that the progeny were produced, novel mutations seem unlikely to have accumulated to account for the widespread transgression in segregating hybrid populations observed here. Likewise, the hypothesis that transgressive segregation can result from the expression of rare recessive alleles that are normally heterozygous in the parental lines is not applicable to this experiment since the parental lines are haploid and such deleterious mutations could be readily purged. Other hypothesized mechanisms such as overdominance and epistatic gene interactions could contribute to the observation. However, since the parental lines are haploid, overdominance and non-additive gene interactions among loci are unlikely to have evolved to contribute to transgressive segregation.

Our results can, however, be explained by the hypothesis of complementary gene action of additive or partially dominant/recessive alleles, similar to what have been observed in plants and animals (Rieseberg, 2003). If genes with positive effects on fitness are distributed across the genome in both parental lines, hybridization could bring them together to create novel combinations with fitness exceeding both parental strains. If this mechanism is the main cause of transgressive segregation, there may be two predictions that can be further tested.

The first is that the frequency of transgressive progeny will be correlated with the genetic divergence of the parental lines (Rieseberg, 1999). The rational for this prediction is that greater genetic divergence will be accompanied by an increase in the number of fixed differences between the parental lines, resulting in transgressive segregation for a larger number of traits. In plants and animals, this hypothesis has not been vigorously tested, primarily because there is a lack of organisms capable of hybridization but show a variety of genetic divergence. Our study system may allow us to test this prediction. The two populations of *C. neoformans var. grubii* and *C. neoformans var. neoformans* have diverged for about 18.5 millions years and serotypes (B) and (C) have been diverged from serotypes (A) and (D) for about twice as long. A systematic analysis of these inter-serotype hybridizations would provide an excellent opportunity to test this prediction.

The second prediction assumes that the more similar the phenotype of the parents, the greater the likelihood transgressive segregation will be observed in the hybrids. This prediction has been confirmed by empirical studies in plants (DeVicente & Tanksley, 1993; Mansur et al., 1993; Rieseberg, 1999). Our result here is consistent with this prediction. For example, in the T25/SD environment where the parents were very similar in their vegetative fitness, progeny with extreme phenotypes were most abundant. In contrast, in the T37/YEPD environment where the parents had a greater difference, few transgressive segregants were observed. Interestingly, among the four tested medium x temperature environments, the T25/SD environment is the most conducive for mating in *C. neoformans.*

A quantitative genetic approach will further our understanding of how extreme phenotypes are generated through hybridization and elucidate the mechanisms responsible for generating such phenotypes. An experiment could be conducted by using molecular markers to cover most of the hybrid genome. The genetic mapping and QTL analysis should allow estimates of the location and relative contributions of individual loci underlying the examined trait. The patterns of interactions between alleles at the same locus (dominance/recessiveness/additivity) as well as among alleles at different loci (epistatis) could also be estimated for individual fitness trait.

The effects of Fluconazole on the vegetative fitness of transgressive hybrids

Regression analysis showed an exponential relationship between vegetative fitness of transgressive hybrids and Fluconazole concentration. This exponential regression function could potentially provide a framework for the general relationship between the vegetative fitness and Fluconazole concentration in *C. neoformans*. A practical benefit could be to incorporate the function into clinical microbiology testing to reduce the number of drug concentrations needed for testing antifungal susceptibility of infectious pathogens. Infection with *C. neoformans* in AIDS patients is rarely cured and Fluconazole has been the drug of choice of lifelong suppressive (maintenance) therapy for these patients (Aller, 2000). Given the prevalence of cryptococcal infection among AIDS patients, reduced testing could potentially have significant practical benefits. Having an exponential model will enable medical researchers to effectively and accurately calculate the dosage needed by each patient based on the fitness of the strain from that patient.

Gene-environment interaction and phenotypic plasticity

Coefficient of variation

Significant interactions were observed between genotype and environmental variables in determining vegetative fitness. Among the four environments, T37/YEPD showed the highest coefficient of variation. The reason for such observation is unclear but one possible explanation is that the availability of abundant supply of nutrient in YEPD medium might contribute to greater variation among progeny, contributing to the variation seen here.

Phenotypic plasticity in different medium and temperature environments

In our temperature and medium comparisons, the two parental strains showed relatively little difference in their phenotypic plasticity. In contrast, their progeny showed significant variation in phenotypic plasticity in all tested environmental conditions. Given the long evolutionary divergence of serotypes (A) and (D) strains, the highly similar phenotypic plasticity of the two parental strains suggests significant constraints natural environmental conditions must have placed on the evolution of phenotypic plasticity in *C. neoformans*. The significant differences in phenotypic plasticity among progeny and between certain progeny and the parental strains suggest that phenotypic plasticity of the parental strains is likely controlled by genes at different loci. Furthermore, the quantitative variation coupled to the skewed distribution in phenotypic plasticity among the progeny indicate the existence of both major and minor genes controlling phenotypic plasticity in this organism. Further genetic mapping analysis may allow us to locate these genes.

One group of genes worth considering encodes the heat-shock proteins. In *C. neoformans*, a study comparing two strains H99 (serotype A) and JEC21 (serotype D) indicated striking differences in transcription profiles when growing at 25 °C versus 37°C. At 37 °C, elevated transcript levels for several genes encoding heat shock proteins and translation machinery were observed in the serotype (D) strain JEC21, but less so in the serotype (A) strain H99 (Steen *et al.*, 2002). Researchers have speculated that *C. neoformans* Hsp90 may interact with the Ras1 signal transduction pathway to control the ability to grow at elevated temperatures (Burnie *et al.*, 2005).

Heat-shock response is one of the most frequently examined trait in phenotypic plasticity research. In almost all cellular organisms, high temperature environments activate the heat-shock response and many heat-shock genes have been identified (e.g.

Feder et al. 1999). The activation of a heat-shock response allows organisms to grow at higher than their optimal growth temperature. Various allelic forms of Hsp70 have been examined for their correlation to phenotypic plasticity. For example, in the common fruit-fly Drosophila melanogaster, allele frequencies of Hsp70 were environmentally-dependent. Populations that experienced frequent warm temperature showed higher frequency of the 56H8 HSp70 allele expression (Bettencourt *et al.,* 2002). In another pathogenic yeast Candida albicans, nutrient limitations have also been found to strongly limit Hsp70 expression (Dabrowa *et al.,* 1990).

Conclusion

In this study, I observed both outbreeding depression and intermediate phenotypes in a hybrid population of *C. neoformans*. Environmental factors were found to have a significant role in determining the relative fitness of the hybrid progeny. In addition, a variable number of progeny showed evidence of extreme phenotypes and transgressive segregation. As the drug concentrations increased, the relative fitness of the transgressive hybrids increased as well. There was a significant interaction between progeny genotype and environmental factors in determining vegetative fitness. My statistical analysis indicated that among drug concentrations, the co-efficient of variation was highest at T37/YEPD, and the phenotypic plasticity was most notable at the T25/SD environment. My results suggest that hybridization can play a significant role in the evolution of this important human fungal pathogen. Further analysis of the progeny using high-density linkage mapping could potentially map the genes and identify their relative contributions to vegetative fitness in the diverse environments examined here. Such an analysis will have broad implications on our understanding about the role of hybridization in biological evolution.

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Appendix

Table A-1 Mean vegetative fitness of JEC20 was calculated at different concentrations of Fluconzole in T37/SD. Total number of concentrations are 10. For each concentration, 45 values of growth rate correspond with JEC20.

Table A-1 summary of vegetative fitness of JEC20 at different Fluconazole concentrations in T37/SD environment

0 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	100 µg/ml
3.50	2.56	2.58	1.82	1.10	0.10	0.10	0.10	0.10	0.10
2.60	2.32	2.14	2.02	1.90	1.80	1.74	1.62	1.48	0.84
1.66	1.32	1.32	1.14	0.54	0.48	0.10	0.10	0.10	0.10
1.28	1.18	1.14	1.00	0.38	0.32	0.10	0.10	0.10	0.10
0.86	0.68	0.66	0.66	0.60	0.58	0.46	0.44	0.32	0.10
0.68	0.64	0.58	0.56	0.52	0.50	0.48	0.40	0.36	0.28
1.60	1.26	1.28	1.02	0.60	0.40	0.10	0.10	0.10	0.10
1.88	1.90	1.70	1.56	1.42	1.28	1.10	1.04	0.56	0.50
1.94	1.60	1.44	0.72	0.56	0.10	0.10	0.10	0.10	0.10
0.76	0.78	0.64	0.66	0.58	0.10	0.10	0.10	0.10	0.10
1.24	1.22	1.14	1.12	0.84	0.10	0.10	0.10	0.10	0.10
1.82	1.16	1.04	0.84	0.68	0.10	0.10	0.10	0.10	0.10
1.00	0.92	0.92	0.86	0.66	0.56	0.48	0.40	0.42	0.10
1.00	0.96	0.98	0.68	0.10	0.10	0.10	0.10	0.10	0.10
1.48	1.28	1.18	1.22	1.02	0.98	0.96	0.80	0.70	0.10
0.82	0.72	0.56	0.54	0.10	0.10	0.10	0.10	0.10	0.10
1.30	1.42	1.08	1.10	0.10	0.10	0.10	0.10	0.10	0.10
1.40	1.96	1.76	1.52	1.48	0.48	0.10	0.10	0.10	0.10
0.96	0.76	0.98	0.74	0.64	0.58	0.36	0.10	0.10	0.10
0.82	1.14	0.42	0.50	0.44	0.34	0.28	0.10	0.10	0.10
1.50	1.30	1.20	1.06	0.90	0.84	0.74	0.64	0.52	0.34
1.58	1.48	1.42	1.40	1.12	0.96	0.88	0.86	0.30	0.10
1.46	1.06	1.02	1.00	0.96	0.88	0.86	0.74	0.74	0.60
1.84	1.42	1.40	1.38	1.34	1.04	1.00	0.82	0.10	0.10
1.62	1.42	1.26	1.08	0.88	0.82	0.74	0.56	0.46	0.26
0.78	0.70	0.60	0.54	0.44	0.10	0.10	0.10	0.10	0.10
0.84	0.62	0.84	0.84	0.76	0.68	0.54	0.46	0.28	0.10
1.06	0.94	0.90	0.76	0.64	0.62	0.44	0.44	0.32	0.26
1.82	1.70	1.44	1.28	1.12	1.10	1.06	0.62	0.46	0.30
1.82	1.78	1.74	1.72	1.64	1.30	1.04	1.00	0.20	0.10
2.08	2.02	1.82	1.58	1.24	1.02	0.10	0.10	0.10	0.10
1.38	1.24	1.08	0.98	0.92	0.60	0.60	0.44	0.26	0.10
1.80	1.74	1.72	1.68	1.28	1.00	0.80	0.50	0.44	0.30
1.18	1.06	0.82	0.78	0.76	0.70	0.62	0.58	0.28	0.10
0.90	0.90	0.80	0.68	0.64	0.56	0.60	0.52	0.46	0.36
1.12	3.36	0.90	0.88	0.78	0.68	0.66	0.68	0.10	0.10
2.00	1.92	1.54	1.20	1.10	1.10	0.96	0.80	0.38	0.30
2.68	2.38	2.20	1.92	1.66	1.06	0.76	0.48	0.10	0.10
2.12	1.98	1.54	1.28	1.08	0.62	0.60	0.46	0.40	0.34
1.26	1.16	0.94	0.80	0.66	0.52	0.44	0.30	0.10	0.10

0.96	0.78	0.64	0.46	0.36	0.30	0.10	0.10	0.10	0.10
1.78	1.66	1.50	1.50	1.14	0.10	0.10	0.10	0.10	0.10
0.78	0.74	0.68	0.60	0.60	0.52	0.84	0.40	0.34	0.34
1.18	1.00	0.92	0.84	0.72	0.50	0.42	0.34	0.26	0.22

Table A-2 Mean vegetative fitness of JEC20 was calculated at different concentrations of Fluconzole in T37/YEPD. Total number of concentrations are 10. For each concentration, 45 values of growth rate correspond with JEC20.

Table A-2 summary of vegetative fitness of JEC20 at different Fluconazole concentrations in T37/YEPD environment

0 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	100 µg/ml
0.74	2.02	2.22	0.26	0.10	0.10	0.10	0.10	0.10	0.10
2.24	1.96	1.48	0.88	0.32	0.10	0.10	0.10	0.10	0.10
2.22	1.92	1.56	0.70	0.10	0.10	0.10	0.10	0.10	0.10
2.62	2.12	1.76	0.10	0.10	0.10	0.10	0.10	0.10	0.10
2.36	1.96	1.74	0.46	0.10	0.10	0.10	0.10	0.10	0.10
2.27	2.44	1.80	1.46	0.10	0.10	0.10	0.10	0.10	0.10
1.68	2.56	2.10	0.48	0.44	0.10	0.10	0.10	0.10	0.10
2.72	2.60	2.60	0.60	0.10	0.10	0.10	0.10	0.10	0.10
2.34	2.22	1.82	0.90	0.10	0.10	0.10	0.10	0.10	0.10
2.92	2.54	2.20	1.40	0.10	0.10	0.10	0.10	0.10	0.10
0.70	0.64	0.64	0.36	0.10	0.10	0.10	0.10	0.10	0.10
1.84	1.80	1.78	0.56	0.10	0.10	0.10	0.10	0.10	0.10
2.14	1.80	1.16	1.12	1.10	1.02	0.92	1.32	0.10	0.10
2.18	2.04	2.16	0.86	1.50	2.92	2.98	1.86	0.10	0.10
2.94	2.88	2.74	2.46	2.38	2.18	1.94	1.48	0.70	0.10
2.46	2.32	2.10	1.62	1.50	1.26	1.06	1.00	0.70	0.10
2.66	2.18	1.92	1.90	1.64	1.56	1.38	1.34	0.10	0.10
2.80	2.26	2.08	1.62	1.78	1.40	1.02	0.80	0.10	0.10
2.40	2.26	1.64	1.20	1.26	1.82	1.66	1.48	0.78	0.10
2.44	2.16	2.14	2.06	2.04	1.84	1.66	0.84	0.76	0.10
1.90	1.64	1.36	1.26	1.02	0.10	0.10	0.10	0.10	0.10
2.48	2.08	1.74	0.98	0.82	0.10	0.10	0.10	0.10	0.10
3.66	2.78	2.66	1.88	1.66	1.50	1.26	1.16	0.86	0.10
1.54	1.34	1.22	0.88	0.54	0.10	0.10	0.10	0.10	0.10
1.54	1.18	1.04	0.92	0.90	0.84	0.62	0.10	0.10	0.10
2.10	1.64	0.58	0.50	0.10	0.10	0.10	0.10	0.10	0.10
1.92	1.74	1.48	1.28	0.10	0.10	0.10	0.10	0.10	0.10
1.56	1.56	1.44	1.24	1.08	0.90	0.10	0.10	0.10	0.10
2.58	1.20	0.58	0.36	2.22	1.82	2.40	0.10	0.10	0.10
2.94	0.50	0.48	0.10	0.10	0.10	0.10	0.10	0.10	0.10
1.54	1.32	1.14	1.04	0.92	0.10	0.10	0.10	0.10	0.10
2.06	1.84	1.60	1.04	0.86	0.70	0.56	0.52	0.10	0.10
0.64	0.60	0.54	0.50	0.10	0.10	0.10	0.10	0.10	0.10
2.12	1.80	1.54	1.42	1.38	0.10	0.10	0.10	0.10	0.10
2.16	1.88	1.38	1.26	1.16	0.78	0.68	0.56	0.46	0.10
2.08	1.74	1.44	1.02	0.10	0.10	0.10	0.10	0.10	0.10
1.56	1.20	1.06	0.78	0.66	0.10	0.10	0.10	0.10	0.10
1.80	1.14	0.92	0.92	0.84	0.80	0.48	0.10	0.10	0.10
2.36	2.14	1.84	1.60	1.26	0.94	0.62	0.44	0.34	0.30

2.82	2.64	2.22	1.96	1.18	0.56	0.44	0.42	0.10	0.10
2.36	2.12	1.76	1.54	1.08	0.60	0.48	0.36	0.30	0.28
2.70	2.40	2.00	1.48	1.18	0.66	0.44	0.36	0.10	0.10
3.02	2.38	1.98	1.68	1.40	1.06	0.76	0.54	0.10	0.10
3.16	2.86	2.60	2.30	2.06	1.38	0.10	0.10	0.10	0.10
2.26	2.10	1.78	1.52	1.08	0.80	0.54	0.40	0.34	0.28

Table A-3 Mean vegetative fitness of JEC20 was calculated at different concentrations of Fluconzole in T25/SD. Total number of concentrations are 10. For each concentration, 45 values of growth rate correspond with JEC20.

