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Ploidy and Allelic Variation Affects Fungal Pathogen Virulence in a Nematode Host

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Abstract

Ploidy and Allelic Variation Affects Fungal Pathogen Virulence in a Nematode Host

By Dorian J. Feistel

Genotype-by-genotype interactions between hosts and pathogens can play a significant role in determining pathogen virulence. In addition to allelic variation, ploidy is an important component of an individual genotype. Thus, the relative contributions of pathogen ploidy and allelic composition to pathogen virulence merit further investigation. Here, we investigated the effects of ploidy and allelic background on virulence in the fungal pathogen *Candida albicans* using the nematode *Caenorhabditis elegans* as a host. Using a near isogenic strain of *C. elegans* as the host permitted us to specifically characterize the pathogen side of this genotype-by-genotype interaction. We measured fecundity and survivability in *C. elegans* exposed to different *C. albicans* isolates that vary in their ploidy and allelic composition and found that both ploidy and allelic composition alter *C. albicans* virulence. We found that *C. albicans* negatively impacts both *C. elegans* survivability and fecundity. However, the degree to which *C. elegans* fitness was reduced varies, depending on the genetic background of *C. albicans*. The diploid laboratory reference strain SC5314 and its relative tetraploid displayed the highest degree of virulence on *C. elegans* fecundity, while the clinical FH tetraploid strain had decreased virulence relative to its diploid. This result suggests that ploidy and allelic contribution interact together to alter *C. albicans* virulence. Nematode survivability was significantly reduced when exposed to SC5314 diploids, but was not effected when exposed to its relative tetraploid. However, both SC5314 diploid and tetraploid delayed the reproductive timing in *C. elegans*, which consequently reduced the population growth. Taken together, our results suggest that the level of virulence expressed by *C. albicans* depends on a strain's ploidy and specific allelic combinations. Future studies that include a variety of host genotypes, such as immunocompromised or a heterozygous population of *C. elegans*, would increase the variance we observed in *C. albicans* virulence and potentially lead to a better understanding of genotype-by-genotype interactions between host and pathogen.

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Introduction

Pathogen virulence is measured as the reduction in host fitness resulting from a host-pathogen interaction (Read, 1994; Lipsitch *et al*., 1999; Cressler *et al.,* 2015). Therefore, virulence is not solely the property of the pathogen, but rather the product of the interaction between a host and its pathogen. While many biotic (Katsir *et al.,* 2008) and abiotic (Mauch-Mani and Mauch, 2005) factors contribute to virulence, the genotype-by-genotype interaction between hosts and pathogens is a primary determinant of host infection and the resulting level of virulence (Schulte *et al*, 2010). One important factor in an organism's genotype is its ploidy or number of chromosome sets it contains. Ploidy has been shown to be critical for genotype-by-genotype interactions between pathogens and hosts (Lambrechts, 2010). For example, increases in ploidy (polyploidy) are associated with elevated host immune response (Osnas and Lively, 2006) and have also been documented in virulent isolates of fungal pathogens such as *Aspergillus nidulans* (Purell and Martin, 1973). However, pathogen polyploidy has also been associated with decreases in virulence (Ibrahim *et al.*, 2005; Lin *et al.,* 2008) and thus, these contrasting virulence properties associated with increased ploidy suggest an interaction between ploidy and allelic composition determines an organism's overall genotype. As such, dissecting the role of ploidy in conjunction with allelic composition in host-pathogen genotype-by-genotype interactions is largely unclear and merit further investigation.

Together, ploidy and allelic composition contribute to interactions between genes to produce an organism's genetic background (Yoshiki & Moriwaki, 2006). Allelic composition refers to not just the specific alleles present in a genome, but the overall degree of allelic heterozygosity throughout the genome. The role of specific alleles has been investigated

extensively regarding pathogen virulence and host-pathogen interactions. For example, Lockhart *et al.* (2005) showed in a murine host infection model infected with pathogenic fungi that heterozygosity at the fungal mating type locus increases virulence compared to strains that are homozygous. This result supports the premise that pathogen virulence depends on the pathogen's specific allelic combination (Manning *et al*., 2007). Ploidy intrinsically impacts allelic composition – haploids contain a single allelic set of genes, whereas diploids and polyploids can either be homozygous or heterozygous for any given locus, and dominance can mask recessive alleles. Allelic composition in polyploids is further complicated by changing allelic ratios, by which in the case of tetraploidy can have one (A_1 A_1 A_1 A_1), two (A_1 A_1 A_2 A_2), three (A_1 A_1 A_2 A_3) or four (A_1 A_2 A_3 A_4) alleles present depending on the mechanism and age of the polyploidization event. Thus, a major challenge in determining the specific role ploidy has on pathogen virulence is disentangling it from allelic composition.

The opportunistic fungal pathogen *Candida albicans* is excellent for studying how both allelic composition and ploidy contribute to genetic background and virulence. *C. albicans* is a commensal in the human gastrointestinal microbiota (Odds, 1988) and various other niches (Underhill & Iliev, 2014). Despite its commensal existence *C. albicans* is the predominant agent in fungal infection in humans, causing a range of disease states that include superficial mucosal infections to life threatening systemic infections (McCullough *et al.*, 1996). The severity of fungal infection is closely linked to the immune status of the host, with superficial infections occurring in healthy individuals and bloodstream infections in immunocompromised hosts (MacCallum, 2011). *C. albicans* is predominantly isolated as a highly heterozygous diploid (~1 SNP per 283 coding bp; Muzzey *et al.,* 2013), however its genome is highly labile, displaying a

range of genomic rearrangements and ploidy states (Morrow & Fraser, 2013). For example, genome analysis between a series *C. albicans* clinical isolates from a single immunocompromised patient reveled extensive allelic and ploidy variation across each isolates genome compared to the laboratory reference strain (Abby *et al.,* 2014). Importantly, clinical isolates have also been found to change their ploidy within an infected host in less than 20 days (Marr *et al.,* 1997). Furthermore, comparisons of sequenced genomes from *C. albicans* clinical isolates not only show substantial genetic variation between these strains but also a wide-range of virulence phenotypes in mammalian and invertebrate hosts (Hirakawa, *et al*., 2015). In addition to the ploidy variation detectable in clinical isolates, we can also directly manipulate *C. albicans* ploidy in the laboratory. Here, our goal is to determine the effects of *C. albicans* ploidy on host fitness, while controlling for the genetic background effects of different strains.

To study the impact of *C. albicans* ploidy on host fitness, we use the hermaphroditic nematode *Caenorhabditis elegans* as a model host system. *C. elegans* has been shown to be a useful for studying host-microbe interactions (Sifri *et al.*, 2005) because many pathogens that cause illness in humans also cause disease in *C. elegans* (Irazoqui *et al.*, 2010). Infecting *C. elegans* is easily achieved by replacing or incorporating its standard laboratory food *E. coli* with a desired pathogen (Irazoqui *et al.*, 2010), which can colonize the gut and causes disease. For instance, Jain *et al.* (2013) showed that *C. albicans* ingested by *C. elegans* colonized the gut and subsequently caused infection in the intestine that resulted in morphological changes and increased mortality. Furthermore, the finite number of offspring, rapid generation time of *C. elegans,* and short lifespan (~4 weeks; Muschiol *et al.*, 2009), and relative to most model hosts (2-3 years for murine hosts; Miller *et al.,* 2005), permits us to address pathogen impact on both host survival and fecundity, two important measures of organismal fitness.

In this work we elucidate on the effects of allelic background and ploidy on *C. albicans* virulence in the *C. elegans* host in order to characterize the pathogen side of this genotype-by-genotype interaction. We measured nematode survivability and fecundity exposed to a diverse collection of *C. albicans* isolates that vary in their ploidy and allelic composition and found that both ploidy and allelic composition alter *C. albicans* virulence. Intriguingly, by assessing fecundity as a fitness measure, we found that exposure to some strains of *C. albicans* significantly delays the reproductive timing of *C. elegans* and this disruption significantly reduces the population growth of *C. elegans*.

Methods

Strains and media

C. albicans strains used in this study are listed in Table 1. *C. elegans* N2 Bristol was used for to test host survival, fecundity and population growth. *C. elegans* populations were maintained at 15°C on 100mm petri dishes with 25 mL of lite nematode growth agar medium (NGM [US Biological]) with *E. coli* OP50 as a food source. Nematodes were transferred to a newly seeded *E. coli* plate every 3-4 days. For survival, fecundity and population growth assays, NGM was supplemented with uridine (0.08g/L), histidine (0.08g/L), and adenine (0.04g/L) to facilitate growth of auxotrophic *C. albicans* strains and streptomycin sulfate (200mg/L) to inhibit *E. coli* overgrowth so fungal strains could proliferate.