Table A-3 summary of vegetative fitness of JEC20 at different Fluconazole concentrations in T25/SD environment

0 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	100 µg/ml
2.34	2.22	1.92	1.88	1.86	1.78	1.50	1.22	1.10	1.26
1.40	1.30	1.16	0.80	0.70	0.58	0.50	0.50	0.10	0.10
1.56	0.96	1.06	0.96	1.00	0.80	0.58	0.38	0.24	0.10
0.76	0.70	0.62	0.54	0.56	0.54	0.52	0.38	0.34	0.30
0.74	0.70	0.62	0.60	0.58	0.56	0.50	0.32	0.22	0.20
1.04	1.04	1.08	1.04	0.98	0.98	0.88	0.66	0.56	0.44
1.28	0.94	0.90	0.82	0.66	0.70	0.54	0.50	0.32	0.30
0.68	0.96	0.86	0.74	0.70	0.58	0.60	0.38	0.38	0.10
0.44	0.38	0.38	0.44	0.42	0.40	0.36	0.38	0.10	0.10
0.40	0.38	0.38	0.36	0.34	0.36	0.34	0.34	0.10	0.10
0.82	0.64	0.60	0.50	0.46	0.46	0.44	0.40	0.38	0.24
1.76	1.54	1.50	1.14	1.14	1.02	0.96	0.10	0.10	0.10
1.02	0.70	0.66	0.72	0.64	0.46	0.44	0.10	0.10	0.10
4.20	4.03	3.38	2.82	2.24	1.58	1.64	1.32	0.10	0.10
0.58	0.44	0.40	0.40	0.42	0.40	0.34	0.10	0.10	0.10
1.16	1.22	0.84	0.62	0.54	0.64	0.10	0.10	0.10	0.10
1.12	1.06	1.02	0.82	0.70	0.72	0.56	0.44	0.40	0.34
0.86	0.84	0.74	0.58	0.54	0.50	0.30	0.10	0.10	0.10
0.88	0.86	0.88	0.84	0.78	0.66	0.62	0.48	0.10	0.10
1.24	1.04	1.02	0.84	0.84	0.70	0.64	0.44	0.42	0.34
1.06	0.94	0.86	0.78	0.74	0.70	0.54	0.46	0.44	0.34
1.10	0.94	0.80	0.68	0.60	0.40	0.34	0.24	0.10	0.10
1.00	0.94	0.68	0.66	0.68	0.64	0.62	0.58	0.44	0.38
1.36	0.66	0.56	0.54	0.54	0.50	0.50	0.42	0.36	0.28
1.34	1.18	1.08	1.00	0.96	0.90	0.76	0.66	0.48	0.42
1.08	1.08	1.06	0.94	0.84	0.76	0.56	0.44	0.42	0.30
1.20	1.20	1.00	1.06	0.84	0.76	0.56	0.44	0.42	0.30
1.16	1.18	1.14	1.06	0.94	0.80	0.78	0.54	0.30	0.26
1.72	1.68	1.62	1.46	1.26	0.82	0.66	0.30	0.10	0.10
1.14	1.06	1.00	0.92	0.76	0.74	0.72	0.70	0.56	0.50
1.24	1.16	0.88	0.84	0.80	0.64	0.56	0.44	0.30	0.24
1.48	1.50	1.38	1.34	1.08	0.90	0.80	0.68	0.44	0.36
1.98	1.90	1.82	1.30	1.12	1.06	1.00	0.94	0.80	0.46
2.18	2.18	1.78	1.52	1.14	1.06	1.02	0.86	0.76	0.64
0.96	0.90	0.82	0.64	0.60	0.36	0.36	0.30	0.30	0.10
1.16	1.02	0.76	0.70	0.64	0.56	0.50	0.46	0.34	0.26
0.94	0.80	0.78	0.74	0.72	0.68	0.56	0.50	0.44	0.30
1.58	1.42	1.14	1.02	0.82	0.56	0.42	0.30	0.28	0.10
1.34	1.24	1.04	0.92	0.76	0.58	0.42	0.34	0.32	0.26
0.92	0.74	0.64	0.54	0.48	0.10	0.10	0.10	0.10	0.10

0.78	0.72	0.60	0.54	0.48	0.10	0.10	0.10	0.10	0.10
1.32	1.12	0.92	0.82	0.72	0.10	0.10	0.10	0.10	0.10
1.44	1.26	1.08	0.90	0.84	0.50	0.34	0.28	0.10	0.10
0.68	0.64	0.56	0.50	0.42	0.10	0.10	0.10	0.10	0.10
1.02	0.88	0.72	0.68	0.64	0.46	0.40	0.10	0.10	0.10

Table A-4 Mean vegetative fitness of JEC20 was calculated at different concentrations of Fluconzole in T25/YEPD. Total number of concentrations are 10. For each concentration, 45 values of growth rate correspond with JEC20.

Table A-4 summary of vegetative fitness of JEC20 at different Fluconazole concentrations in T25/YEPD environment

0 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	100 µg/ml
0.88	0.52	0.78	0.58	0.52	0.20	0.10	0.10	0.10	0.10
0.70	0.88	0.58	0.52	0.48	0.52	0.24	0.10	0.10	0.10
2.44	2.08	1.68	1.56	1.30	1.18	0.50	0.10	0.10	0.10
1.22	1.02	0.98	0.64	0.54	0.40	0.10	0.10	0.10	0.10
1.14	1.14	1.02	0.70	0.42	0.10	0.10	0.10	0.10	0.10
1.18	1.22	1.12	0.82	0.46	0.44	0.10	0.10	0.10	0.10
0.90	0.78	0.74	0.58	0.46	0.22	0.10	0.10	0.10	0.10
1.30	1.14	1.10	1.10	0.90	0.26	0.10	0.10	0.10	0.10
1.16	1.14	1.18	0.74	0.73	0.62	0.10	0.10	0.10	0.10
1.18	1.16	1.04	0.96	0.90	0.78	0.10	0.10	0.10	0.10
1.12	1.22	1.14	1.04	0.82	0.76	0.10	0.10	0.10	0.10
1.30	1.16	1.32	1.14	1.00	0.68	0.58	0.54	0.10	0.10
1.44	1.38	1.30	1.22	0.78	0.56	0.52	0.46	0.26	0.24
1.42	1.38	1.24	1.10	1.28	1.34	0.84	0.36	0.32	0.32
1.04	0.94	0.84	0.72	0.66	0.48	0.46	0.10	0.10	0.10
1.04	1.02	0.94	0.90	0.78	0.68	0.62	0.10	0.10	0.10
1.64	1.46	1.24	0.78	0.56	0.46	0.42	0.38	0.26	0.10
1.66	0.96	0.88	0.80	0.60	0.42	0.38	0.36	0.34	0.10
1.42	0.92	0.78	0.54	0.48	0.56	0.38	0.30	0.10	0.10
1.10	1.02	0.96	0.92	0.84	0.60	0.10	0.10	0.10	0.10
1.36	1.26	1.12	1.00	0.86	0.80	0.76	0.52	0.42	0.10
0.96	0.76	0.74	0.50	0.40	0.36	0.30	0.10	0.10	0.10
1.00	0.86	0.68	0.50	0.30	0.28	0.10	0.10	0.10	0.10
0.80	0.72	0.58	0.52	0.50	0.44	0.38	0.10	0.10	0.10
1.60	1.30	1.02	0.10	0.10	0.10	0.10	0.10	0.10	0.10
1.54	1.26	1.06	0.54	0.46	0.44	0.40	0.10	0.10	0.10
0.96	0.82	0.76	0.68	0.68	0.48	0.44	0.38	0.10	0.10
1.44	1.36	1.32	1.26	1.06	0.44	0.38	0.10	0.10	0.10
1.54	1.30	1.20	0.96	0.74	0.10	0.10	0.10	0.10	0.10
0.70	0.62	0.54	0.50	0.46	0.10	0.10	0.10	0.10	0.10
0.66	0.56	0.52	0.50	0.48	0.34	0.10	0.10	0.10	0.10
1.68	1.50	1.20	1.06	0.92	0.56	0.10	0.10	0.10	0.10
1.84	1.54	1.38	1.06	0.94	0.64	0.36	0.32	0.26	0.10
1.78	1.44	1.26	1.06	0.84	0.72	0.66	0.52	0.36	0.10
1.58	1.38	1.18	1.04	1.00	0.86	0.72	0.64	0.56	0.42
2.14	2.02	1.88	1.62	1.36	1.00	0.88	0.60	0.10	0.10
2.38	2.12	2.00	1.70	1.50	1.06	0.88	0.54	0.10	0.10
2.60	2.14	2.00	1.86	0.94	0.86	0.80	0.76	0.46	0.10
1.00	0.68	0.54	0.82	0.50	0.52	0.52	0.64	0.42	0.10
2.78	2.50	2.34	1.02	1.04	0.10	0.10	0.10	0.10	0.10
1.58	1.74	1.40	1.04	1.10	0.52	0.46	0.10	0.10	0.10

2.12	1.40	1.22	1.12	0.84	0.62	0.52	0.10	0.10	0.10
2.02	1.68	1.48	1.32	1.18	0.76	0.64	0.66	0.36	0.10
2.38	2.14	1.82	1.54	1.28	0.76	0.60	0.38	0.10	0.10
1.36	1.10	0.98	0.74	0.60	0.50	0.40	0.38	0.30	0.10

Table B-1 Mean vegetative fitness of CDC15 was calculated at different concentrations of Fluconzole in T37/SD. Total number of concentrations are 10. For each concentration, 45 values of growth rate correspond with CDC15.

Table B-1 summary of vegetative fitness of CDC15 at different Fluconazole concentrations in T37/SD environment

0 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	100 µg/ml
2.82	2.58	2.34	2.04	1.68	1.86	1.56	1.44	0.10	0.10
3.30	2.90	2.60	2.40	0.10	0.10	0.10	0.10	0.10	0.10
2.40	2.30	1.72	1.60	1.36	1.20	1.08	0.98	0.56	0.36
1.38	1.98	1.48	1.36	1.16	1.64	2.00	1.74	1.72	0.84
1.10	0.82	0.76	0.72	0.70	0.64	0.62	0.62	0.60	0.50
0.80	0.86	0.74	0.72	0.64	0.10	0.10	0.10	0.10	0.10
0.84	0.88	0.88	0.62	0.62	0.64	0.62	0.60	0.50	0.44
2.76	2.50	2.16	1.88	1.06	0.10	0.10	0.10	0.10	0.10
1.70	1.66	1.50	1.36	1.18	1.14	1.04	1.00	0.36	0.38
0.92	1.12	0.94	0.96	0.96	1.26	0.70	0.48	0.50	0.40
1.44	1.44	1.50	1.36	1.32	1.24	1.08	0.94	0.82	0.58
1.38	1.12	0.96	0.98	0.94	0.98	0.92	0.86	0.46	0.36
1.44	1.32	1.42	1.16	0.90	0.90	0.92	0.80	0.70	0.42
1.54	1.36	1.08	1.04	0.94	0.94	0.92	0.74	0.52	0.48
1.62	1.36	1.26	1.18	1.12	1.20	0.96	0.90	0.72	0.32
0.94	0.94	0.76	0.94	0.72	0.82	0.92	0.44	0.10	0.10
1.46	1.30	1.20	1.34	1.12	1.14	1.12	0.94	0.80	0.10
1.44	2.88	2.06	1.64	2.96	2.54	2.50	1.86	1.12	0.58
1.04	0.90	1.06	0.94	0.98	0.80	0.84	0.70	0.66	0.44
0.68	1.06	0.38	0.44	0.42	0.40	0.34	0.10	0.10	0.10
3.22	2.96	2.68	2.26	2.10	1.82	1.46	1.20	0.90	0.64
1.62	1.30	1.32	1.34	1.08	1.00	1.10	0.82	0.62	0.10
1.16	0.92	0.88	0.86	0.84	0.82	0.84	0.80	0.70	0.30
1.78	1.68	1.68	1.72	1.62	1.64	1.36	1.06	0.10	0.10
1.46	1.42	1.34	0.96	0.94	0.92	0.86	0.58	0.44	0.24
0.96	1.02	0.96	0.90	0.88	0.82	0.76	0.62	0.52	0.32
1.02	1.00	0.94	0.90	0.88	0.72	0.48	0.44	0.34	0.10
1.30	1.24	1.08	0.94	0.76	0.74	0.74	0.66	0.48	0.26
1.00	0.96	0.94	0.88	0.86	0.82	0.82	0.82	0.44	0.38
2.02	1.76	1.72	1.70	1.63	1.36	1.04	1.02	0.10	0.10
2.32	2.16	2.06	1.80	1.62	1.34	1.02	0.72	0.62	0.42
1.24	1.18	1.10	0.94	0.92	0.68	0.60	0.52	0.44	0.38
1.82	1.74	1.50	1.34	1.32	1.10	0.96	0.80	0.64	0.60
1.20	0.98	0.86	0.86	0.84	0.72	0.64	0.52	0.48	0.34
2.04	1.90	1.74	1.60	1.50	1.38	1.14	0.96	0.74	0.64
0.96	0.84	0.78	0.62	0.60	0.58	0.50	0.50	0.24	0.20
1.26	1.26	1.28	1.06	0.98	0.86	0.76	0.74	0.30	0.26
3.06	2.90	2.68	2.28	1.82	1.30	1.04	0.84	0.54	0.36
3.08	3.06	2.98	2.64	2.22	1.64	1.28	0.94	0.74	0.62
1.78	1.64	1.54	1.40	1.18	0.78	0.62	0.54	0.48	0.40

1.38	1.26	1.12	0.94	0.74	0.62	0.54	0.48	0.38	0.30
1.88	1.58	1.36	1.16	0.96	2.76	0.58	0.54	0.46	0.40
0.88	0.80	0.72	0.62	0.50	0.36	0.30	0.28	0.26	0.22
1.50	1.38	1.20	0.98	0.94	0.70	0.60	0.48	0.44	0.40

Table B-2 Mean vegetative fitness of CDC15 was calculated at different concentrations of Fluconzole in T37/YEPD. Total number of concentrations are 10. For each concentration, 45 values of growth rate correspond with CDC15.

Table B-2 summary of vegetative fitness of CDC15 at different Fluconazole concentrations in T37/YEPD environment

0 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	100 µg/ml
1.26	3.42	3.46	1.02	1.04	2.12	0.92	0.48	0.44	0.62
3.02	2.96	2.76	2.74	2.58	2.58	2.44	1.50	1.40	0.78
3.42	3.14	2.84	2.74	2.66	2.70	2.56	2.10	1.60	0.70
3.26	3.54	3.30	3.06	2.84	2.72	2.68	2.06	1.36	0.86
3.06	3.04	3.16	3.12	2.86	2.90	2.44	1.96	1.10	0.80
2.86	3.16	3.12	3.02	2.66	2.50	1.04	1.14	0.62	0.10
2.74	3.16	3.16	2.88	2.52	1.86	1.84	0.82	0.76	0.82
3.26	3.28	3.18	2.36	2.30	2.00	1.92	1.96	1.76	0.62
3.00	3.72	3.42	2.06	2.42	3.22	2.70	1.78	0.97	0.28
2.38	2.52	2.32	2.12	1.84	1.56	1.52	1.44	1.02	0.60
0.96	0.66	0.64	1.12	0.96	0.84	0.88	0.40	0.10	0.36
3.44	3.38	3.16	2.32	2.50	3.26	3.02	1.50	0.88	0.10
3.16	2.46	1.92	1.88	1.70	1.70	1.60	1.30	0.10	0.10
3.08	3.34	3.04	2.90	2.86	2.94	3.00	2.14	0.44	0.42
2.88	2.80	2.58	2.30	2.14	2.10	1.94	1.84	0.92	0.82
2.46	2.34	2.28	2.14	1.86	1.74	1.36	1.12	0.96	0.64
2.58	2.26	2.08	2.06	1.68	1.64	1.42	1.30	0.56	0.10
2.12	1.64	3.84	1.60	1.20	1.00	0.72	0.30	0.10	0.10
1.90	2.10	2.02	1.92	1.88	1.86	1.90	1.86	1.26	0.94
2.92	2.86	2.66	2.54	2.50	2.48	2.42	2.26	1.10	0.10
3.38	3.02	2.76	2.12	2.10	2.00	1.88	1.48	1.32	1.06
3.58	3.46	3.42	2.62	2.24	2.00	1.88	1.36	1.32	0.56
6.54	4.40	3.46	3.18	3.06	2.94	2.80	1.52	0.82	0.64
2.66	3.50	3.48	3.02	2.80	2.24	1.28	0.82	0.10	0.10
5.14	4.50	4.46	4.00	3.90	3.82	3.30	2.50	1.68	0.92
1.24	4.12	3.84	3.54	3.04	2.80	1.96	1.06	0.86	0.52
2.32	2.32	2.28	2.18	2.08	1.94	1.78	1.06	0.26	0.10
2.80	2.70	2.56	2.16	1.92	1.68	1.46	1.06	0.78	0.58
3.04	2.98	2.52	2.44	2.04	2.00	1.86	0.30	0.10	0.10
2.80	2.72	2.86	2.74	1.70	1.80	1.70	0.96	0.78	0.10
2.56	2.48	2.54	2.24	2.06	1.82	1.50	1.14	0.72	0.54
2.92	2.76	2.56	2.18	2.02	1.90	1.70	1.18	0.62	0.32
1.20	1.10	1.08	1.02	0.94	0.86	0.82	0.62	0.50	0.24
3.08	2.58	2.28	2.20	2.06	1.90	1.64	1.14	0.60	0.34
3.34	2.80	2.44	2.26	2.00	1.62	1.44	1.10	0.76	0.44
3.06	2.92	2.82	2.56	2.36	2.04	1.08	0.62	0.10	0.10
2.62	2.28	2.14	1.96	1.62	1.34	1.18	0.80	0.54	0.36
2.32	2.22	2.16	1.92	1.92	1.48	1.40	1.14	0.42	0.10
3.48	3.20	3.00	2.76	2.46	2.10	1.70	1.36	0.88	0.54
4.34	4.12	3.30	2.68	2.26	1.66	1.14	0.94	0.68	0.52

2.70	2.58	2.52	2.28	1.94	1.34	0.92	0.76	0.62	0.52
3.60	3.44	3.06	2.76	2.30	1.58	1.20	0.86	0.56	0.44
3.26	3.00	2.72	2.38	2.16	1.98	1.62	1.30	0.80	0.56
3.44	3.18	2.70	6.32	1.70	1.04	0.92	0.88	0.58	0.44
3.32	3.06	2.72	2.24	1.76	1.56	1.26	1.04	0.88	0.72

Table B-3 Mean vegetative fitness of CDC15 was calculated at different concentrations of Fluconzole in T25/SD. Total number of concentrations are 10. For each concentration, 45 values of growth rate correspond with CDC15.