Seeding NGM plates for survival, fecundity, and population growth assays

C. albicans and *E. coli* OP50 strains were inoculated in 3 mL of YPD or 5 mL of LB respectively, and cultured at 30°C for 1-2 days. *C. albicans* culture densities were measured with

a spectrophotometer and diluted to a final volume of 3.0 OD_{600} per mL (~6 x 10^7 cells per ml). *E. coli* cultures were harvested and washed twice with 1 mL of ddH_2O. The supernatant was removed and the pellet was centrifuged for 60 sec at maximum to remove any excess liquid. The pellet was weighed and concentrated with sterilized water to a final volume of 200 mg/mL. NGM plates were seeded with 6.25 µL *E. coli*, 1.25 µL *C. albicans* (3.75 µL for MH126), and brought to a final volume of 50 µL with ddH_2O. The entire 50 µL was spotted onto the center of a 35mm supplemented-NGM plate and incubated at room temperature overnight before addition of eggs or transferring nematode. *E. coli* OP50 was used as a control at the same concentration describe above.

Egg preparation and synchronization for survival, fecundity and population growth assays

For survival, fecundity and population growth assays, approximately 100 nematodes at the L3/L4 stage were transferred to a 100mm NGM plate seeded with *E. coli* OP50 and maintained at 20°C for 2-3 days prior to the start of an experiment. On the first day of an experiment, these NGM plates were washed with M9 and contents (live nematodes and eggs) transferred to 15 mL conical tube and collected by centrifugation (2 min at 1500 rpm). The pellet was re-suspended in a 1:4 bleach (5.25%) solution and transferred to a micro-centrifuge tube. The suspension was mixed via inversion for 90-120 sec and subsequently centrifuged (30 sec at 1500 rpm). The pellet was washed with 1 mL M9 and centrifuged three consecutive times to remove excess bleach solution and brought to a final suspension with 500 µL M9. To determine the concentration of eggs, 10 µL was placed on a concaved slide and eggs counted and the egg suspension diluted with M9 to a final concentration of 10 eggs/µL. All assays were treated equally on the first day (Day 0) by adding roughly 100 eggs to a treatment or *E. coli* plate (described above).

Survival assay

This experimental procedure is a modified version of Jain *et al*. (2013). Briefly, 72h (Day 3) after adding nematode eggs to a plate, 40 adult nematodes were randomly selected and transferred in replicates of three to newly seeded plates with the same concentration/volume of food as describe above and incubated at 20°C. From there, nematodes were transferred to freshly seeded plates every over day until all nematode populations went extinct. The number of living, dead, and censored worms were scored daily.

Fecundity assay

48h (Day 2) after adding nematode eggs to a plate, a single L3/L4 reproductively immature hermaphroditic nematode was randomly selected and transferred (10 independent biological replicate per treatment per block) to a newly seeded 35mm plates treatment or *E. coli* plates (described above) containing one-fifth the volume of food and incubated at 20°C. *C. elegans* were transferred to freshly seeded plates in 24 time intervals from day two until the end of the experiment. Eggs remained undisturbed on the plate and were incubated at 20°C for an additional 24 h to provide enough time to hatch, at which the number of viable progeny per day were scored. Nematodes that died during the assay were scored dead at the time of transfer. Censored nematodes were excluded from the analysis.

Population growth Assay

48h after adding nematode eggs to a plate (Day 2), a single L3/L4 reproductively immature hermaphroditic nematode was randomly selected and transferred (6 independent biological replicates per treatment) to a freshly seeded 100mm treatment or *E. coli* plate (described above) containing a 6-fold increase in food. On day 7 of the experiment, each population was rinsed with M9 until the majority of nematodes were displaced and transferred to

a 15mL conical tubes. Tubes were placed at 4°C for 1h to allow the nematodes to settle at the bottom. All tubes were concentrated to a final volume of 10mL M9. Six 20uL samples were taken from each population and counted.