Table B-3 summary of vegetative fitness of CDC15 at different Fluconazole concentrations in T25/SD environment

0 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	100 µg/ml
2.22	2.04	1.82	1.72	1.62	1.46	1.32	0.90	0.10	0.10
2.08	1.88	1.64	1.20	1.08	1.00	0.86	0.82	0.72	0.64
1.96	1.72	1.78	1.84	1.64	1.64	1.48	1.18	1.06	0.64
0.76	0.68	0.64	0.66	0.60	0.56	0.10	0.10	0.10	0.10
0.70	0.66	0.64	0.60	0.58	0.58	0.52	0.46	0.40	0.30
0.94	1.10	1.08	1.06	0.88	0.92	0.84	0.74	0.60	0.44
1.10	1.04	0.88	0.76	0.74	0.62	0.60	0.50	0.36	0.30
1.32	1.10	0.94	0.80	0.50	0.48	0.46	0.46	0.50	0.24
0.54	0.42	0.68	0.48	0.48	0.44	0.44	0.54	0.42	0.42
0.38	0.38	0.40	0.40	0.40	0.28	0.30	0.40	0.22	0.20
1.64	1.12	1.08	1.02	0.76	0.80	0.70	0.74	0.64	0.50
1.70	1.46	1.40	1.14	1.12	1.08	1.00	0.72	0.66	0.50
1.36	1.14	1.14	1.10	0.94	0.92	0.88	0.88	0.58	0.52
3.94	3.32	2.42	1.90	1.30	1.16	1.24	1.00	1.34	1.12
0.68	0.48	0.40	0.54	0.44	0.32	0.40	0.10	0.10	0.10
1.42	1.14	0.82	0.78	0.80	0.84	0.66	0.78	0.34	0.10
1.18	1.08	0.94	0.82	0.74	0.92	0.90	0.74	0.64	0.48
0.90	1.16	0.86	0.78	0.58	0.58	0.48	0.40	0.36	0.10
1.38	1.14	0.92	0.76	0.76	0.80	0.82	0.64	0.48	0.44
1.32	1.12	1.08	1.00	0.94	0.96	0.90	0.88	0.82	0.72
1.56	1.54	1.46	1.38	1.18	1.06	0.90	0.80	0.66	0.48
1.36	1.24	1.10	1.04	0.94	0.64	0.58	0.48	0.44	0.34
0.98	1.38	1.30	1.10	0.88	0.76	0.76	0.90	0.58	0.34
1.00	0.88	0.86	0.86	0.86	0.82	0.76	0.56	0.46	0.34
1.96	1.86	1.82	1.72	1.66	1.50	1.42	0.86	0.66	0.60
0.86	0.84	0.80	0.76	0.74	0.72	0.68	0.68	0.60	0.32
1.34	1.18	1.20	1.18	1.16	1.08	0.90	0.82	0.72	0.50
1.42	1.22	1.20	1.18	1.08	1.08	0.96	0.88	0.72	0.52
0.94	0.92	0.90	0.84	0.72	0.64	0.62	0.54	0.46	0.30
1.24	1.18	1.12	1.00	0.98	0.84	0.82	0.80	0.68	0.48
1.38	1.02	0.88	0.82	0.72	0.52	0.48	0.48	0.46	0.38
1.36	1.02	1.00	0.96	0.84	0.80	0.76	0.72	0.36	0.30
2.18	2.12	2.04	1.46	1.30	1.26	1.20	0.92	0.50	0.46
2.34	2.20	1.92	1.82	1.44	1.14	0.98	0.78	0.68	0.50
1.34	1.18	1.02	1.00	0.92	0.82	0.72	0.56	0.54	0.40
1.24	1.12	0.92	0.84	0.82	0.72	0.68	0.58	0.54	0.38
1.00	0.96	0.94	0.92	0.74	0.60	0.58	0.56	0.54	0.30
2.74	2.58	2.18	2.02	1.52	1.10	0.86	0.74	0.62	0.46
1.62	1.44	1.22	1.08	0.94	0.80	0.76	0.76	0.76	0.50
1.16	1.04	0.86	0.80	0.64	0.50	0.48	0.44	0.42	0.40

1.08	0.98	0.80	0.74	0.74	0.66	0.54	0.48	0.42	0.40
1.36	1.26	1.02	0.84	0.70	0.54	0.48	0.42	0.34	0.30
1.66	1.50	1.32	1.14	1.04	0.84	0.72	0.62	0.54	0.34
0.78	0.74	0.62	0.54	0.48	0.44	0.42	0.32	0.30	0.26
1.38	1.22	1.12	1.16	0.94	0.94	0.82	0.74	0.58	0.52

Table B-4 Mean vegetative fitness of CDC15 was calculated at different concentrations of Fluconzole in T25/YEPD. Total number of concentrations are 10. For each concentration, 45 values of growth rate correspond with CDC15.

Table B-4 summary of vegetative fitness of CDC15 at different Fluconazole concentrations in T25/SD environment

0 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	100 µg/ml
1.16	0.56	1.08	0.92	0.98	0.52	0.54	0.46	0.26	0.20
1.14	1.26	0.76	0.96	1.18	1.24	0.78	0.24	0.28	0.22
3.18	2.42	2.28	2.12	1.88	1.76	1.54	1.30	0.52	0.46
1.74	1.36	1.30	1.24	1.20	1.12	0.92	0.80	0.62	0.36
2.14	2.08	2.04	1.88	1.74	1.30	1.10	0.96	0.78	0.76
2.38	2.28	2.10	1.74	1.90	1.74	1.46	1.16	0.86	0.40
1.56	1.40	0.96	1.00	1.08	1.04	0.74	0.48	0.46	0.30
1.96	1.50	1.88	1.82	1.80	1.46	1.30	0.82	0.48	0.10
1.72	1.74	1.68	1.70	1.28	1.52	0.86	0.66	0.46	0.36
1.50	1.56	1.42	1.36	1.20	1.08	1.00	0.66	0.52	0.10
2.00	1.52	1.38	1.28	1.24	1.26	0.90	0.78	0.60	0.52
2.38	2.44	2.50	2.38	2.36	2.08	2.02	1.96	0.66	0.50
1.80	1.76	1.72	1.72	1.54	1.30	1.18	1.02	0.62	0.54
1.42	1.20	1.10	1.08	1.06	1.02	0.92	0.38	0.28	0.10
1.82	1.88	1.80	1.56	1.36	1.24	1.02	0.88	0.84	0.74
1.68	1.64	1.52	1.36	1.28	1.10	1.08	1.06	0.52	0.28
2.52	2.22	2.26	1.78	1.72	1.24	0.86	0.78	0.42	0.36
2.70	1.16	0.96	1.50	1.72	1.56	0.74	0.46	0.30	0.10
1.90	1.60	1.64	1.50	1.38	1.06	0.90	0.66	0.46	0.36
1.68	1.52	1.36	1.28	1.24	0.94	0.54	0.50	0.42	0.10
2.60	2.22	2.02	1.92	1.80	1.62	1.34	0.92	0.58	0.42
1.52	1.36	0.84	0.82	0.80	0.76	0.52	0.22	0.10	0.10
1.38	1.22	1.06	0.98	1.00	0.70	0.72	0.56	0.10	0.10
1.32	1.24	1.08	1.00	0.82	0.70	0.66	0.60	0.48	0.32
4.14	3.94	3.84	3.76	3.62	3.08	2.54	1.32	0.94	0.50
1.56	1.44	1.38	1.48	1.44	0.82	0.64	0.42	0.30	0.26
1.42	1.24	1.04	0.90	0.86	0.64	0.64	0.54	0.48	0.34
1.80	1.78	1.76	1.76	1.62	1.32	1.28	0.44	0.40	0.20
2.20	1.90	1.62	1.34	1.22	1.12	1.06	0.80	0.56	0.42
1.28	1.18	1.10	0.98	0.84	0.66	0.64	0.56	0.46	0.36
1.36	1.18	1.04	1.00	0.94	0.82	0.70	0.60	0.48	0.42
2.22	1.96	1.62	1.36	1.08	0.62	0.48	0.40	0.32	0.30
2.20	1.90	1.56	1.12	1.02	0.64	0.54	0.38	0.36	0.28
1.90	1.86	1.62	1.40	1.36	1.18	0.82	0.54	0.44	0.34
1.78	1.72	1.54	1.36	1.28	0.96	0.76	0.60	0.50	0.44
2.40	2.22	1.98	1.80	1.52	1.04	0.94	0.80	0.54	0.48
3.02	2.78	2.32	2.08	1.96	1.56	1.42	0.98	0.66	0.54
2.84	2.60	2.52	2.30	2.22	2.16	2.08	1.64	1.04	0.46
1.04	1.12	1.10	1.08	0.50	1.24	0.96	0.74	0.52	0.46
2.88	2.40	2.08	1.26	1.76	1.34	1.12	0.66	0.48	0.10
2.22	2.14	1.96	1.64	1.60	1.28	1.14	0.54	0.42	0.10

1.94	1.84	1.70	1.78	1.62	0.62	0.52	0.10	0.10	0.10
2.30	2.14	2.00	1.92	1.86	1.70	1.60	1.32	0.48	0.38
2.66	2.40	2.14	1.66	1.40	1.00	0.78	0.60	0.40	0.34
1.50	1.40	1.18	1.00	0.82	0.68	0.66	0.54	0.44	0.32

Table C-1 Mean vegetative fitness of each of 269 progeny was calculated at different concentrations of Fluconzole in T37/SD. Total number of concentrations are 10. For each concentration, 269 values of growth rate correspond with 269 progeny.

Table C-1 summary of vegetative fitness of *C. neoformans* hybrids at different Fluconazole concentrations in T37/SD environment

* 0 µg/mL

0.10	1.74	0.76	1.26	2.58	1.70	1.18	1.34	0.78	1.74
2.86	2.10	1.46	1.72	0.92	1.18	0.94	1.76	0.76	1.68
1.76	1.22	2.06	1.08	1.16	2.24	1.36	1.34	1.02	0.80
1.10	2.28	1.08	2.12	1.22	1.94	1.30	1.04	1.18	1.16
1.10	0.52	1.16	0.82	1.22	1.80	2.06	1.40	1.84	1.22
1.16	2.12	1.40	1.94	1.02	1.78	0.10	1.78	2.52	2.74
1.18	1.18	1.82	1.56	1.94	1.34	1.88	0.70	0.84	1.66
1.22	2.62	1.14	3.02	1.10	0.84	0.78	1.24	1.98	1.24
1.90	1.50	0.66	1.34	1.66	1.52	1.40	0.88	1.88	0.70
0.98	1.58	1.12	1.38	1.16	2.00	1.40	3.20	0.86	1.08
1.04	1.44	1.02	1.34	1.84	0.82	2.00	1.46	0.30	1.10
1.42	2.38	0.62	1.08	0.70	1.36	1.38	0.44	1.30	1.42
1.10	2.32	0.88	1.52	0.60	1.32	1.32	1.70	1.00	1.84
1.16	1.68	2.02	1.80	2.10	1.46	1.02	0.44	2.22	1.82
0.90	1.76	0.46	0.88	2.52	2.34	0.82	0.82	1.34	1.08
1.22	1.12	0.92	1.16	1.30	1.88	1.44	0.34	1.18	2.04
0.94	1.76	0.46	1.38	1.24	0.58	2.60	1.74	2.94	2.04
2.46	2.06	2.18	2.26	1.76	0.80	3.00	2.06	1.60	1.42
0.58	0.82	1.42	3.14	1.46	0.92	1.52	0.54	1.34	1.02
0.72	1.00	1.76	1.88	0.98	2.46	1.02	1.86	0.48	0.98
1.22	1.42	1.72	0.98	1.48	1.84	2.62	1.98	2.06	0.78
2.78	3.30	1.30	1.12	1.14	1.38	1.14	1.20	2.74	0.98
1.38	2.30	1.18	0.60	1.74	1.56	1.30	1.72	1.28	1.90
1.40	1.14	0.94	2.16	0.86	2.28	2.32	1.68	0.72	0.82
1.60	0.78	1.52	1.92	2.66	0.80	1.64	0.82	1.30	1.12
1.76	1.84	1.94	1.80	1.94	0.88	1.44	1.22	0.96	1.26
1.78	1.20	2.84	1.62	1.36	1.90	1.74	1.74	1.02	

* 0.5 µg/mL

0.10	1.68	0.70	1.14	2.38	1.66	1.42	1.22	0.88	1.52
2.84	1.90	1.42	1.62	0.80	1.02	0.86	1.54	1.34	1.62
1.52	1.16	1.64	1.04	1.06	1.88	0.88	1.24	0.82	0.68
0.88	1.90	0.84	1.66	0.90	1.92	1.18	0.84	1.00	1.02
0.82	0.46	1.14	0.80	1.14	1.58	2.16	1.16	1.62	1.06
1.08	2.02	1.12	1.86	0.78	1.58	0.10	1.64	1.84	2.52
1.18	1.10	1.48	1.24	1.92	1.26	1.92	0.66	0.66	1.60
1.44	2.10	0.54	2.86	0.94	0.64	1.00	1.00	1.74	1.08
1.72	1.28	0.46	1.08	2.06	1.36	1.24	0.80	1.56	1.18
1.28	1.54	1.36	1.34	2.02	1.46	1.60	2.94	0.76	1.02
0.92	1.38	0.82	1.24	1.36	0.80	1.74	1.32	0.48	0.94
1.12	2.34	0.44	1.00	0.44	1.22	1.04	0.38	1.16	1.06
0.64	2.02	0.74	1.36	0.52	0.98	1.26	1.54	0.82	1.62
1.30	1.64	1.66	1.62	2.16	1.12	0.90	0.34	2.06	1.74

0.98	1.66	0.58	0.86	1.62	2.08	0.74	0.74	1.32	1.12
1.08	1.02	0.82	1.06	1.12	1.88	1.34	0.10	1.08	1.54
0.74	1.52	0.36	1.14	1.14	0.74	2.52	1.46	2.62	1.44
1.74	1.90	2.12	2.20	1.68	0.82	2.88	2.02	1.38	1.38
0.56	0.76	1.22	2.78	1.44	0.78	1.34	0.54	1.20	1.32
0.46	0.96	1.68	1.86	0.92	1.84	0.92	1.82	0.38	0.94
0.94	1.24	1.62	1.34	1.26	1.46	2.34	2.20	1.86	0.66
2.66	2.86	1.28	0.92	1.12	1.32	0.86	1.12	2.66	0.96
1.08	2.22	1.10	0.48	1.58	1.48	1.38	1.36	1.22	2.10
1.38	0.94	0.88	1.74	0.82	1.90	1.80	1.54	0.58	0.76
1.56	0.70	1.36	1.54	2.42	0.80	1.82	1.02	1.24	1.08
1.60	1.68	1.64	1.74	1.80	0.78	1.30	1.16	1.00	1.24
1.72	1.16	2.32	1.3	1.3	1.8	1.74	1.64	0.94	

* 1 μg/mL

0.10	1.42	0.68	1.10	2.20	1.54	1.18	1.34	0.98	1.52
2.86	1.74	1.26	1.48	0.96	1.00	0.68	0.72	0.54	0.60
1.38	1.06	1.46	0.96	0.44	1.76	0.98	0.76	0.86	0.96
0.72	1.86	0.78	1.56	0.96	1.86	1.02	1.08	1.56	1.06
0.84	0.42	0.94	0.46	1.26	1.44	1.66	1.52	1.80	2.18
1.00	1.92	1.06	1.76	0.74	1.38	0.10	0.56	0.52	1.44
1.10	1.00	1.40	1.16	1.46	1.04	1.64	0.90	1.72	0.10
1.32	2.00	0.10	2.84	0.88	0.56	0.84	0.76	1.50	1.18
1.44	0.96	0.10	1.00	1.84	1.16	1.06	3.20	0.54	1.00
1.26	1.36	1.12	1.02	0.86	1.34	1.44	1.06	0.10	0.82
0.84	0.88	0.96	1.10	1.48	0.70	1.82	0.10	1.08	1.16
1.24	2.22	0.54	0.94	0.42	1.04	1.02	1.22	0.68	1.50
0.56	1.76	0.68	1.16	0.38	0.90	1.22	0.36	2.00	1.56
1.32	1.48	1.48	1.48	2.10	1.10	0.84	0.76	1.16	1.20
0.64	1.44	0.38	0.66	1.36	1.94	0.72	0.10	0.94	1.24
1.18	0.88	0.64	0.98	1.08	1.62	1.22	1.34	2.53	1.24
0.72	1.26	0.28	1.04	1.12	0.66	2.36	1.92	0.92	1.42
1.62	1.70	2.12	2.10	1.38	0.80	2.80	0.46	1.00	1.38
0.48	0.66	1.16	2.60	1.42	0.70	1.20	1.48	0.34	0.76
0.10	0.64	1.64	1.60	0.90	1.70	0.86	2.02	1.84	0.56
0.78	1.20	1.24	1.30	0.98	1.38	1.98	1.08	2.56	1.24
2.64	2.02	1.16	0.86	1.00	1.02	0.48	1.12	1.02	1.16
2.32	2.04	1.06	0.42	1.50	1.46	1.26	1.44	0.46	0.84
1.30	0.78	0.82	1.50	0.74	1.50	1.66	1.22	1.04	1.02
1.56	0.60	1.38	1.46	2.18	0.76	1.78	0.66	1.44	1.61
1.56	1.42	1.64	1.68	0.66	1.16	1.06	0.96	1.06	1.62
1.04	1.94	0.98	1.24	1.38	1.72	1.54	0.94	1.14	

* 2 μg/mL

0.10	1.12	0.66	1.04	1.60	1.46	1.04	1.08	0.42	1.28
2.48	0.10	1.18	1.20	0.86	2.48	0.54	1.14	1.26	1.30
1.38	0.94	1.46	0.90	0.10	1.58	0.74	0.70	0.48	0.52
0.80	1.76	0.68	1.24	0.56	1.86	1.32	0.72	0.68	0.70
0.80	0.40	0.50	0.36	0.68	1.38	1.32	0.92	1.44	0.96
0.96	1.68	0.96	1.12	0.10	1.26	0.10	1.24	1.74	1.98
0.72	0.98	1.26	1.12	1.38	0.86	1.84	0.52	0.10	1.26
1.22	1.84	0.10	2.60	1.10	0.52	0.66	0.82	1.64	0.10
1.22	0.94	0.10	0.92	1.62	1.02	0.94	0.62	0.78	1.24
1.16	0.86	1.18	0.64	1.54	1.10	1.62	3.20	0.52	0.92
0.78	0.92	0.72	0.92	1.52	0.60	1.72	0.80	0.10	0.80
1.24	1.90	0.40	0.74	0.42	0.96	0.98	0.10	0.60	1.04
0.50	1.58	0.54	1.02	0.32	0.78	1.12	1.14	0.68	1.36
0.98	1.18	1.02	1.30	2.12	0.88	0.80	0.30	1.94	1.44
0.64	1.18	0.10	0.50	1.26	1.86	0.74	0.72	1.04	1.06
0.56	0.74	0.64	0.86	0.98	1.56	1.08	0.10	0.88	1.60
0.62	1.04	0.24	0.80	0.66	0.42	2.24	1.18	2.13	1.36
1.58	1.48	1.92	1.66	1.20	0.78	2.14	1.56	0.84	1.16
0.10	0.54	0.10	2.44	0.30	0.70	1.10	0.40	0.96	0.94
0.10	0.44	1.58	1.32	0.78	1.32	0.80	1.46	0.10	0.62
0.72	1.14	1.12	1.36	0.98	1.28	1.92	1.94	1.80	0.52
2.40	1.44	1.02	0.80	0.92	0.64	0.46	1.04	2.38	1.08
0.98	1.92	0.10	0.36	1.42	1.34	1.10	1.06	0.84	1.12
1.22	0.74	0.10	1.56	0.38	1.26	1.64	1.28	0.36	0.90
1.52	0.58	1.40	1.36	2.06	0.66	1.80	1.32	0.96	0.82
1.42	1.48	1.44	1.62	0.74	0.54	1.04	1.00	0.92	0.90
1.38	0.86	1.86	0.96	0.88	1.42	1.72	1.36	0.1	

* 4 μg/mL

0.10	0.90	0.64	0.86	1.34	1.34	0.60	0.10	0.98	1.04
2.08	0.10	1.12	1.02	0.76	3.46	0.44	1.22	0.64	1.10
1.24	0.76	1.20	0.82	0.10	1.42	0.70	0.10	0.70	0.46
0.46	1.76	0.64	0.70	0.44	1.76	1.00	0.50	0.56	0.50
0.72	0.38	0.44	0.10	0.62	1.36	0.10	1.24	0.84	0.88
0.88	1.44	1.24	0.98	0.10	0.80	0.10	1.62	1.10	1.74
0.48	0.82	1.10	1.10	1.28	0.80	1.80	0.10	0.44	1.10
1.12	1.80	0.10	2.40	0.72	0.46	0.40	1.48	0.66	0.10
1.00	0.92	0.10	2.78	1.10	0.88	0.90	0.78	0.46	0.70
1.06	0.10	0.90	0.48	1.68	1.04	1.48	0.54	3.36	0.84
0.80	0.86	0.68	0.84	1.32	0.58	1.68	0.10	0.10	0.66
0.98	1.80	0.58	0.62	0.32	0.88	0.46	0.44	0.10	1.00
0.50	1.24	0.36	0.88	0.30	0.68	1.08	0.68	0.40	0.58
0.86	0.98	1.02	1.20	1.62	0.10	0.78	1.86	0.24	1.30
0.10	1.02	0.10	0.38	1.20	1.62	0.66	0.94	0.56	1.10
0.10	0.70	0.48	0.78	0.72	0.80	0.94	0.78	0.10	1.32
0.56	0.92	0.10	0.76	0.10	0.10	2.12	1.60	1.06	0.42

1.30	1.30	1.88	1.16	1.00	0.10	1.18	0.66	1.00	0.70
0.10	0.44	0.10	2.20	0.30	0.68	0.94	0.86	0.30	0.66
0.10	0.34	1.50	0.10	0.10	1.42	0.74	0.10	1.16	0.50
0.66	0.98	1.08	1.06	0.86	1.24	1.50	1.74	1.38	0.40
2.02	0.10	0.90	0.64	0.84	0.52	0.42	2.12	0.86	0.96
0.98	1.58	0.10	0.10	1.06	1.26	0.94	0.82	0.62	1.24
0.50	0.68	0.10	1.26	0.32	1.22	1.52	0.10	1.06	0.96
1.26	0.54	1.44	0.10	1.54	0.46	1.72	0.76	0.84	0.62
1.24	1.38	1.36	0.90	0.36	0.50	0.90	0.94	0.90	0.86
1.18	0.74	1.52	0.90	0.10	1.38	1.76	1.28	0.10	

* 8 μg/mL

1.10	0.78	0.66	0.84	1.12	1.18	0.92	0.98	0.10	0.58
1.88	0.10	1.06	0.88	0.64	0.64	0.48	0.54	1.18	0.76
1.16	0.74	1.02	0.64	0.10	1.16	0.66	0.10	0.10	0.42
0.26	1.66	0.64	0.50	0.68	1.16	1.36	0.50	0.54	0.10
0.70	0.24	0.10	0.10	0.68	1.42	0.10	0.62	1.04	0.80
0.84	1.04	1.22	0.86	0.10	0.54	0.10	0.76	1.66	1.52
0.38	0.50	1.06	1.04	1.06	0.50	1.42	0.34	0.10	1.04
1.08	1.54	0.10	1.80	1.06	0.40	0.40	0.48	1.06	0.10
1.00	0.90	0.10	0.72	1.18	0.74	0.10	0.10	0.74	0.72
1.08	0.10	0.88	0.10	1.08	0.74	1.34	3.24	0.10	0.82
0.76	0.84	0.64	0.52	1.12	0.50	1.58	0.10	0.10	0.62
0.94	1.60	0.46	0.42	0.28	0.62	0.10	0.10	0.10	0.90
0.52	1.10	0.10	0.72	0.26	0.68	0.98	0.44	0.62	0.48
0.92	0.90	0.64	0.98	1.68	0.10	0.56	0.24	1.70	1.28
0.10	0.54	0.10	0.10	1.18	1.52	0.58	0.50	0.72	1.72
0.10	0.52	0.42	0.54	0.46	0.62	0.56	0.10	0.56	1.20
0.44	0.74	0.10	0.74	0.10	0.10	2.00	0.94	1.48	0.38
0.84	0.80	1.76	0.10	0.86	0.10	0.50	0.68	0.62	0.50
0.10	0.38	0.10	2.18	0.28	0.66	0.74	0.10	0.76	0.62
0.10	0.10	1.44	0.10	0.10	1.28	0.50	1.00	0.10	0.50
0.62	0.94	1.06	0.98	0.76	1.20	1.12	1.24	1.38	0.10
1.88	0.10	0.82	0.64	0.84	0.54	0.10	0.72	1.98	0.58
0.94	1.44	0.10	0.10	0.76	1.20	0.78	1.30	0.68	0.60
0.46	0.62	0.10	1.04	0.10	1.20	1.50	1.12	0.10	0.84
1.12	0.26	1.46	0.10	1.22	0.50	1.58	1.16	0.56	0.56
1.20	1.24	1.36	0.10	0.10	0.46	0.66	0.90	0.90	0.80
0.92	0.62	1.20	0.92	0.10	1.20	1.22	1.18	0.10	

* 16 μg/mL

1.30	0.76	0.64	0.76	1.00	1.08	0.62	0.80	0.10	0.46
1.76	0.10	1.04	0.78	0.60	0.62	0.48	0.44	0.92	0.62
1.14	0.74	1.00	0.58	0.10	0.88	0.76	0.10	0.10	0.30
0.30	1.58	0.60	0.10	0.72	0.66	0.98	0.38	0.42	0.10
0.72	0.10	0.10	0.10	0.34	1.04	0.10	0.44	0.84	0.74

0.82	0.90	0.10	0.76	0.10	0.56	0.10	0.64	1.26	0.86
0.10	0.34	0.90	0.92	0.88	0.36	1.02	0.28	0.10	1.00
1.02	1.16	0.10	1.44	0.68	0.34	0.50	0.44	0.88	0.10
0.92	0.86	0.10	0.62	0.98	0.52	0.10	0.10	0.60	0.72
0.86	0.10	0.64	0.10	1.10	0.88	1.12	2.00	0.10	0.82
0.68	0.68	0.58	0.44	0.70	0.40	0.96	0.10	0.10	0.58
0.76	1.14	0.50	0.40	0.28	0.50	0.10	0.10	0.10	0.88
0.62	0.94	0.10	0.52	0.20	0.66	0.76	0.10	0.52	0.10
0.86	0.82	0.10	0.74	0.88	0.10	0.48	0.22	1.28	1.20
0.10	0.52	0.10	0.10	0.86	1.38	0.54	0.48	0.62	1.44
0.10	0.10	0.30	0.46	0.40	0.26	0.10	0.10	0.42	1.02
0.38	0.56	0.10	0.56	0.10	0.10	1.88	0.88	1.36	0.40
0.10	0.74	1.60	0.10	0.66	0.10	0.10	0.10	0.56	0.38
0.10	0.34	0.10	2.02	0.10	0.54	0.58	0.10	0.64	0.32
0.10	0.10	1.04	0.10	0.10	1.16	0.36	1.12	0.10	0.38
0.38	0.76	0.98	0.56	0.52	1.10	1.12	1.12	1.22	0.10
1.62	0.10	0.68	0.58	0.74	0.54	0.10	0.76	1.08	0.62
0.92	1.22	0.10	0.10	0.54	1.24	0.68	0.64	0.42	0.10
0.40	0.60	0.10	0.82	0.10	1.16	1.28	1.02	0.10	0.68
1.02	0.32	1.52	0.10	0.62	0.46	1.36	0.84	0.46	0.52
1.02	1.00	1.02	0.10	0.10	0.40	0.52	0.62	0.86	0.10
0.72	0.60	0.82	0.88	0.10	0.94	0.96	0.88	0.60	

* 32 μg/mL

1.30	0.60	0.64	0.60	0.96	0.88	0.52	0.66	0.10	0.42
1.40	0.10	1.00	0.40	0.68	0.60	0.54	0.40	1.18	0.48
0.76	0.66	0.94	0.44	0.10	0.78	0.42	0.10	0.10	0.10
0.10	1.42	0.50	0.10	0.54	0.64	0.82	0.30	0.36	0.10
0.64	0.10	0.10	0.10	0.10	0.56	0.10	0.42	0.74	0.64
0.74	0.66	0.10	0.68	0.10	0.34	0.10	0.52	0.88	0.64
0.10	0.28	0.96	0.70	0.88	0.10	0.98	0.24	0.10	0.90
0.58	0.54	0.10	1.22	0.34	0.28	0.50	0.36	0.70	0.10
0.92	0.80	0.10	0.30	0.94	0.50	0.10	0.10	0.50	0.46
0.74	0.10	0.64	0.10	1.48	0.64	0.98	1.60	0.10	0.66
0.70	0.48	0.50	0.10	0.50	0.10	0.10	0.10	0.10	0.46
0.72	0.84	0.10	0.32	0.30	0.46	0.10	0.10	0.10	0.86
0.60	0.72	0.10	0.50	0.10	0.54	0.72	0.10	0.42	0.10
0.50	0.64	0.10	0.60	0.88	0.10	0.44	0.20	0.42	1.12
0.10	0.44	0.10	0.10	0.78	1.20	0.50	0.40	0.54	0.82
0.10	0.10	0.10	0.38	0.32	0.10	0.10	0.10	0.40	0.90
0.34	0.50	0.10	0.42	0.10	0.10	1.40	0.10	1.00	0.10
0.10	0.56	1.32	0.10	0.58	0.10	0.10	0.10	0.10	0.32
0.10	0.26	0.10	1.88	0.10	0.46	0.10	0.10	0.52	0.10
0.10	0.10	0.74	0.10	0.10	0.98	0.28	1.00	0.10	0.30
0.36	0.72	0.80	0.52	0.46	1.02	1.12	1.08	0.54	0.10
0.84	0.10	0.10	0.46	0.68	0.40	0.10	0.56	0.76	0.62
0.80	1.06	0.10	0.10	0.34	1.42	0.60	0.64	0.38	0.10
0.36	0.52	0.10	0.10	0.10	0.92	0.66	0.62	0.10	0.52

0.76	0.10	1.11	0.10	0.56	0.42	1.02	0.46	0.46	0.46
0.82	0.68	0.68	0.10	0.10	0.30	0.44	0.50	0.76	0.10
0.52	0.48	0.60	0.54	0.10	0.66	0.36	0.64	0.70	

* 64 µg/mL

0.50	0.46	0.36	0.42	0.40	0.30	0.44	0.48	0.10	0.34
0.83	0.10	0.50	0.10	0.60	2.06	0.36	0.10	0.76	0.42
0.46	0.48	0.60	0.44	0.10	0.48	0.10	0.10	0.10	0.10
0.10	0.78	0.42	0.10	0.10	0.64	0.56	0.10	0.10	0.10
0.50	0.10	0.10	0.10	0.10	0.44	0.10	0.38	0.44	0.48
0.62	0.52	0.10	0.54	0.10	0.30	0.10	0.46	0.44	0.50
0.10	0.26	0.10	0.38	0.10	0.10	0.56	0.22	0.10	0.58
0.58	0.36	0.10	0.50	0.10	0.10	0.10	0.30	0.34	0.10
0.44	0.56	0.10	0.10	0.80	0.48	0.10	0.10	0.44	0.44
0.54	0.10	0.10	0.10	0.90	0.10	0.80	0.10	0.10	0.50
0.54	0.30	0.10	0.10	0.44	0.10	0.10	0.10	0.10	0.10
0.66	0.46	0.10	0.10	0.10	0.34	0.10	0.10	0.10	0.64
0.48	0.10	0.10	0.44	0.10	0.34	0.40	0.10	0.34	0.10
0.32	0.54	0.10	0.10	0.52	0.10	0.10	0.10	0.10	1.08
0.10	0.36	0.10	0.10	0.46	0.82	0.28	0.10	0.10	0.60
0.10	0.10	0.10	0.34	0.10	0.10	0.10	0.10	0.28	0.86
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.70	0.10
0.10	0.10	0.42	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.22	0.10	0.62	0.10	0.36	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.76	0.26	0.50	0.10	0.30
0.10	0.66	0.52	0.72	0.10	0.56	0.66	0.62	0.38	0.10
0.58	0.10	0.10	0.28	0.44	0.40	0.10	0.10	0.62	0.44
0.68	0.72	0.10	0.10	0.22	0.92	0.10	0.10	0.32	0.10
0.26	0.10	0.10	0.10	0.10	0.10	0.60	0.10	0.10	0.44
0.52	0.10	0.10	0.10	0.38	0.34	0.84	0.10	0.10	0.10
0.46	0.10	0.46	0.10	0.10	0.10	0.26	0.38	0.56	0.10
0.10	0.32	0.10	0.10	0.10	0.76	0.10	0.50	0.50	

* 100 µg/mL

0.10	0.10	0.20	0.10	0.36	0.10	0.26	0.10	0.10	0.26
0.26	0.10	0.38	0.10	0.30	1.16	0.10	0.10	0.68	0.10
0.10	0.26	0.36	0.10	0.10	0.36	0.10	0.10	0.10	0.10
0.10	0.28	0.36	0.10	0.10	0.28	0.10	0.10	0.10	0.10
0.28	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.46
0.42	0.32	0.10	0.40	0.10	0.26	0.10	0.10	0.36	0.44
0.10	0.10	0.10	0.36	0.10	0.10	0.34	0.20	0.10	0.65
0.56	0.26	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.32	0.36	0.10	0.10	0.10	0.10	0.10	0.10	0.26	0.10
0.24	0.10	0.10	0.10	0.60	0.10	0.30	0.10	0.10	0.26
0.38	0.28	0.10	0.10	0.40	0.10	0.10	0.10	0.10	0.10
0.54	0.10	0.10	0.10	0.10	0.30	0.10	0.10	0.10	0.38

0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.24	0.10
0.32	0.46	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.94
0.10	0.24	0.10	0.10	0.10	0.60	0.10	0.10	0.10	0.38
0.10	0.10	0.10	0.26	0.10	0.10	0.10	0.10	0.20	0.38
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.50	0.10
0.10	0.10	0.30	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.64	0.10	0.32	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.36	0.22	0.36	0.10	0.10
0.10	0.65	0.36	0.42	0.10	0.28	0.46	0.58	0.30	0.10
0.10	0.10	0.10	0.20	0.10	0.34	0.10	0.10	0.10	0.10
0.10	0.60	0.10	0.10	0.10	0.84	0.10	0.10	0.24	0.10
0.10	0.10	0.10	0.10	0.10	0.10	0.26	0.10	0.10	0.34
0.36	0.10	0.10	0.10	0.28	0.30	0.10	0.10	0.10	0.10
0.10	0.10	0.22	0.10	0.10	0.10	0.10	0.10	0.46	0.10
0.10	0.22	0.10	0.10	0.10	0.28	0.10	0.38	0.10	

Table C-2 Mean vegetative fitness of each of 269 progeny was calculated at different concentrations of Fluconzole in T25/SD. Total number of concentrations are 10. For each concentration, 269 values of growth rate correspond with 269 progeny.

Table C-2 summary of vegetative fitness of *C. neoformans* hybrids at different Fluconazole concentrations in T25/SD environment

* 0 μg/mL

1.96	0.88	1.10	1.62	1.18	0.54	0.70	1.28	1.06	1.34
1.76	1.36	0.92	2.34	0.74	1.10	0.90	0.92	0.96	1.23
1.36	1.50	0.44	1.96	0.92	1.40	0.88	0.66	0.90	1.20
0.76	2.28	0.64	1.84	2.84	0.84	1.16	0.72	0.96	1.10
1.00	1.94	0.48	1.00	0.50	0.92	0.86	1.90	0.84	1.30
1.02	1.62	1.28	0.36	1.14	1.24	0.98	0.46	1.20	1.50
2.04	2.30	2.04	0.88	0.94	0.88	1.16	1.22	1.72	1.40
0.78	1.88	0.76	0.80	0.48	0.84	1.70	0.82	1.64	1.00
0.68	3.04	3.44	1.24	0.68	0.98	1.92	0.68	0.94	0.70
0.50	0.94	0.10	0.94	1.48	0.72	0.98	0.28	0.80	1.30
1.20	1.16	0.66	1.30	0.64	2.50	0.88	1.52	1.74	1.10
1.14	1.06	0.98	1.24	0.96	1.74	1.26	1.00	1.20	1.00
1.32	1.58	1.16	1.16	1.20	1.30	3.44	0.32	2.24	0.90
0.10	1.20	0.76	0.82	1.34	0.74	1.98	0.32	1.00	0.90
0.58	0.86	0.66	0.66	1.16	0.92	1.00	0.84	0.90	0.87
1.02	1.10	0.54	3.28	1.14	1.44	2.56	2.14	1.42	0.90
0.70	1.26	0.98	1.10	1.18	1.32	1.04	1.04	1.34	1.50
1.40	1.44	1.26	1.12	1.06	0.46	1.68	3.82	0.96	1.10
0.26	1.40	1.48	0.88	3.12	0.96	1.00	0.50	1.04	1.40
0.44	0.68	1.38	0.84	1.54	1.96	0.86	0.66	0.68	1.00
0.66	1.62	1.06	1.10	1.66	1.52	0.80	0.70	1.00	0.60
0.86	0.94	1.34	1.34	1.66	0.64	0.98	1.06	1.00	1.10
1.46	1.30	0.94	0.82	1.90	2.36	0.80	1.02	1.16	0.70
1.46	1.04	2.72	0.46	1.78	0.84	0.84	0.68	0.46	1.40
0.88	1.12	1.70	0.52	1.06	1.40	0.76	1.28	1.23	1.00
1.70	1.30	1.70	1.80	1.00	0.90	0.80	1.10	1.50	1.00
0.90	0.76	0.70	1.10	1.00	1.20	1.10	1.30	1.10	

* 0.5 μg/mL

1.52	0.86	0.98	1.58	0.86	0.50	0.76	1.22	0.84	0.60
1.64	1.18	0.82	2.02	0.70	0.92	0.38	0.86	0.86	0.70
1.12	1.36	0.84	1.64	0.84	1.18	0.92	0.52	0.54	0.90
0.72	2.24	0.58	1.66	2.62	0.76	1.00	0.68	0.90	0.70
1.00	1.78	0.44	0.88	0.38	0.74	0.66	1.58	2.46	0.90
1.04	1.58	0.90	0.32	1.10	1.12	0.86	0.32	1.14	1.00
1.92	1.66	1.70	0.80	0.78	0.84	1.14	1.46	1.64	1.90
0.76	1.76	0.70	0.70	0.38	0.84	1.64	0.70	1.46	1.60
0.58	2.92	3.26	1.16	0.64	0.86	1.74	0.70	0.92	1.20
0.36	0.78	0.10	0.88	1.30	0.64	0.96	0.10	0.72	1.20
0.84	0.88	0.56	0.92	0.50	2.02	0.80	1.48	1.48	0.60
1.08	1.02	0.82	1.04	0.86	1.56	1.20	0.90	1.16	0.80
0.68	1.32	1.10	1.04	1.02	1.02	3.34	0.34	2.16	0.70