Statistical analysis

All statistical analyses were performed using the statistical software RStudio (R Core Team, 2013). All graphical representations of our data were generated using the software Prism 7. To analyze nematode survivability, we used a Cox proportional hazardous model (Therneau and Grambsch, 2000; Therneau, 2015). Nematodes that died on the plate at a specific time point during the experiment were scored as a "1", indicating the event of death took place from fungal infection. Nematodes that were found dead on the rim of the plate were scored as "0", indicating that death occurred from a non-related reason and censored in subsequent analysis. We used a Gehan-Breslow test at a significant p-value < 0.05 to assess survivability which assumes that survivability at earlier time points are more accurate than later survival time points and weighs these data accordingly. Data were combined from two biological replicates with three technical replicates per treatment.

To assess the effects of ingesting *C. albicans* on *C. elegans* total, early and late fecundity, we parsed the data into three different statistical tests: genetic background (GB) (which pooled diploids and tetraploids from the same background), diploids (D-GB), and tetraploids (T-GB). We performed a one-way ANOVA for each test and tested for significance at p-value < 0.05. When statistically comparing the effect of BG, D-BG, or T-BG on nematode fecundity to the control, we performed a post hoc analysis using Dunnet's multiple comparisons test. When analyzing BG, BG-D, or BG-T among *C. albicans* strains on nematode fecundity, we followed up the test with a post hoc Tukey multiple comparisons test. To examine the factors that

influence *C. elegans* fecundity, we ran a two-way ANOVA with genetic background, ploidy, and their interaction term as explanatory variables and total, early, or late fecundity size as the response variable. Similar statistical analyses were used when comparing the effects of *C. albicans* isogenic strains on fecundity and SC5314 diploid and tetraploid on nematode population growth.

Results

Exposure to *C. albicans* reduces *C. elegans* fitness

Pathogens can affect host fitness either by decreasing lifespan and/or reducing fecundity. To determine how *C. albicans* (SC5314) impacts *C. elegans* host fitness, we first measured nematode lifespan when exposed to *C. albicans*. We observed a significant reduction in survival when *C. elegans* was reared on *C. albicans* compared to when it was not (Figure 1A), consistent with previously published results (Jain *et al.*, 2013). The median time to death was reduced by half when nematodes were exposed to *C. albicans* (8 days, $P << 0.001$, Gehan-Breslow logrank test) compared to unexposed nematodes (16 days), indicating that *C. elegans* lifespan is impacted by *C. albicans* infection.

While overall survival was reduced, nematode death was never observed earlier than 5 days post-infection, the period in which adult nematodes produce the majority of their offspring (Muschiol et al 2009). Given this information, we wanted to investigate whether *C. albicans* alters *C. elegans* reproduction. To test this, we measured the total number of viable progeny produced within 7 days from exposed and unexposed nematodes. Exposure to *C. albicans* reduced the total number of viable progeny compared to the control (Fig. 1B: $F_{2,155} = 4.7$, $P < 0.05$, One-way ANOVA) by ~9%. We were curious if the reduction in fecundity was dependent

on live *C. albicans* cells or if foreign cellular material in general causes this distress. To distinguish between these two possibilities, we measured the total number of viable progeny from nematodes exposed to heat-killed *C. albicans* (HK) and observed no detectable difference in this treatment compared to the control (Figure 1B). Therefore, *C. albicans* only reduced host fitness as a live agent.

In addition to total viable progeny produced, we also followed the number of progeny produced per day. Intriguingly, we found the largest reduction in reproduction on Day3 in nematodes exposed to *C. albicans* (105 ± 4.69) relative to the control and HK treatments (187 ± 2.88 and 170 ± 5.84, respectively) ($F_{2,155}$ = 123.1, P < 0.001) (Figure 1C). Inversely, there was increased reproduction on Days4-6 from these nematodes (Figure 1C). These results together suggest that there is a delay in reproductive timing, in addition to an overall reduction in viable progeny. To simplify analysis of reproductive timing, we partitioned total viable progeny into two categories: early (Days 0-3) and late (Days 4-7). Early reproduction was 35% reduced when exposed to live *C. albicans* ($F_{2,155}$ = 70.63, P < 0.001, One-way ANOVA) whereas late reproduction was increased in this treatment ($F_{2,155}$ = 29.64, P < 0.001, One-way ANOVA) compared to control and HK treatments (Figure 1D). Taken together, our data indicate that *C. albicans* severely delays and reduces reproduction in addition to impacting overall survival in *C. elegans*.