0.10	1.14	0.48	0.66	0.98	0.72	1.98	0.26	0.94	0.80
0.36	0.76	0.62	0.58	1.12	0.86	0.98	1.06	0.82	0.60
0.78	1.02	0.44	2.74	1.14	1.40	1.84	1.72	1.34	1.20
0.60	1.06	0.94	1.08	1.00	1.24	0.86	0.84	1.18	1.00
1.14	1.34	1.24	1.18	1.06	0.40	1.44	2.46	0.76	1.00
0.10	1.18	1.14	0.68	2.92	0.84	0.86	0.42	0.86	1.60
0.10	0.60	1.20	0.80	1.50	1.44	0.82	1.06	0.62	1.00
0.60	1.54	1.04	1.08	1.66	1.12	0.76	0.56	1.06	0.70
0.72	0.84	1.20	1.24	1.54	0.44	0.74	1.02	1.26	1.50
1.42	1.32	0.96	0.82	1.68	2.34	0.74	0.84	1.08	1.00
0.54	1.00	2.64	0.38	1.76	0.48	0.60	0.66	0.44	0.90
0.90	1.02	1.66	0.38	0.94	1.46	0.74	1.16	0.70	0.70
1.20	0.60	1.10	0.70	0.60	0.60	1.00	1.00	1.20	1.00
0.90	0.80	0.70	0.90	0.90	1.30	1.50	0.80	0.80	

* 1 μg/mL

1.62	0.90	0.98	1.24	0.78	0.34	0.72	1.04	0.64	0.76
1.52	1.08	0.34	1.84	0.46	0.92	0.38	0.80	0.84	0.52
1.28	1.34	0.56	1.68	0.72	1.06	0.96	0.34	0.46	0.98
0.68	2.22	0.74	1.46	2.84	0.66	1.00	0.66	0.84	0.70
0.92	1.74	0.46	0.74	0.30	0.66	0.54	1.28	0.82	0.80
1.04	1.24	0.92	0.10	1.12	1.02	0.74	0.30	0.92	0.80
1.36	1.66	1.36	0.78	0.74	0.82	1.04	1.24	1.62	0.50
0.76	1.70	0.62	0.60	0.40	0.68	1.62	0.68	1.30	0.80
0.60	2.00	3.28	1.02	0.62	0.78	1.42	0.68	0.86	0.40
0.42	0.68	0.10	0.74	1.20	0.58	0.92	0.10	0.60	0.43
0.76	0.10	0.54	1.06	0.36	1.92	0.78	1.00	0.96	0.70
0.96	1.02	0.62	0.96	0.74	1.48	1.20	0.62	1.12	0.80
1.06	1.18	1.00	0.78	0.98	0.96	2.62	0.54	1.64	0.50
0.10	1.00	0.46	0.62	0.84	0.68	1.88	0.24	0.84	0.70
0.38	0.74	0.54	0.58	1.00	0.84	0.90	0.92	0.64	0.30
0.62	0.86	0.44	2.88	1.16	1.36	1.74	1.50	1.04	0.80
0.56	2.18	0.84	0.96	0.86	1.00	0.74	0.90	1.10	0.80
0.88	1.10	1.18	0.90	1.02	0.30	1.28	2.14	0.64	0.80
0.10	1.04	1.02	0.62	2.70	1.02	0.64	0.40	0.76	0.70
0.10	0.54	1.10	0.74	1.48	1.12	0.78	1.02	0.52	0.70
0.52	1.38	0.92	1.02	1.26	1.10	0.76	0.78	0.98	0.40
0.56	0.78	1.18	1.06	1.46	0.26	0.66	0.78	1.08	0.60
1.24	1.30	0.96	0.70	1.68	2.18	0.54	0.80	0.90	0.70
0.52	0.94	2.50	0.50	1.40	0.44	0.58	0.50	0.38	0.80
0.76	1.02	1.64	0.38	0.82	1.32	0.58	1.00	0.64	0.40
0.70	0.90	1.10	1.30	1.40	1.50	1.10	1.10	1.40	0.90
0.90	0.90	1.10	1.40	1.30	1.40	1.10	0.90	0.70	

* 2 μg/mL

1.48	0.86	0.94	1.24	0.82	0.30	0.62	0.82	0.42	0.90

1.00	1.10	0.30	1.74	0.30	0.88	0.34	0.66	0.84	1.56
0.92	1.32	0.48	1.26	0.80	0.94	0.80	0.32	0.42	0.70
0.68	1.66	0.60	1.36	1.74	0.60	0.94	0.56	0.82	0.58
0.86	1.68	0.36	0.72	0.34	0.62	0.52	1.16	0.80	0.92
1.00	1.24	0.70	0.10	1.08	0.84	0.70	0.10	0.96	0.54
1.04	1.34	1.04	0.62	0.92	0.72	1.04	1.12	1.50	0.84
0.68	1.60	0.62	0.50	0.10	0.64	1.58	0.60	1.16	0.90
0.52	1.88	3.06	0.78	0.54	0.74	1.22	0.64	0.72	0.44
0.48	0.64	0.10	0.64	1.00	0.54	0.86	0.10	0.50	0.60
0.60	0.10	0.46	0.96	0.32	1.72	0.76	0.86	0.94	0.80
1.02	0.90	0.64	0.76	0.62	1.26	1.18	0.86	0.90	1.20
1.00	0.98	0.78	0.64	0.76	0.88	2.60	0.44	1.56	1.30
0.10	0.80	0.46	0.60	0.84	0.64	1.84	0.34	0.70	0.30
0.50	0.68	0.54	0.50	0.90	0.76	0.88	0.62	0.58	0.50
0.56	0.84	0.38	2.46	1.16	1.30	1.56	1.20	0.92	0.60
0.62	1.96	0.74	0.76	0.84	0.94	0.74	0.70	0.92	0.40
0.82	1.02	1.14	0.76	1.00	0.10	1.20	2.06	0.52	0.70
0.10	0.96	0.92	0.62	2.64	0.94	0.58	0.44	0.72	0.40
0.10	0.50	0.84	0.70	1.06	1.08	0.62	0.54	0.44	0.80
0.48	1.32	0.88	1.00	1.16	0.86	0.66	0.54	0.82	0.70
0.52	0.60	1.10	0.42	1.42	0.10	0.54	0.68	0.82	0.60
1.22	1.16	0.96	0.68	1.45	2.06	0.48	0.72	0.72	0.40
0.46	0.78	2.26	0.44	1.18	0.44	0.52	0.46	0.36	0.70
0.66	0.98	1.56	0.32	0.74	1.28	0.52	0.98	0.94	0.70
0.80	0.80	0.70	0.60	1.30	1.34	1.40	1.10	0.90	0.80
0.70	0.80	0.90	0.70	0.80	0.90	0.50	0.70	0.68	

* 4 µg/mL

1.42	0.86	0.82	1.16	0.68	0.26	0.60	0.74	0.40	0.82
0.88	0.98	0.22	1.40	0.26	0.78	0.10	0.58	0.92	0.36
0.94	1.16	0.62	1.22	0.46	0.88	0.72	0.30	0.40	0.54
0.68	1.54	0.60	1.14	1.12	0.54	0.82	0.52	0.82	0.34
0.80	1.64	0.16	0.58	0.36	0.56	1.36	1.08	0.80	0.36
0.86	1.16	0.38	0.10	0.82	0.74	0.54	0.10	0.92	0.64
0.70	1.04	0.88	0.32	0.80	0.60	1.00	0.94	0.82	0.70
0.58	1.50	0.50	0.46	0.10	0.52	1.52	0.54	0.86	0.70
0.54	1.06	2.94	0.70	0.52	0.52	1.04	0.56	0.70	0.80
0.38	0.54	0.10	0.58	0.88	0.46	0.84	0.10	0.46	0.76
0.50	0.10	0.32	0.84	0.84	1.64	0.78	0.82	0.94	0.50
0.82	0.84	0.64	0.58	0.60	0.96	1.08	0.78	0.84	0.70
0.86	0.92	0.70	0.58	0.74	0.84	1.82	0.42	1.14	0.50
0.10	0.60	0.44	0.50	0.74	0.62	1.76	0.40	0.54	0.76
0.40	0.58	0.44	0.46	0.84	0.68	0.86	0.58	0.54	0.50
0.46	0.74	0.32	2.18	1.14	1.26	1.38	0.94	0.84	1.10
0.54	1.64	0.66	0.70	0.94	0.78	0.72	0.24	0.90	1.20
0.52	0.86	0.96	0.74	0.80	0.10	1.06	1.60	0.44	1.60
0.10	0.84	0.88	0.62	1.70	0.96	0.62	0.38	0.60	1.20
0.10	0.50	0.76	0.52	1.04	0.90	0.54	0.56	0.42	1.20

0.44	1.16	0.88	1.00	1.04	0.78	0.30	0.48	0.64	1.10
0.42	0.58	1.18	0.28	1.32	0.10	0.44	0.64	0.70	0.70
1.14	0.98	0.82	0.70	1.38	1.50	0.50	0.62	0.50	0.70
0.46	0.76	1.36	0.50	0.72	0.52	0.44	0.10	0.30	0.80
0.60	0.94	1.56	0.50	0.68	0.76	0.44	0.92	0.76	0.50
0.40	0.50	0.70	0.60	0.40	0.70	0.70	0.80	0.70	0.50
0.70	0.80	0.40	0.50	0.70	0.80	0.90	0.90	0.60	

* 8 μg/mL

1.40	0.84	0.74	0.42	0.58	0.22	0.56	0.56	0.34	0.80
0.82	1.04	0.10	1.34	0.10	0.62	0.10	0.36	0.88	0.60
0.44	1.10	0.54	1.20	0.48	0.70	0.70	0.10	0.36	0.40
0.62	1.46	0.56	1.10	1.18	0.40	0.78	0.38	0.76	0.30
0.72	1.48	0.16	0.60	0.34	0.50	0.38	0.98	0.66	0.50
0.10	0.42	0.34	0.10	0.70	0.66	0.10	0.10	0.74	0.70
0.62	0.88	0.90	0.10	0.76	0.44	0.88	0.88	0.78	0.54
0.54	1.32	0.40	0.40	0.10	0.40	1.46	0.46	0.76	0.86
0.60	1.04	2.32	0.10	0.48	0.52	0.94	0.56	0.66	0.34
0.44	0.50	0.10	0.50	0.74	0.44	0.80	0.10	0.44	0.40
0.36	0.10	0.10	0.76	0.24	1.48	0.68	0.82	0.80	0.28
0.74	0.76	0.80	0.40	0.32	0.76	1.00	0.80	0.82	0.28
0.94	0.76	0.64	0.54	0.72	0.70	1.62	0.36	1.08	0.56
0.10	0.48	0.42	0.38	0.78	0.46	1.74	0.24	0.50	0.48
0.28	0.52	0.10	0.30	0.74	0.50	0.72	0.60	0.48	0.76
0.38	0.64	0.28	2.24	1.06	1.16	1.34	0.90	0.76	0.80
0.50	0.40	0.42	0.26	0.76	0.74	0.64	0.56	0.82	0.58
0.54	0.82	0.84	0.74	0.76	0.10	1.04	1.52	0.36	0.90
0.10	0.82	0.84	0.58	1.44	1.00	0.60	0.44	0.54	0.60
0.10	0.44	0.72	0.50	1.02	0.64	0.48	0.54	0.34	0.50
0.10	0.98	0.72	0.80	0.96	0.70	0.10	0.58	0.36	0.44
0.38	0.52	1.08	0.10	1.20	0.10	0.40	0.58	0.64	0.32
0.98	0.70	0.80	0.60	1.22	1.42	0.42	0.62	0.56	0.26
0.42	0.62	1.32	0.42	0.76	0.46	0.42	0.10	0.80	0.42
0.52	0.92	1.52	0.34	0.60	0.98	0.38	0.72	0.70	0.38
0.30	0.70	0.70	0.70	0.50	0.50	0.60	0.80	0.80	0.50
0.50	0.80	0.60	0.80	0.90	0.50	0.80	0.80	0.50	

* 16 μg/mL

1.28	0.84	0.70	0.10	0.54	0.10	0.58	0.40	0.26	0.66
0.78	0.94	0.10	1.12	0.10	0.54	0.10	0.28	0.80	0.74
0.34	0.92	0.10	1.20	0.46	0.56	0.74	0.10	0.36	0.78
0.54	1.44	0.52	1.02	0.10	0.30	0.76	0.38	0.68	0.76
0.62	1.40	0.10	0.40	0.26	0.42	0.34	0.94	0.68	0.66
0.10	0.10	0.10	0.10	0.62	0.86	0.10	0.10	0.58	0.44
0.58	0.82	0.86	0.10	0.68	0.36	0.60	0.72	0.78	0.80
0.52	1.20	0.30	0.36	0.10	0.38	0.90	0.42	0.20	0.82

0.60	0.98	0.66	0.10	0.48	0.46	0.80	0.52	0.66	1.08
0.34	0.46	0.10	0.44	0.64	0.36	0.10	0.10	0.42	0.50
0.30	0.10	0.10	0.82	0.20	1.30	0.58	0.76	0.78	0.48
0.76	0.56	0.76	0.36	0.28	0.66	1.00	0.34	0.76	0.76
0.94	0.62	0.34	0.46	0.66	0.60	1.52	0.10	1.06	0.50
0.10	0.40	0.38	0.30	0.78	0.10	1.62	0.28	0.48	0.60
0.24	0.42	0.10	0.24	0.74	0.44	0.62	0.58	0.38	0.50
0.28	0.54	0.26	2.00	0.78	1.10	0.90	0.78	0.54	0.70
0.34	0.32	0.32	0.10	0.70	0.72	0.56	0.56	0.86	0.80
0.38	0.74	0.76	0.60	0.10	0.10	1.02	1.28	0.34	0.60
0.10	0.94	0.72	0.54	1.42	1.02	0.50	0.40	0.40	0.60
0.10	0.40	0.58	0.44	0.98	0.60	0.40	0.10	0.28	0.50
0.10	0.76	0.30	0.74	0.70	0.56	0.10	0.74	0.28	0.70
0.28	0.52	1.06	0.10	0.90	0.10	0.46	0.42	0.56	0.60
0.60	0.56	0.56	0.46	1.20	0.70	0.24	0.46	0.48	0.80
0.40	0.60	1.30	0.26	0.46	0.36	0.38	0.10	0.10	0.60
0.46	0.82	1.18	0.42	0.46	0.10	0.28	0.68	0.76	0.50
0.40	0.50	0.50	0.40	0.30	0.60	0.40	0.40	0.40	0.50
0.40	0.30	0.30	0.30	0.50	0.40	0.40	0.30	0.44	

* 32 μg/mL

0.94	0.58	0.58	0.10	0.50	0.10	0.52	0.32	0.22	0.44
0.68	0.78	0.10	0.78	0.10	0.44	0.10	0.24	0.74	0.32
0.10	0.58	0.10	0.96	0.54	0.50	0.68	0.10	0.32	0.26
0.44	1.34	0.34	1.00	0.10	0.10	0.64	0.10	0.60	0.42
0.52	1.38	0.10	0.34	0.10	0.34	0.26	0.76	0.10	0.38
0.10	0.10	0.10	0.10	0.56	0.68	0.10	0.10	0.38	0.30
0.50	0.48	0.86	0.10	0.60	0.28	0.78	0.52	0.64	0.32
0.50	0.78	0.10	0.32	0.10	0.30	0.82	0.42	0.10	0.10
0.54	0.92	0.10	0.10	0.44	0.44	0.70	0.42	0.64	0.50
0.28	0.42	0.10	0.32	0.58	0.10	0.10	0.10	0.10	0.40
0.32	0.10	0.10	0.46	0.20	1.04	0.34	0.68	0.76	0.30
0.72	0.56	0.54	0.28	0.24	0.10	0.96	0.10	0.68	0.40
0.68	0.54	0.30	0.44	0.48	0.36	1.38	0.10	0.46	0.30
0.10	0.34	0.36	0.24	0.58	0.10	1.58	0.32	0.40	0.50
0.10	0.34	0.10	0.28	0.70	0.28	0.44	0.40	0.30	0.60
0.28	0.44	0.10	1.75	0.54	0.78	0.86	0.72	0.48	0.40
0.10	0.24	0.30	0.10	0.72	0.68	0.56	0.70	0.70	0.30
0.34	0.66	0.48	0.48	0.10	0.10	0.30	1.06	0.32	0.50
0.10	0.68	0.34	0.52	1.06	0.92	0.44	0.10	0.36	0.60
0.10	0.34	0.56	0.28	0.74	0.58	0.10	0.10	0.10	0.43
0.10	0.60	0.10	0.56	0.38	0.54	0.10	0.54	0.26	0.65
0.22	0.46	0.90	0.10	0.60	0.10	0.40	0.34	0.58	0.40
0.68	0.38	0.56	0.44	1.02	0.10	0.10	0.40	0.48	0.50
0.32	0.52	0.88	0.30	0.10	0.10	0.32	0.10	0.10	0.30
0.10	0.76	1.02	0.32	0.36	0.10	0.10	0.46	0.50	0.40
0.30	0.40	0.30	0.40	0.50	0.30	0.40	0.50	0.30	0.30
0.30	0.30	0.40	0.30	0.30	0.30	0.40	0.20	0.40	