Genetic background affects *C. albicans* virulence

C. albicans strains isolated from patient infections are genetically diverse (Hirakawa *et al.*, 2015) when compared to each other and the laboratory reference strain (SC5314). We were curious if different genetic backgrounds of *C. albicans* differ in virulence, as determined by

impact on fecundity in *C. elegans*. To test this, we measured both total reproductive output and timing in nematodes exposed to SC5314 and two different sets of clinical strains (FH and PN, see table 1) of *C. albicans*. While there was significant reduction in total viable progeny produced among *C. albicans* treatments compared to the control ($F_{3,253}$ = 6.312, P < 0.001, One-way ANOVA), this result was driven by SC5314 (P < 0.001, Dunnet's test) and not the FH (P = 0.34) and PN (P = 0.22) treatments (Figure 2A). However, reproductive delays are observed for all three *C. albicans* genetic backgrounds compared to the control (Figure 2B). Specifically, early reproduction was reduced among *C. albicans* treatments ($F_{3,253}$ = 35.59, P < 0.001, ANOVA) and all three genetic backgrounds independently reduced early reproduction relative to the control (Fig. 3B; SC5314: P < 0.001; FH: P < 0.001; PN: P < 0.05, Dunnet's test) in contrast to total reproductive output. Furthermore, there were significant differences between genetic backgrounds in regards to the impact on early reproduction ($F_{2,184}$ = 22.7, P < 0.001, ANOVA). SC5314 reduced early reproduction the most compared to the FH and PN backgrounds (SC5314–FH: P < 0.001; SC5314–PN: P < 0.001, Tukey's test) whereas there was no significant difference between FH and PN (P = 0.721, Tukey's test). Similar differences between genetic backgrounds were observed for late reproduction ($F_{3,253}$ = 12.3, P < 0.001, ANOVA). Our results indicate that each of the different *C. albicans* genetic backgrounds delay reproduction but vary in their capacity to reduce total viable progeny produced.

***C. albicans* ploidy and allelic background impact virulence properties**

We were interested in determining the relationship between *C. albicans* ploidy and virulence, as measured by reductions in *C. elegans* fecundity. To test this, we measured both total reproductive output and timing in nematodes exposed to diploid or tetraploid *C. albicans* using the previously described genetic backgrounds (Table 1). When we analyzed diploids from

different allelic backgrounds, only SC5314 reduced the total fecundity of *C. elegans* relative to the control ($P < 0.001$, Dunnet's test) (Figure 3A). Early reproduction, however, was significantly reduced by SC5314 and FH diploids (SC5314: $P < 0.001$; FH: $P < 0.001$, Dunnet's test) (Figure 3B). Comparing across *C. albicans* strains, SC5314 and FH diploids were similar in reducing nematode early reproduction and were significantly different from the PN diploid (WT–PN: $P < 0.001$; FH–PN: $P < 0.01$, Tukey's test) (Figure 3B). Late reproduction was also significantly affected by SC5314 and FH diploids compared to the control (SC5314: $P < 0.001$, FH: $P < 0.001$, Dunnet's test) (Figure 3B).

In contrast to diploids, the total fecundity of *C. elegans* was more substantially reduced by tetraploids. SC5314 and PN tetraploids significantly reduced total viable progeny when compared to the control (SC5314: $P < 0.001$, PN: <0.05, Dunnet's test) (Figure 3C). Differences in virulence among *C. albicans* tetraploids were also observed, showing that SC5314 was significantly different from the FH tetraploid ($P < 0.001$, Tukey's test) (Figure 3A). However, early reproduction was significantly impacted by only the SC5314 tetraploid when compared to the control (SC5314: $P < 0.001$, PN, $P < 0.05$, Dunnet's test), reducing early fecundity by >50% (Figure 3D). Additionally, SC5314 was significantly different among *C. albicans* FH and PN tetraploids (WT–FH: $P < 0.001$, WT–PN: $P < 0.001$, Tukey's test) (Figure 3D). Late reproduction was only significantly impacted by SC5314 tetraploids relative to the control ($P < 0.01$, Dunnet's test) (Figure 3D).

Interestingly, we found a significant interaction between ploidy and genetic background when comparing the effect of *C. albicans* diploids and tetraploids on total fecundity ($P < 0.01$, Two-way ANOVA) and early reproduction ($P < 0.001$, Two-way ANOVA) (Figure 3E). Looking at the total and early panels in Figure 3E, the number of progeny decreases as ploidy

increases for SC5314, whereas the ploidy and virulence has an inverse relationship for FH. SC5314 and FH diploids are almost equivalent in their effect to reduce total fecundity (Figure 3A) and early reduction (Figure 3B); however, virulence significantly diverged between the two genetic backgrounds when ploidy increased to tetraploid (Figure 3C&D). Importantly, although the FH and PN tetraploids generally share the same genetic background as their respective diploid strains, previous studies have identified allelic variation between the tetraploid and diploid strains (Abbey et al 2014 and unpublished data). Therefore, this interaction effect may not solely be the result of the shift from diploidly to tetraploidy. Nonetheless, our results demonstrate that ploidy directly affects the virulence of SC5314 and suggest that the effects of ploidy may vary between genetic backgrounds.

_C._ albicans reduces the population size of _C. elegans_

The genetic background and ploidy of *C. albicans* significantly impacted early reproduction of *C. elegans* in our study. *C. albicans* SC5314 diploid reduced the total fecundity by 9% relative to the control (Figures 1B&3A), but severely reduced early reproduction by 35% (Figures 1D&3B). We were curious if a delay in reproduction would impact the population growth of nematodes. To test this, we exposed a single nematode to the laboratory reference strain SC5314 at different ploidies and measured the population density relative to a control after seven days. This was enough time for a *C. elegans* population to grow substantially large, and consist of multiple overlapping generations. SC5314 diploid and tetraploid significantly reduced the population size of *C. elegans* by 32% and 35%, respectively, relative to the control (diploid: $P < 0.001$; tetraploid: $P < 0.001$, Dunnett's test) (Figure 4A).

We were concerned that the result generated by the SC5315 tetraploid in the population growth assay was caused by increased nematode mortality. Thus, we also tested *C. elegans*

survivability when exposed to SC5314 tetraploids. Nematodes' survival was not affected when exposed to SC5314 tetraploids and we observed a similar effect when compared to the control (P=0.3982, Gehan-Breslow logrank test) (Figure 4B). Similar to Figure 1A, SC5314 diploids had the greatest reduction in survivability relative to the control ($P < 0.01$, Gehan-Breslow test) (Figure 4B). Since survivability was not decreased in the tetraploid strain, our results give added support that effecting the early reproduction of *C. elegans* has a severe impact on population fitness.

Discussion

Here, we sought to understand how the genetic background of the human fungal opportunistic pathogen *Candida albicans* contributes to virulence phenotypes in the nematode host *C. elegans* by disentangling ploidy and allelic composition. We found that *C. albicans* negatively impacts both *C. elegans* survivability and fecundity. However, the degree to which *C. elegans* fitness is reduced varies, depending on the genetic background of *C. albicans*, with SC5314 strains displaying the highest degree of virulence on fecundity (Figure 3A-D). In contrast to the SC5314 strains, the FH tetraploid had decreased virulence relative to the diploid (Figure 4E), showing that ploidy and allelic composition interact to produce varying levels of virulence. By including fecundity in our assessment of host fitness, we show that *C. albicans* can significantly delay reproductive timing in *C. elegans* and reduce population growth (Figure 4A), thus severely decrease *C. elegans* fitness. Taken together, these results suggest that the level of virulence expressed by *C. albicans* depends on a strain's ploidy and specific allelic combinations.