* 64 μg/mL

0.68	0.44	0.46	0.10	0.40	0.10	0.38	0.10	0.20	0.32
0.50	0.66	0.10	0.10	0.10	0.36	0.10	0.20	0.32	0.10
0.10	0.50	0.10	0.68	0.50	0.42	0.32	0.10	0.10	0.46
0.34	0.96	0.10	0.72	0.10	0.10	0.48	0.10	0.46	0.10
0.34	0.80	0.10	0.26	0.10	0.30	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	1.50	0.46	0.10	0.10	0.10	0.76
0.50	0.26	0.56	0.10	0.50	0.24	0.36	0.10	0.76	0.10
0.46	0.28	0.10	0.28	0.10	0.26	0.78	0.40	0.10	0.58
0.24	0.76	0.10	0.10	0.40	0.48	0.48	0.22	0.58	0.10
0.10	0.36	0.10	0.10	0.46	0.10	0.10	0.10	0.10	0.60
0.40	0.10	0.10	0.40	0.10	0.78	0.10	0.56	0.60	0.50
0.42	0.50	0.38	0.30	0.22	0.10	0.72	0.10	0.50	0.44
0.42	0.44	0.32	0.36	0.34	0.28	0.10	0.10	0.44	0.34
0.10	0.30	0.34	0.10	0.46	0.10	0.84	0.22	0.32	0.10
0.10	0.28	0.10	0.10	0.50	0.10	0.10	0.34	0.26	0.32
0.10	0.36	0.10	1.56	0.46	0.62	0.70	0.50	0.42	0.36
0.10	0.22	0.28	0.10	0.20	0.56	0.50	0.54	0.38	0.10
0.30	0.36	0.46	0.60	0.10	0.10	0.10	0.58	0.30	0.10
0.10	0.42	0.30	0.38	0.96	0.76	0.34	0.10	0.32	0.28
0.10	0.30	0.44	0.10	0.54	0.58	0.10	0.10	0.10	0.28
0.10	0.56	0.10	0.30	0.32	0.10	0.10	0.40	0.24	0.10
0.10	0.40	0.24	0.10	0.48	0.10	0.34	0.30	0.44	0.10
0.64	0.10	0.50	0.48	0.90	0.10	0.10	0.32	0.40	0.10
0.10	0.34	0.78	0.24	0.10	0.10	0.28	0.10	0.10	0.10
0.10	0.26	0.66	0.24	0.32	0.10	0.10	0.42	0.20	0.10
0.10	0.10	0.30	0.30	0.40	0.30	0.40	0.30	0.20	0.30
0.40	0.30	0.30	0.20	0.30	0.20	0.30	0.20	0.26	

* 100 μg/mL

0.42	0.30	0.34	0.10	0.10	0.10	0.34	0.10	0.10	0.28
0.28	0.46	0.10	0.10	0.10	0.34	0.10	0.10	0.30	0.10
0.10	0.44	0.10	0.46	0.46	0.32	0.32	0.10	0.10	0.10
0.22	0.30	0.10	0.54	0.10	0.10	0.36	0.10	0.10	0.22
0.20	0.64	0.10	0.22	0.10	0.10	0.10	0.10	0.10	0.40
0.10	0.10	0.10	0.10	0.40	0.34	0.10	0.10	0.10	0.22
0.34	0.22	0.42	0.10	0.28	0.22	0.28	0.10	0.30	0.48
0.34	0.24	0.10	0.22	0.10	0.10	0.28	0.38	0.10	0.10
0.26	0.46	0.10	0.10	0.26	0.10	0.10	0.10	0.56	0.10
0.10	0.24	0.10	0.10	0.32	0.10	0.10	0.10	0.10	0.40
0.10	0.10	0.10	0.30	0.10	0.54	0.10	0.28	0.44	0.40
0.30	0.36	0.10	0.22	0.10	0.10	0.48	0.10	0.46	0.40
0.40	0.42	0.24	0.22	0.26	0.10	0.10	0.10	0.42	0.10
0.10	0.30	0.28	0.10	0.10	0.10	0.80	0.16	0.24	0.10
0.10	0.10	0.10	0.10	0.30	0.10	0.10	0.26	0.10	0.46
0.10	0.32	0.10	0.50	0.28	0.46	0.10	0.46	0.34	0.10
0.10	0.10	0.22	0.10	0.10	0.28	0.10	0.46	0.28	0.10

0.24	0.24	0.40	0.40	0.10	0.10	0.10	0.40	0.28	0.10
0.10	0.40	0.22	0.22	0.46	0.48	0.26	0.10	0.10	0.10
0.10	0.24	0.48	0.10	0.10	0.30	0.10	0.10	0.10	0.10
0.10	0.38	0.10	0.10	0.20	0.10	0.10	0.10	0.10	0.20
0.10	0.10	0.10	0.10	0.26	0.10	0.28	0.20	0.10	0.20
0.38	0.10	0.40	0.38	0.76	0.10	0.10	0.30	0.44	0.20
0.10	0.20	0.40	0.24	0.10	0.10	0.10	0.10	0.10	0.20
0.10	0.10	0.40	0.16	0.24	0.10	0.10	0.24	0.24	0.40
0.40	0.30	0.20	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.19	

Table C-3 Mean vegetative fitness of each of 269 progeny was calculated at different concentrations of Fluconzole in T25/YEPD. Total number of concentrations are 10. For each concentration, 269 values of growth rate correspond with 269 progeny.

Table C-3 summary of vegetative fitness of *C. neoformans* Hybrids at different Fluconazole concentrations in T25/YEPD environment

* 0 μg/mL

0.58	1.00	1.60	2.02	1.84	2.10	4.26	2.82	1.36	0.84
0.80	2.44	0.52	2.34	1.80	3.22	3.04	1.00	1.82	1.40
1.10	2.62	0.82	1.46	1.78	1.00	1.16	1.04	1.68	2.06
1.10	0.98	1.34	1.64	0.96	2.52	2.54	1.36	1.66	3.30
2.44	1.88	0.74	2.14	2.18	1.10	1.30	0.82	1.02	0.74
2.30	1.28	1.92	2.02	1.74	1.62	1.04	1.64	1.34	1.64
1.40	1.46	0.92	2.16	0.76	1.00	1.20	1.34	2.44	1.94
1.10	0.94	1.92	2.98	1.74	0.70	1.34	1.34	3.08	2.52
1.24	1.82	2.06	0.70	1.76	3.92	1.42	1.10	2.06	1.56
1.26	0.96	0.58	2.04	1.68	1.00	0.88	2.42	1.44	0.96
1.62	2.00	2.36	1.32	1.24	1.10	0.98	1.60	1.24	1.32
2.04	1.50	3.02	0.96	3.10	2.00	1.10	0.88	0.10	1.72
0.88	0.58	1.88	1.18	2.16	1.10	2.66	2.54	1.84	1.52
2.44	2.06	2.18	1.02	1.30	1.22	1.02	2.72	2.74	0.58
1.48	2.98	1.58	1.36	2.30	1.00	0.72	1.20	1.18	0.76
1.00	1.18	1.56	1.46	1.24	0.84	2.64	1.80	1.32	1.22
1.92	1.20	0.86	1.42	1.10	1.20	2.74	1.48	1.00	1.54
2.10	1.60	1.20	0.96	0.92	1.00	1.12	1.28	0.76	2.08
2.24	1.92	3.58	1.60	1.72	0.74	2.20	1.34	1.06	1.20
2.34	1.38	1.28	1.06	1.46	0.10	2.44	1.30	1.36	1.32
2.40	0.94	0.64	1.22	0.92	1.26	1.34	0.76	1.64	1.32
2.00	4.08	2.16	0.44	1.72	1.26	1.24	0.10	1.98	0.66
1.28	1.44	1.38	1.32	1.12	1.68	0.40	1.76	2.74	0.68
0.98	0.84	1.18	0.98	2.20	2.40	1.36	1.60	0.44	0.58
1.36	2.00	1.28	1.30	1.00	1.66	1.20	1.20	1.12	1.30
1.42	1.28	2.30	2.26	1.62	1.38	2.68	1.32	1.58	1.84
0.80	0.80	1.34	1.10	2.48	2.46	1.10	1.10	0.84	

* 0.5 μg/mL

0.52	2.86	0.86	0.92	1.20	1.64	1.46	1.22	1.92	1.26
0.66	1.04	2.36	0.98	0.32	1.40	1.68	1.74	1.04	2.14
1.08	2.02	2.40	1.12	0.68	1.56	1.38	2.96	1.56	2.32
1.36	1.48	0.76	0.84	1.12	0.98	1.42	0.48	0.86	1.48
2.28	0.96	1.36	1.38	0.68	1.12	1.90	1.34	1.50	1.18
1.98	1.04	1.10	1.04	1.78	2.28	1.76	2.22	1.56	2.26
1.50	1.26	1.20	1.24	0.92	2.80	1.80	1.86	0.76	1.18
1.84	1.28	0.74	0.98	1.86	1.28	1.58	1.30	1.66	1.30
1.66	0.92	1.64	1.68	1.96	1.18	0.76	0.72	1.66	1.90
1.10	0.92	0.96	1.20	0.52	1.36	2.00	1.16	1.52	0.68
1.40	0.94	1.92	0.90	1.80	0.10	1.22	1.32	1.08	0.74

1.76	2.48	1.52	2.24	1.76	1.68	0.88	1.44	2.90	0.96
0.70	0.76	0.58	2.52	1.42	2.58	0.74	0.50	2.16	1.14
2.38	0.48	2.04	1.04	1.84	1.04	0.98	0.84	1.14	2.38
1.18	2.22	1.58	1.50	1.54	1.16	1.24	1.06	2.28	2.46
0.90	2.68	0.70	1.38	1.00	1.06	1.44	1.30	1.08	1.46
1.52	0.56	1.04	1.32	0.72	0.64	1.24	1.96	1.02	1.46
0.54	2.00	1.54	1.28	1.02	1.12	0.88	1.18	0.78	0.86
1.84	1.94	1.40	1.30	3.28	1.30	1.34	0.96	1.54	1.28
1.94	1.20	1.66	0.56	1.18	1.42	0.90	0.96	1.24	0.74
2.30	1.10	0.74	0.72	0.58	1.94	1.12	0.64	0.86	1.26
1.44	0.36	3.88	1.66	1.98	2.62	0.32	0.32	1.58	1.65
1.06	0.48	1.42	1.26	1.24	0.24	1.16	0.44	0.94	1.68
0.94	0.10	0.78	1.64	1.02	1.58	0.82	1.04	1.86	1.88
3.78	1.06	2.74	2.28	1.24	1.28	0.72	1.12	1.16	1.20
1.52	1.58	1.44	2.14	1.16	1.50	1.74	0.66	3.74	2.32
0.84	2.00	1.32	1.08	1.14	0.76	1.04	1.70	1.22	

* 1 μg/mL

0.54	2.82	0.76	0.80	1.20	1.48	1.16	1.02	1.62	0.86
0.48	0.98	2.28	0.84	0.28	1.20	1.68	1.52	0.98	1.84
1.14	2.02	2.36	0.94	0.66	1.46	1.30	2.70	1.54	2.34
1.30	1.10	0.72	0.68	1.10	0.80	1.98	0.46	0.78	0.64
2.16	0.90	1.50	1.32	0.68	1.02	1.72	1.32	1.02	1.02
1.86	0.90	1.18	0.90	1.88	1.96	1.46	1.70	1.24	2.12
1.32	1.16	1.10	1.14	0.92	2.62	1.59	1.86	0.72	1.04
1.66	1.24	0.88	0.74	1.82	1.20	1.48	1.12	1.50	1.26
1.42	0.82	1.36	1.52	1.66	0.10	0.70	0.56	1.22	1.88
1.00	0.80	0.92	0.88	0.46	1.48	1.96	1.02	1.16	0.60
1.34	0.68	1.98	2.18	1.66	0.10	1.12	1.14	0.92	0.60
1.58	2.20	1.46	2.08	2.06	1.28	0.88	1.44	2.78	0.84
0.48	0.66	0.46	2.24	1.40	2.50	0.52	0.40	2.26	1.06
2.30	0.46	1.66	1.00	1.84	0.88	0.76	0.46	1.00	2.32
1.18	2.20	1.50	1.34	1.44	1.08	1.06	0.82	2.16	2.40
0.72	2.14	0.60	1.46	0.96	1.04	1.92	1.26	1.00	1.50
1.30	0.78	0.98	1.04	0.60	0.52	1.02	1.92	0.80	1.50
0.58	1.66	1.38	1.18	0.90	0.56	0.86	1.16	0.64	0.70
1.68	1.76	1.24	1.18	3.18	1.32	1.36	0.92	1.32	1.08
1.88	1.02	1.18	0.52	1.00	1.32	0.82	0.92	1.06	0.78
2.22	0.86	0.62	0.50	0.50	1.86	1.04	0.66	0.62	0.98
1.20	0.30	3.66	1.62	1.98	2.32	0.28	0.10	1.28	1.32
0.80	0.90	2.56	1.00	1.04	0.10	1.04	0.48	0.86	1.50
0.82	0.10	0.62	1.32	0.82	1.54	0.52	0.94	1.52	1.72
3.64	1.04	2.56	2.18	1.18	1.20	0.64	1.02	1.34	1.12
1.46	2.28	1.16	1.90	0.98	1.38	1.54	0.52	3.42	2.04

0.70	2.00	1.14	0.86	0.98	0.62	0.94	1.30	1.02

* 2 μg/mL

0.58	1.94	0.64	0.66	1.10	1.24	1.12	0.78	1.60	1.08
0.44	0.84	2.18	0.78	0.28	1.02	1.72	1.34	0.88	1.62
1.08	2.00	2.10	0.74	0.62	1.14	1.26	2.36	1.36	2.10
1.18	0.64	0.64	0.56	0.82	0.72	1.46	0.44	0.46	0.10
1.88	0.84	1.56	1.12	0.52	0.82	1.70	1.30	0.94	0.84
1.66	0.88	0.46	0.70	1.62	1.94	1.20	1.42	0.86	1.98
1.00	1.04	1.16	0.92	0.96	2.58	1.26	1.78	0.60	0.98
1.64	1.10	0.80	0.64	1.12	1.20	1.68	0.86	1.26	1.04
1.42	0.74	1.32	1.24	1.70	0.10	0.58	0.46	1.06	1.80
0.76	0.72	0.88	0.82	0.36	1.46	1.86	0.90	1.00	0.50
1.28	0.60	5.68	2.64	1.26	0.10	0.98	1.00	0.78	0.56
1.22	1.86	1.30	1.82	2.56	1.02	0.88	1.38	2.26	0.72
0.38	0.62	0.10	2.04	1.32	2.28	0.40	0.50	1.60	0.66
2.12	0.46	1.32	1.02	1.84	0.78	0.64	0.66	0.90	2.32
0.80	1.58	1.48	0.96	1.20	0.94	0.96	0.78	1.92	2.20
0.64	1.90	0.56	1.14	0.86	0.96	1.66	1.12	0.86	1.46
1.24	0.70	0.92	0.86	0.60	0.60	0.80	1.94	0.66	1.46
0.62	1.14	1.22	0.98	0.82	0.66	0.76	1.12	0.56	0.32
1.50	1.64	0.94	1.54	3.00	1.16	1.16	0.98	1.18	1.02
1.78	0.90	1.32	0.60	0.64	1.22	0.66	0.98	0.96	0.66
2.06	0.78	0.64	0.52	0.44	1.74	0.94	0.72	0.56	1.08
1.14	0.30	3.16	1.50	1.86	1.94	0.30	0.10	1.06	1.14
0.68	0.42	1.58	0.52	0.76	0.10	0.96	0.44	0.68	1.36
0.72	0.10	0.50	1.14	0.74	1.48	0.10	0.82	1.24	1.58
3.12	0.98	2.32	2.18	1.02	1.14	0.10	0.86	1.18	0.96
1.12	2.38	0.86	0.82	0.90	1.26	1.20	0.54	3.16	1.26
0.56	2.00	1.02	0.76	0.86	0.58	0.76	1.16	0.92	

* 4 μg/mL

0.44	1.88	0.52	0.54	1.02	1.04	0.94	0.56	1.58	0.50
0.32	0.74	1.82	0.72	0.10	0.66	1.56	1.10	0.72	1.56
0.82	1.58	2.06	0.56	0.48	1.02	0.64	2.22	1.28	1.52
1.16	0.44	0.54	0.58	0.74	0.64	1.52	0.34	0.30	0.10
1.18	0.80	1.62	0.92	0.10	0.72	1.56	1.30	0.80	0.74
1.38	0.58	0.32	0.64	1.64	1.14	0.84	1.04	0.52	1.36
1.00	0.92	1.00	0.74	0.88	2.54	1.16	1.80	0.50	0.82
1.44	0.94	0.74	0.54	1.26	0.36	1.12	0.74	1.02	1.00
0.86	0.76	1.24	1.08	1.62	0.10	0.56	0.44	1.02	1.86
0.10	0.64	0.84	0.10	0.32	1.26	1.56	0.78	0.80	0.38
1.24	0.46	1.60	6.40	1.16	0.10	0.90	0.94	0.72	0.44
0.94	1.64	1.22	1.66	1.92	0.78	0.84	0.72	2.04	0.74
0.10	0.56	0.10	1.90	0.66	2.14	0.32	0.28	1.52	0.62
1.68	0.10	1.16	0.72	1.78	0.72	0.58	0.80	0.86	1.84
0.68	1.90	1.46	0.72	1.10	0.82	0.88	0.68	1.90	2.12

0.54	1.66	0.34	0.86	0.10	0.72	1.52	1.04	0.72	1.46
0.70	0.54	0.90	0.78	0.68	0.24	0.72	1.76	0.64	1.46
0.44	0.90	1.12	0.84	0.74	0.94	0.64	1.06	0.54	0.10
1.50	1.34	0.82	0.70	2.58	1.04	1.12	0.56	1.00	0.90
0.94	0.84	1.26	0.24	0.52	1.10	0.64	0.56	0.70	0.62
1.80	0.64	0.56	0.78	0.40	1.64	0.78	0.62	0.58	1.18
1.08	0.10	2.80	1.32	1.78	0.58	0.40	0.10	0.96	0.92
0.60	0.20	1.58	0.50	0.60	0.10	0.66	0.40	0.54	1.02
0.72	0.10	0.44	0.88	0.74	1.42	0.10	0.72	1.10	1.44
2.98	0.90	1.56	2.18	0.60	1.04	0.10	0.78	0.10	0.86
0.86	2.28	0.54	0.68	0.78	1.22	0.50	0.48	2.92	1.24
0.50	1.82	0.88	0.68	0.70	0.52	0.62	1.02	0.80	