We were surprised that virulence not only varied between different ploidy states, but also in the relative direction between different clinical groups. For example, both SC5314 and FH diploids had similar early reproduction virulence phenotypes (Figure 3B), yet virulence increased in the SC5314 tetraploid and decreased in the FH tetraploid (Figure 3E). One factor that may explain these contrasting results is that the SC5314 strains were laboratory derived (Bennett and Johnson 2003) and thus are nearly isogenic and only differ in ploidy (i.e. contain the exact same set of alleles in a 1:1 ratio) whereas the FH strains differ in allelic composition in addition to ploidy. The FH strains were isolated in a time series from a single immunocompromised patient who received a bone marrow transplant (Marr *et al.,* 1997). Sequence analysis for the FH strains show extensive allelic variation between diploid and tetraploid (Abbey *et al.,* 2014) and these allelic differences may impact virulence phenotypes to a greater degree than ploidy does in this genetic background. To more comprehensively test the precise role of *C. albicans* ploidy on virulence, we need a series of isogenic strains that vary in ploidy from diverse genetic backgrounds This is now possible to perform with the discovery of *C. albicans* haploids (Hickman *et al.,* 2013) that can undergo a process called autodiploidization, which duplicates the entire genome. Tetraploids could be generated by mating isogenic diploids. A series of isogenic strains varying in ploidy could add more information in assessing the effects of ploidy on virulence.

Here, we found that the SC5314 tetraploid *C. albicans* did not impact overall survival of *C. elegans* hosts (Figure 4B), consistent with previous studies showing *C. albicans* tetraploids have decreased virulence in murine models (Ibrahim *et al.*, 2005; Lin *et al.,* 2008). However, we did discover that *C. elegans* fecundity was severely impacted by *C. albicans* SC5314 tetraploid

(Figure 3C&D). Further, we were able to get a more comprehensive view of *C. albicans* virulence by testing the growth rate of single host lineage. Overall, we found that *C. albicans* can reduce *C. elegans* host fitness, apart from inducing greater host mortality (Figure 4). Therefore, in some cases survivability is not the most accurate predictor of pathogen virulence. By assessing both survival and fecundity as fitness measures in *C. elegans*, we can reveal cryptic virulence phenotypes that are difficult to determine in other host models such as mice. Our study not only showed that *C. elegans* fecundity can be used to assess virulence in *C. albicans*, but that it should be coupled with survivability assays to increases the power of studying *C. albicans* virulence. Thus, despite the fact that host survival can be unaffected with exposure to *C. albicans*, it does not necessarily imply that overall host fitness is not impacted.

In this work we found that ploidy and allelic background together contribute to *C. albicans* virulence, and that strains isolated from different infections impact nematode survival and fecundity differently. Given that virulence phenotypes manifest from host-pathogen genotype-by-genotype interactions, one consideration of our results is that the low virulence displayed in some of our strains may be more virulent in a different host genetic background. Fungal infections in general, and life-threatening *C. albicans* infections specifically arise in hosts with compromised immune systems. In healthy individuals, the host uses its immune defenses to minimizes or stop infection from a pathogen, which consequently alters the pathogen's virulence phenotype (Casadevall & Pirofski, 2001). We elected to use healthy, highly inbred and homozygous *C. elegans* hosts in order to reduce any effects from host genetic background to facilitate investigation only pathogen virulence phenotypes. However, if we used an immunocompromised or a heterozygous population of *C. elegans* exposed to the same *C.*

albicans strains used here, we expect the level of virulence for each strain would be different from our current results due to different host genetic background. Therefore, incorporating a variety of host genotypes would most likely increase the variance we observed in virulence and potentially lead to a better understanding of genotype-by-genotype interactions between host and pathogen, such that some host genotypes would be more resistant to infection from certain pathogen genotypes and that some pathogen genotypes may be better at infecting certain host genotypes.

Table 1. Strains used in this study

Strain	Alias	Ploidy	Background	Refrence
FH 2N	FH1	Diploid	Clinical isolate from a patient with a marrow transplant	Marr et al (1997), Abbey et al (2014)
SC5314 2N	SC5314	Diploid	Laboratory reference strain originally isolated from a patient with candidiasis	Gillum et al. (1984)
PN 2N	PN	Diploid	Clinical isolate obtaned from an immunocompromised patient	This study
FH 4N	FH 6	Polyploid 4 × Chr1, Chr2, Chr3, Chr7, ChrR; 3 × Chr4, Chr5, Chr6; 2 × isochromosome(5L)	Clinical isolate (same patient as MH11)	Marr et al (1997), Abbey et al (2014)
PN 4N	PN1	Tetraploid	Clinical isloate (same patient as MH126)	Gerstein et al. (2017)
SC5314 4N	SC5314	Tetraploid	Mating product between two SC5314-derived diploid strains	Bennett and Johnson (2003)