* 8 μg/mL

0.24	1.22	0.48	0.52	0.92	0.74	0.10	0.10	1.50	1.00
0.10	0.58	1.22	0.64	0.10	0.54	0.76	0.78	0.56	1.04
0.68	1.50	1.88	0.40	0.52	0.82	0.48	1.30	1.20	0.96
0.90	0.10	0.22	0.50	0.44	0.56	1.42	0.74	0.10	0.10
0.10	0.62	1.12	0.10	0.10	0.52	0.96	1.32	0.64	0.48
1.24	0.44	0.10	0.62	1.52	0.10	0.30	0.76	0.50	0.84
0.82	0.86	0.98	0.66	0.74	1.14	0.92	1.48	0.46	0.68
0.74	0.60	0.60	0.46	1.30	1.00	1.02	1.04	0.62	0.50
0.66	0.72	0.84	0.68	1.46	0.10	0.64	0.38	0.96	1.32
0.10	0.66	0.74	0.10	0.26	0.10	1.08	0.62	0.56	0.10
1.16	0.10	1.54	1.48	0.92	0.10	0.82	0.52	0.54	0.10
0.54	1.14	1.16	1.70	1.50	0.58	0.84	0.52	1.86	0.70
0.10	0.48	0.10	1.14	0.58	1.94	0.28	0.24	1.38	0.44
1.38	0.10	1.02	1.44	1.70	0.48	0.48	1.04	0.56	1.18
0.58	1.74	1.52	0.52	0.80	0.48	0.72	0.10	1.68	1.88
0.52	1.32	0.10	0.64	0.10	0.52	1.24	1.00	0.56	1.34
0.64	0.10	0.86	0.64	0.48	0.10	0.10	1.38	0.64	1.34
0.94	0.64	0.88	0.36	0.62	1.26	0.56	0.96	0.40	0.10
0.76	0.70	0.80	0.62	2.44	0.96	1.00	0.52	0.54	0.86
0.10	0.66	0.76	0.10	0.44	1.00	0.48	0.52	0.54	0.10
1.60	0.48	0.46	0.72	0.10	1.18	0.48	0.60	0.52	0.96
0.84	0.10	2.60	1.18	0.62	0.10	0.24	0.10	0.86	0.66
0.66	0.10	1.46	0.24	0.46	0.10	0.46	0.10	0.44	0.94
0.62	0.10	0.34	0.10	0.48	1.42	0.10	0.56	0.72	0.88
2.28	0.88	1.54	2.04	0.56	0.90	0.10	0.78	0.10	0.86
0.54	1.22	0.10	0.54	0.64	0.50	0.30	0.46	2.38	1.22
0.10	1.16	0.72	0.58	0.10	0.44	0.46	1.00	0.76	

* 16 μg/mL

0.24	0.74	0.46	0.44	0.84	0.66	0.10	0.10	0.44	0.82
0.10	0.52	0.76	0.52	0.10	0.48	0.46	0.52	0.54	0.76
0.54	1.28	1.52	0.10	0.44	0.58	0.10	1.18	0.74	0.60

0.78	0.10	0.10	0.40	0.42	0.50	0.98	0.68	0.10	0.10
0.10	0.48	0.10	0.10	0.10	0.44	0.72	1.00	0.54	0.42
1.16	0.32	0.10	0.54	1.16	0.10	0.10	0.54	0.10	0.58
0.60	0.76	0.64	0.56	0.68	1.14	0.56	1.56	0.40	0.52
0.44	0.52	0.40	0.36	0.64	0.66	0.82	0.94	0.60	0.40
0.10	0.62	0.62	0.50	1.00	0.10	0.42	0.10	0.74	0.76
0.10	0.56	0.64	0.10	0.28	0.10	0.78	0.56	0.48	0.10
0.80	0.10	1.44	0.10	0.56	0.10	0.78	0.40	0.10	0.10
0.38	0.92	0.84	1.24	0.94	0.46	0.46	0.46	1.66	0.64
0.10	0.10	0.10	1.28	0.50	1.08	0.10	0.10	0.80	0.10
1.48	0.10	0.76	0.68	1.64	0.44	0.42	0.10	0.52	0.84
0.52	1.30	0.10	0.46	0.74	0.44	0.62	0.10	0.90	1.40
0.54	1.28	0.10	0.10	0.10	0.46	0.92	0.84	0.44	1.06
0.58	0.10	0.84	0.46	0.10	0.10	0.10	1.06	0.54	1.06
0.62	0.54	0.68	0.26	0.52	0.68	0.10	0.96	0.10	0.10
0.80	0.66	0.56	0.74	2.08	0.94	0.80	0.10	0.48	0.84
0.10	0.60	0.36	0.10	0.28	0.68	0.42	0.10	0.48	0.10
1.24	0.46	0.42	0.10	0.10	1.06	0.30	0.52	0.42	0.96
0.40	0.10	1.98	0.86	0.46	0.10	0.20	0.10	0.52	0.52
0.56	0.10	0.84	0.10	0.36	0.10	0.42	0.10	0.42	0.48
0.54	0.10	0.30	0.10	0.40	1.34	0.10	0.36	0.56	0.88
2.04	0.78	0.76	2.00	0.52	0.74	0.10	0.58	0.10	0.78
0.50	1.00	0.10	0.36	0.56	0.46	0.20	0.44	2.02	1.08
0.10	0.96	0.72	0.46	0.10	0.36	0.10	0.76	0.62	

* 32 μg/mL

0.26	0.54	0.44	0.40	0.46	0.54	0.10	0.10	0.36	0.42
0.10	0.50	0.60	0.36	0.10	0.42	0.32	0.36	0.10	0.48
0.10	0.54	0.88	0.10	0.40	0.52	0.10	0.76	0.44	0.10
0.64	0.10	0.10	0.32	0.10	0.42	0.76	0.48	0.10	0.10
0.10	0.40	0.10	0.10	0.10	0.42	0.10	0.94	0.42	0.36
0.92	0.28	0.10	0.46	1.04	0.10	0.10	0.10	0.10	0.10
0.46	0.68	0.10	0.48	0.40	1.06	0.10	0.70	0.10	0.50
0.10	0.46	0.38	0.36	0.56	0.44	0.40	0.50	0.10	0.32
0.10	0.52	0.42	0.48	0.58	0.10	0.34	0.10	0.36	0.42
0.10	0.44	0.46	0.10	0.24	0.10	0.10	0.10	0.34	0.10
0.68	0.10	0.36	0.10	0.10	0.10	0.62	0.36	0.10	0.10
0.34	1.02	0.80	0.76	0.46	0.42	0.46	0.44	1.46	0.50
0.10	0.10	0.10	0.48	0.50	0.98	0.10	0.10	0.64	0.10
0.30	0.10	0.54	0.40	0.80	0.40	0.10	0.10	0.10	0.66
0.10	0.76	0.10	0.38	0.58	0.42	0.52	0.10	0.48	1.02
0.48	1.04	0.10	0.10	0.10	0.44	0.80	0.60	0.34	0.78
0.46	0.10	0.74	0.36	0.10	0.10	0.10	0.74	0.44	0.78
0.10	0.44	0.54	0.22	0.44	0.24	0.10	0.80	0.10	0.10
0.36	0.64	0.54	0.54	1.26	0.80	0.68	0.10	0.36	0.64
0.10	0.10	0.35	0.10	0.10	0.54	0.10	0.10	0.42	0.10
0.62	0.40	0.10	0.10	0.10	0.86	0.10	0.38	0.10	0.88

0.30	0.10	1.48	0.64	0.30	0.10	0.20	0.10	0.46	0.10
0.34	0.10	0.46	0.10	0.10	0.10	0.36	0.10	0.38	0.38
0.10	0.10	0.82	0.10	0.26	1.22	0.10	0.28	0.48	0.46
0.10	0.64	0.56	1.22	0.10	0.54	0.10	0.34	0.10	0.66
0.46	0.44	0.10	0.10	0.50	0.34	0.10	0.36	0.90	0.50
0.10	0.48	0.62	0.10	0.10	0.30	0.10	0.60	0.52	

* 64 μg/mL

0.10	0.30	0.26	0.36	0.20	0.40	0.10	0.10	0.26	0.10	0.26
0.10	0.40	0.52	0.10	0.10	0.10	0.10	0.32	0.10	0.10	0.42
0.10	0.40	0.66	0.10	0.40	0.32	0.10	0.54	0.34	0.10	0.10
0.44	0.10	0.10	0.34	0.10	0.32	0.56	0.46	0.10	0.10	0.10
0.10	0.36	0.10	0.10	0.10	0.36	0.10	0.62	0.10	0.32	0.44
0.74	0.10	0.10	0.40	0.54	0.10	0.10	0.10	0.10	0.10	0.10
0.36	0.54	0.10	0.10	0.10	0.76	0.10	0.50	0.10	0.10	0.10
0.10	0.24	0.34	0.32	0.32	0.38	0.20	0.38	0.10	0.10	0.28
0.10	0.48	0.10	0.36	0.10	0.10	0.36	0.10	0.10	0.28	0.10
0.10	0.10	0.34	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.44
0.60	0.10	0.30	0.10	0.10	0.10	0.46	0.10	0.10	0.10	0.10
0.10	0.58	0.44	0.62	0.20	0.34	0.50	0.36	1.22	0.26	0.26
0.10	0.10	0.10	0.34	0.10	0.44	0.10	0.10	0.48	0.10	0.52
0.10	0.10	0.10	0.30	0.52	0.32	0.10	0.10	0.10	0.50	0.10
0.10	0.62	0.10	0.10	0.38	0.10	0.44	0.10	0.10	0.64	0.10
0.34	0.46	0.10	0.10	0.10	0.36	0.46	0.50	0.32	0.50	0.28
0.10	0.10	0.30	0.32	0.10	0.10	0.10	0.54	0.10	0.50	0.10
0.10	0.10	0.36	0.10	0.10	0.26	0.10	0.10	0.10	0.10	0.50
0.46	0.10	0.42	0.46	1.00	0.24	0.54	0.10	0.32	0.52	0.44
0.10	0.10	0.10	0.10	0.10	0.28	0.10	0.10	0.34	0.10	
0.34	0.32	0.10	0.10	0.10	0.80	0.10	0.38	0.10	0.10	
0.10	0.10	1.20	0.10	0.10	0.10	0.10	0.10	0.44	0.10	
0.10	0.10	0.44	0.10	0.10	0.10	0.22	0.10	0.30	0.10	
0.10	0.10	0.26	0.10	0.10	1.32	0.10	0.10	0.34	0.38	
0.10	0.22	0.46	0.10	0.10	0.32	0.10	0.28	0.10	0.36	
0.26	0.42	0.10	0.10	0.44	0.10	0.10	0.28	0.10	0.44	
0.10	0.26	0.52	0.10	0.10	0.28	0.10	0.50	0.44		

* 100 μg/mL

0.10	0.10	0.20	0.26	0.10	0.34	0.10	0.10	0.10	0.10
0.10	0.10	0.22	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.36	0.30	0.10	0.26	0.28	0.10	0.30	0.28	0.10
0.10	0.10	0.10	0.24	0.10	0.26	0.32	0.10	0.10	0.10
0.10	0.24	0.10	0.10	0.10	0.32	0.10	0.10	0.10	0.24
0.42	0.10	0.10	0.32	0.40	0.10	0.10	0.10	0.10	0.10
0.24	0.50	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.30	0.10	0.10	0.10	0.30	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.30	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.26	0.10	0.28	0.10	0.10	0.10	0.10	0.10	0.10	0.10

0.10	0.52	0.38	0.10	0.10	0.24	0.10	0.10	0.10	0.22
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.38	0.10
0.10	0.10	0.10	0.10	0.28	0.10	0.10	0.10	0.10	0.22
0.10	0.10	0.10	0.10	0.10	0.10	0.30	0.10	0.10	0.30
0.10	0.10	0.10	0.10	0.10	0.10	0.30	0.30	0.28	0.20
0.10	0.10	0.26	0.26	0.10	0.10	0.10	0.40	0.10	0.20
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.26	0.10	0.30	0.10	0.10	0.22	0.36	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.22	0.10	0.10	0.22	0.10
0.10	0.10	0.10	0.10	0.10	0.76	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.30	0.10
0.10	0.10	0.34	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.22	0.10	0.10	0.86	0.10	0.10	0.34	0.36
0.10	0.10	0.36	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.34	0.10	0.10	0.10	0.10	0.10
0.10	0.20	0.42	0.10	0.10	0.22	0.10	0.42	0.10	

Table C-4 Mean vegetative fitness of each of 269 progeny was calculated at different concentrations of Fluconzole in T37/YEPD. Total number of concentrations are 10. For each concentration, 269 values of growth rate correspond with 269 progeny.

Table C-4 summary of vegetative fitness of *C. neoformans* hybrids at different Fluconazole concentrations in T37/YEPD environment

* 0 μg/mL

0.60	3.26	2.68	3.26	1.84	2.24	2.78	3.12	0.92	3.12
1.86	3.14	3.90	3.72	3.44	1.02	0.88	1.68	3.52	2.82
2.76	2.32	2.38	2.08	2.62	2.16	2.58	1.84	2.88	2.92
3.66	2.54	2.92	2.64	1.60	0.98	3.42	1.54	2.46	2.14
3.62	3.22	3.88	1.28	1.16	3.06	3.42	3.02	2.28	3.12
3.52	2.26	3.30	2.34	2.68	1.94	1.92	0.84	1.58	3.12
2.94	2.86	2.32	3.02	0.82	3.12	2.74	1.22	3.26	2.90
1.82	1.52	0.72	2.84	1.38	1.94	3.24	1.60	2.72	1.54
3.36	2.10	2.78	1.96	3.68	1.94	1.30	3.14	1.40	2.92
3.52	3.24	2.58	2.60	2.44	1.94	3.00	2.82	3.44	2.10
0.98	2.84	3.14	2.66	3.10	1.94	3.02	2.30	1.08	2.50
3.26	2.22	2.72	0.86	1.24	1.94	3.40	2.86	0.68	2.60
1.28	2.68	2.78	2.10	2.96	1.94	2.10	2.46	2.42	2.60
3.88	2.70	1.80	3.18	0.80	1.94	4.80	0.94	2.42	2.50
2.56	4.22	2.02	3.18	3.52	1.94	2.32	1.44	1.34	2.70
1.24	2.22	3.40	3.16	0.78	1.94	5.16	2.72	1.30	2.70
1.98	2.24	2.50	2.16	5.28	1.94	2.96	1.66	2.96	2.92
1.92	2.80	3.68	3.16	2.84	2.92	3.10	2.54	3.16	3.54
3.78	2.66	0.68	2.50	5.26	2.86	2.16	3.12	2.18	2.92
0.56	1.68	2.34	1.00	2.24	2.64	2.70	2.06	2.82	2.96
2.16	3.22	3.50	2.24	3.20	2.70	2.68	0.60	1.50	3.24
1.52	2.68	4.20	2.82	0.92	0.10	2.36	0.70	2.18	2.24
1.08	0.88	1.46	3.54	2.78	0.10	2.80	1.72	2.78	2.70
3.68	0.94	1.84	1.44	3.34	3.04	0.82	0.84	3.58	2.92
3.10	1.22	2.74	3.46	2.28	2.00	2.42	2.18	1.16	3.74
2.66	2.60	2.82	2.56	2.38	2.90	2.28	2.80	2.18	2.13
2.08	2.03	2.50	2.75	2.34	2.78	2.72	2.72	2.08	

* 0.5 μg/mL

1.44	3.04	2.36	2.72	0.78	2.08	2.70	2.72	0.90	3.00
1.60	2.90	3.68	3.10	3.66	1.00	1.12	1.64	3.54	2.14
2.38	2.24	1.56	2.00	3.02	1.72	2.58	1.80	2.70	2.74
3.46	1.66	2.34	2.26	1.26	0.78	3.28	1.50	2.44	1.94
3.48	3.10	3.70	1.22	0.82	2.88	3.38	2.74	2.12	2.38
3.60	2.06	3.60	1.62	2.34	1.78	1.40	0.76	1.46	2.98
1.98	2.54	1.66	2.54	0.80	3.02	2.56	2.74	2.38	2.70
1.32	1.44	0.58	2.90	0.96	2.64	3.02	2.74	2.04	1.42
3.36	1.72	2.52	1.62	3.34	3.00	1.04	2.74	1.06	2.48
3.40	2.44	2.72	2.24	2.36	1.18	2.88	2.74	3.20	2.48
0.60	2.80	3.00	2.56	2.86	2.20	2.94	2.74	0.92	2.48
3.20	2.00	2.30	0.76	1.10	2.66	3.30	2.74	0.42	2.22
0.80	2.66	2.66	2.00	2.92	3.68	1.96	2.74	2.42	2.22
3.64	2.44	1.72	2.76	0.72	2.44	4.38	2.74	2.24	2.50
2.40	3.70	1.76	3.04	3.40	2.38	2.64	2.74	1.32	2.50
1.06	2.06	3.36	2.94	0.72	2.58	4.62	2.74	1.30	2.50

1.94	1.96	2.46	1.92	4.94	1.20	2.18	1.34	2.56	2.40
1.64	2.80	3.70	2.90	2.96	2.50	3.00	2.38	2.40	2.34
3.40	2.60	0.10	2.34	5.08	2.62	2.06	3.22	1.80	1.62
0.42	1.52	2.32	3.10	2.72	2.62	1.74	1.98	2.64	2.32
1.70	2.90	3.70	1.98	3.16	2.04	2.52	0.36	1.52	1.32
1.62	2.46	3.92	2.46	0.94	0.92	2.10	0.88	1.82	1.64
0.94	1.86	0.56	3.34	1.88	2.66	2.62	1.58	2.62	2.40
3.50	0.90	1.82	1.10	2.90	3.18	0.78	1.18	2.98	2.82
2.66	1.12	2.64	3.30	2.18	2.00	1.80	1.90	0.92	1.50
1.64	1.57	1.51	1.44	1.38	1.31	1.25	1.18	1.12	1.05
1.90	2.60	1.86	1.70	0.73	1.66	1.59	1.53	2.20	

* 1 μg/mL

1.96	2.44	2.26	2.04	1.06	1.84	2.60	2.60	2.20	2.66
1.54	2.58	3.60	2.50	3.52	0.90	1.32	1.44	2.20	1.96
2.00	2.10	1.30	1.82	2.38	1.28	1.96	1.66	2.30	2.30
3.32	1.00	0.70	2.04	1.28	0.74	3.14	0.80	2.50	1.66
3.42	2.70	3.38	1.12	1.44	2.80	3.32	2.24	1.70	1.72
3.24	2.00	3.06	1.32	2.50	1.38	1.16	0.72	1.38	2.72
1.90	2.28	1.50	2.26	0.56	2.58	6.20	0.92	2.32	1.72
1.00	1.40	0.72	2.84	0.88	2.14	2.62	1.10	2.74	1.28
3.32	1.10	2.36	1.46	3.12	2.74	1.02	2.70	0.80	1.72
2.60	2.24	2.30	2.04	2.30	1.04	2.10	2.16	3.12	1.00
0.60	2.78	2.84	2.20	2.74	2.02	2.90	1.84	0.68	1.00
3.06	1.74	2.16	0.76	1.02	2.56	3.16	2.08	0.34	1.50
0.78	2.62	2.42	1.76	2.78	2.90	1.96	1.82	1.58	0.90
3.16	2.20	1.70	2.54	0.68	2.12	4.14	3.18	1.30	1.50
2.26	3.28	1.78	2.62	3.22	2.20	2.64	1.08	1.24	0.90
0.80	1.84	2.96	2.30	0.72	2.34	4.46	2.20	1.28	1.72
1.72	1.88	2.38	1.60	3.38	1.08	2.44	2.20	2.40	2.72
1.40	2.42	3.32	2.48	3.10	2.46	2.98	1.00	2.34	1.72
3.26	1.74	0.10	1.74	4.12	2.46	1.94	2.40	1.62	1.28
0.50	1.16	1.52	3.44	0.10	1.94	1.32	2.20	2.32	1.72
1.42	2.74	3.24	1.82	3.16	1.98	2.58	2.10	1.32	1.00
1.24	2.26	3.86	2.28	0.84	0.70	1.90	2.20	1.64	1.00
0.10	2.14	0.82	3.02	1.30	0.88	2.36	1.32	2.40	1.50
1.84	0.78	1.58	0.86	2.32	2.92	0.74	2.20	2.82	0.90
2.48	0.86	2.56	3.18	1.94	2.00	1.48	2.30	0.84	1.50
1.28	2.34	2.40	1.02	0.10	0.76	1.30	1.20	1.74	0.78
3.04	2.34	2.26	1.98	2.00	2.00	2.00	1.00	2.40	

* 2 μg/mL

2.60	1.60	2.10	2.14	0.86	1.62	2.18	2.32	0.10	2.40
1.20	1.60	3.18	2.22	1.95	0.86	1.52	0.90	3.42	1.64
2.08	1.92	2.34	1.62	2.12	1.14	1.94	1.44	1.66	2.16

3.22	0.90	0.62	1.60	1.16	0.64	3.12	0.52	1.64	1.56
2.98	2.74	2.66	1.06	1.60	2.58	2.70	1.48	1.92	1.26
3.16	1.88	2.38	1.22	2.00	1.18	0.74	0.66	1.08	2.16
1.58	1.92	1.36	1.92	0.52	2.40	2.12	0.74	2.02	1.42
0.50	1.20	0.72	2.52	0.10	1.72	1.96	0.90	2.56	1.14
3.28	0.98	2.34	1.36	2.70	2.34	0.76	2.24	0.62	1.42
1.82	2.04	1.98	2.00	1.92	0.82	2.14	1.54	2.44	1.42
0.64	2.48	2.72	1.50	2.40	1.80	2.80	1.60	0.44	1.42
2.78	1.38	1.04	0.68	0.82	2.18	2.98	1.58	0.10	1.34
0.10	1.92	1.94	1.56	1.72	2.06	0.88	1.56	1.38	1.34
2.72	1.92	1.50	2.06	0.82	2.04	3.46	1.04	1.10	2.50
1.78	3.04	1.44	2.52	3.04	1.10	2.56	0.78	0.94	2.50
0.54	1.62	2.72	1.80	0.62	2.08	4.36	1.72	1.12	2.50
1.38	1.64	2.46	1.48	2.92	0.10	2.06	1.18	2.22	2.50
1.24	2.10	2.92	2.14	1.52	2.16	2.52	2.02	2.16	2.50
2.82	1.44	0.10	1.58	4.76	2.36	1.80	1.90	1.44	2.50
0.42	0.92	0.86	2.10	0.10	0.84	0.84	0.50	2.06	1.20
1.26	2.24	1.52	1.92	2.62	1.62	2.60	0.10	1.16	1.20
0.92	1.86	3.78	1.26	0.80	0.66	1.42	0.66	1.60	1.00
0.10	0.56	0.10	2.84	0.80	0.84	2.14	1.04	2.10	1.00
1.28	0.70	1.74	0.58	0.50	2.44	0.70	1.22	2.64	1.00
2.02	0.72	2.20	3.00	1.82	1.00	1.32	1.04	0.76	1.00
1.50	1.70	1.90	1.90	2.00	2.50	1.90	1.50	1.40	2.30
1.60	1.70	2.20	1.30	1.50	1.70	1.30	1.20	1.00	

* 4 μg/mL

0.46	1.58	2.00	2.06	0.84	1.44	1.94	1.98	0.10	2.28
0.92	0.10	3.14	1.98	1.20	0.74	0.72	0.78	2.58	1.40
2.02	1.70	2.32	1.60	0.78	0.98	0.10	1.22	0.10	1.76
2.94	0.84	0.98	1.36	1.06	0.54	2.70	0.42	1.42	1.24
2.90	1.80	2.54	0.98	0.52	2.42	2.64	1.26	1.72	1.16
3.02	1.64	0.42	0.98	1.78	0.98	0.10	0.54	0.86	1.74
1.12	1.66	1.06	1.72	0.10	2.34	0.78	0.64	1.96	1.24
0.10	1.10	0.36	2.50	0.10	1.22	1.60	0.68	2.82	0.88
0.10	0.92	1.28	1.08	2.44	2.18	0.74	1.90	0.10	1.22
1.38	1.76	1.98	1.90	1.58	0.72	2.00	1.38	2.32	1.76
0.10	2.50	2.46	0.10	1.96	1.54	2.88	1.44	0.10	1.24
2.68	1.12	0.10	0.62	0.10	1.80	2.74	1.38	0.10	1.16
0.10	1.90	1.84	1.24	1.22	1.52	0.80	1.44	1.26	1.74
2.22	1.74	1.48	1.50	0.84	1.74	3.18	1.52	0.70	1.24
0.10	2.56	1.14	1.58	2.68	1.00	2.16	0.10	0.92	1.24
0.10	1.48	2.68	1.22	0.54	1.36	4.10	1.20	0.98	1.24
1.02	1.30	2.24	1.30	2.64	0.10	1.66	1.10	2.10	1.24
1.20	1.64	2.62	1.62	0.10	1.70	2.46	2.00	1.82	1.16
2.74	1.30	0.10	1.30	4.30	1.68	1.50	0.10	0.10	1.74
0.40	0.60	0.76	0.40	0.10	0.74	0.64	0.44	1.70	1.24
0.10	1.72	0.10	0.72	2.42	1.50	1.62	0.10	1.04	2.84
0.82	1.60	3.46	0.76	0.74	0.60	1.18	0.80	1.26	0.84

0.10	0.96	0.10	2.64	0.60	0.78	1.96	0.74	1.64	1.20
0.56	0.70	1.68	0.56	0.10	1.88	0.56	0.72	2.42	0.78
1.42	0.66	1.88	2.84	1.76	1.00	1.12	0.10	0.60	1.06
0.52	1.78	2.00	2.10	2.10	2.70	2.50	2.50	1.40	1.60
1.30	1.60	1.70	1.50	1.60	1.70	1.40	1.40	1.40	

* 8 μg/mL

1.20	1.52	2.00	1.98	0.74	1.00	1.60	1.80	0.10	2.20
0.86	0.10	3.12	1.36	0.88	0.72	1.10	0.60	2.10	1.06
1.88	1.16	2.50	1.46	1.26	0.66	0.10	1.08	0.10	1.58
2.80	0.72	0.70	0.10	0.80	0.10	1.92	0.10	1.26	1.06
2.88	1.66	2.42	0.88	0.62	1.74	2.38	0.96	1.34	0.92
2.10	1.32	0.28	0.66	2.30	0.74	0.10	0.52	0.82	1.44
2.10	1.34	0.70	1.50	0.10	2.22	0.66	0.62	1.46	0.86
0.10	0.96	0.28	2.30	0.10	0.96	1.38	0.10	2.40	0.50
0.10	0.78	0.10	0.98	2.30	1.68	0.52	1.52	0.10	0.84
0.88	1.32	1.74	1.84	1.42	0.56	1.86	1.00	2.24	1.02
0.10	2.40	2.04	0.10	1.74	1.02	2.78	1.04	0.10	1.06
2.06	0.80	0.10	0.52	0.10	1.60	2.52	1.02	0.10	2.02
0.10	1.88	1.70	0.80	0.78	1.20	0.10	0.98	1.08	0.52
2.02	0.82	1.08	1.06	0.40	1.34	2.98	1.88	0.10	2.20
0.10	1.68	0.84	1.16	1.72	2.66	1.74	0.10	0.80	1.06
0.10	1.04	2.66	1.04	0.44	0.10	3.96	0.88	0.10	1.58
0.10	0.84	2.10	0.96	2.52	0.10	1.52	0.92	1.60	1.06
0.70	1.38	2.26	1.16	0.10	1.46	2.22	1.94	1.84	0.92
2.80	1.12	0.10	1.04	4.02	1.00	1.46	0.10	0.10	1.02
0.10	0.32	0.46	0.10	0.10	0.10	0.44	0.10	0.10	1.06
0.10	1.40	0.10	0.78	2.24	1.42	1.86	0.10	0.94	2.02
0.74	1.16	2.30	0.36	0.72	0.76	1.04	0.54	1.02	0.52
0.10	0.10	0.10	2.64	0.50	0.76	1.74	0.58	1.06	2.20
0.10	0.66	1.60	0.50	0.10	1.14	0.54	1.08	2.02	1.06
1.20	0.60	1.58	1.26	1.62	1.00	0.10	0.10	0.52	1.58
1.06	0.92	1.06	2.02	0.52	2.20	1.06	1.58	1.06	0.92
1.06	2.02	0.52	2.20	1.06	1.58	1.06	0.92	0.60	

* 16 μg/mL

0.50	1.24	2.10	1.38	0.10	0.58	0.90	0.56	1.28	0.82
0.82	0.10	2.76	1.08	0.84	0.10	0.10	0.96	0.10	1.10
1.82	0.10	0.10	0.10	0.64	0.10	1.30	0.10	1.12	0.74
2.74	0.60	0.66	0.84	0.86	0.88	1.78	0.60	0.66	0.84
2.54	1.44	1.82	0.50	1.48	0.52	0.10	0.42	0.34	1.24
1.10	1.20	0.10	1.06	0.10	1.36	0.10	0.50	0.78	0.64
1.10	1.18	0.66	1.78	0.10	0.66	1.00	0.10	0.80	0.44
0.10	0.90	0.32	0.84	2.20	1.08	0.40	1.44	0.10	0.62
0.10	0.56	0.10	1.64	1.18	0.46	1.34	0.84	2.10	2.38
0.68	1.12	1.46	0.10	1.42	0.74	2.76	0.64	0.10	0.10
0.10	0.96	1.98	0.44	0.10	1.46	1.72	0.88	0.10	2.44

1.62	0.10	0.10	0.56	0.62	1.08	0.10	0.58	0.88	0.10
0.10	0.94	1.52	0.72	0.42	1.10	2.86	1.24	0.10	1.24
1.74	0.66	1.04	1.14	1.08	0.46	1.22	0.10	0.60	0.66
0.10	1.24	0.64	0.88	0.10	0.10	3.26	0.64	0.10	0.10
0.10	0.74	1.64	0.78	2.38	0.10	1.02	0.44	1.52	0.10
0.10	0.60	2.04	1.00	0.10	1.34	2.06	1.86	1.68	1.32
0.36	1.24	2.14	0.84	2.44	0.80	1.18	0.10	0.10	0.72
1.10	0.94	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.58
0.10	0.10	0.10	0.78	1.24	1.22	1.44	0.10	0.84	1.10
0.10	1.00	0.10	0.10	0.66	0.72	0.96	0.54	0.68	0.74
0.68	1.02	1.92	1.58	0.10	0.66	1.26	0.10	0.96	0.84
0.10	0.10	0.10	0.10	0.10	0.96	0.48	1.08	1.88	1.24
0.10	0.64	1.30	0.62	1.32	1.00	0.10	0.10	0.44	0.64
0.86	0.48	1.02	0.70	0.72	1.44	1.00	0.10	0.80	0.44
0.62	2.38	1.30	1.30	1.70	1.90	1.70	2.10	2.10	1.10
1.10	1.10	1.10	1.50	0.40	0.60	0.40	0.50	1.76	

* 32 μg/mL

0.10	0.68	1.22	1.00	0.62	0.10	1.22	0.74	0.10	0.10
0.76	0.10	2.42	0.46	0.10	0.48	0.58	0.10	1.28	0.68
1.20	0.10	0.10	0.74	0.70	0.10	0.10	0.78	0.10	0.98
2.36	0.30	0.62	0.10	0.38	0.10	0.36	0.10	1.06	0.10
2.12	0.80	1.78	0.54	0.40	0.10	0.10	0.46	0.10	0.64
0.68	0.10	0.10	0.10	1.32	0.44	0.10	0.38	0.24	0.98
0.68	0.66	0.48	0.84	0.10	0.10	0.10	0.10	0.10	0.54
0.10	0.10	0.34	1.52	0.10	0.48	0.10	0.10	0.56	0.34
0.10	0.50	0.10	0.72	2.02	0.78	0.32	1.14	0.10	0.62
0.10	1.00	0.44	1.32	0.96	0.42	1.32	0.60	0.64	0.38
0.10	0.40	1.84	0.10	0.10	0.52	1.52	0.38	0.64	0.10
1.34	0.10	0.10	0.10	0.10	1.14	1.26	0.62	0.64	0.68
0.10	0.70	1.14	0.44	0.10	0.86	0.10	0.38	0.64	0.98
0.74	0.46	0.10	0.54	0.40	0.64	1.22	0.46	0.64	0.38
0.10	0.10	0.10	0.10	0.10	0.56	0.40	0.10	0.10	0.10
0.10	0.48	0.48	0.74	0.10	0.10	2.64	0.46	0.10	0.68
0.10	0.10	1.56	0.66	1.22	0.10	0.48	0.10	0.42	0.98
0.34	1.08	1.40	0.58	0.10	0.92	1.30	0.68	1.10	0.42
0.66	0.80	0.10	0.72	1.58	0.52	0.98	0.10	0.10	1.10
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.80
0.10	0.58	0.10	0.10	0.94	1.00	1.08	0.10	0.60	0.90
0.34	0.82	1.24	0.10	0.10	0.50	0.76	0.58	0.10	0.60
0.10	0.10	0.10	0.70	0.10	0.70	0.10	0.10	0.78	0.60
0.10	0.54	0.96	0.10	0.10	0.64	0.46	0.28	0.10	1.10
0.76	0.38	0.82	0.80	1.02	1.00	0.10	0.10	0.38	0.48
0.48	0.48	0.50	0.44	0.78	0.48	0.72	0.42	0.52	1.14
1.40	1.40	1.20	2.36	2.12	2.20	0.68	0.50	0.70	

* 64 µg/mL

0.10	0.10	1.00	0.10	0.58	0.30	0.98	0.56	0.10	0.10
0.10	0.10	2.30	0.30	0.10	0.30	0.10	0.10	0.10	0.52
1.00	0.10	0.10	0.48	0.10	0.30	0.10	0.66	0.10	0.74
1.60	0.22	0.44	0.10	0.10	0.30	0.10	0.10	0.50	0.10
1.90	0.10	1.72	0.10	0.10	0.30	0.10	0.10	0.10	0.40
0.36	0.10	0.10	0.10	1.02	0.44	0.10	0.10	0.10	0.10
0.36	0.60	0.36	0.62	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.38	0.10	0.10	0.48	0.28
0.10	0.38	0.10	0.42	0.74	0.64	0.10	0.74	0.10	0.46
0.10	0.64	0.10	1.06	0.78	0.10	0.34	0.10	0.54	0.10
0.10	0.30	0.46	0.10	0.10	0.42	0.92	0.26	0.10	0.52
1.04	0.10	0.10	0.10	0.10	0.10	0.82	0.10	0.10	0.74
0.10	0.10	0.54	0.40	0.10	0.72	0.10	0.26	0.10	0.10
0.70	0.40	0.10	0.44	0.10	0.52	0.82	0.54	0.10	0.40
0.10	0.10	0.10	0.20	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.42	0.10	0.62	0.10	0.10	1.62	0.38	0.10	0.52
0.10	0.10	0.78	0.56	0.68	0.10	0.50	0.10	0.38	0.74
0.24	0.10	0.80	0.10	0.10	0.72	0.60	0.42	0.10	0.10
0.10	0.66	0.10	0.10	1.02	0.48	0.78	0.10	0.10	0.40
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.50	0.10	0.10	0.40	0.78	0.10	0.10	0.10	0.10
0.32	0.62	0.74	0.10	0.10	0.28	0.10	0.36	0.10	0.10
0.10	0.10	0.10	0.60	0.10	0.54	0.10	0.10	0.58	0.10
0.10	0.10	0.48	0.10	0.10	0.50	0.24	0.10	0.10	0.10
0.54	0.34	0.54	0.76	0.52	0.70	0.10	0.10	0.32	0.58
0.10	0.10	0.10	0.10	0.10	0.10	0.50	0.60	0.70	0.70
0.50	0.40	0.30	0.40	0.30	0.60	0.20	0.20	0.50	

* 100 µg/mL

0.10	0.10	0.10	0.10	0.42	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.44
0.62	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.46
0.10	0.10	0.40	0.10	0.10	0.10	0.10	0.10	0.34	0.10
0.92	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.38
0.10	0.10	0.10	0.10	0.10	0.32	0.10	0.10	0.10	0.10
0.10	0.36	0.10	0.30	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.42	0.50	0.10	0.10	0.10	0.32
0.10	0.10	0.10	0.96	0.44	0.10	0.10	0.10	0.10	0.30
0.10	0.10	0.10	0.10	0.10	0.32	0.10	0.10	0.10	0.10
0.78	0.10	0.10	0.10	0.10	0.28	0.10	0.10	0.10	0.34
0.10	0.10	0.48	0.10	0.10	0.58	0.10	0.10	0.10	0.34
0.10	0.10	0.10	0.32	0.10	0.32	0.10	0.10	0.10	0.34
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.34
0.10	0.10	0.10	0.10	0.10	0.10	0.70	0.10	0.10	0.34
0.10	0.10	0.10	0.38	0.44	0.10	0.10	0.10	0.10	0.10

0.10	0.10	0.10	0.10	0.10	0.10	0.48	0.32	0.10	0.10
0.10	0.64	0.10	0.10	0.62	0.38	0.48	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.36	0.56	0.10	0.10	0.10	0.10
0.10	0.50	0.46	0.10	0.10	0.10	0.10	0.24	0.10	0.10
0.10	0.10	0.10	0.10	0.10	0.38	0.10	0.10	0.10	0.10
0.10	0.10	0.36	0.10	0.10	0.10	0.20	0.10	0.10	0.10
0.36	0.30	0.32	0.10	0.28	0.30	0.10	0.10	0.10	0.10
0.10	0.10	0.10	0.10	0.20	0.20	0.50	0.40	0.30	0.30
0.30	0.40	0.30	0.30	0.20	0.20	0.20	0.20	0.20	