Figure 1

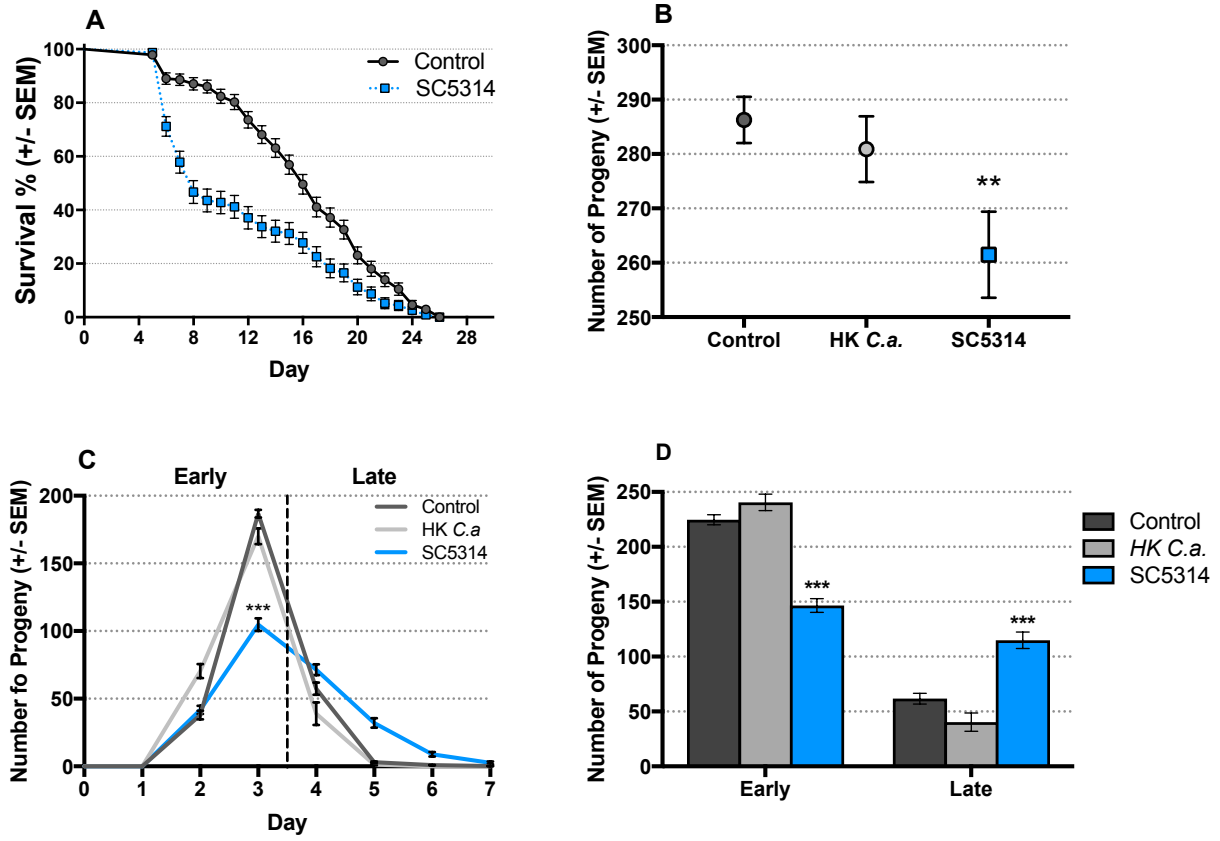

Figure 1. *C. albicans* reduces both survivability and fecundity of *C. elegans*. A) *C. elegans* survival curves when exposed to an *E. coli* control (black circles) or *C. albicans* SC5314 (blue squares) treatment. **B)** Total number of viable *C. elegans* progeny after seven days of exposure to *E. coli*, heat-killed SC5314 (HK *C.a.*; grey circles), or live SC5314. **C)** *C. elegans* progeny produced per day during exposure to *E. coli*, HK *C.a.*, or live SC5314. Dashed line designates the period of Early (days 0-3) and Late (days 4-7) reproduction. **D)** *C. elegans* progeny produced during Early and Late periods of reproduction in control, HK *C.a.*, or SC5315 treatments. All data plotted represent the mean (±SE) and ** ($P < 0.01$) or *** ($P < 0.001$) indicates a statistical difference between SC5314 and the control.

Figure 2

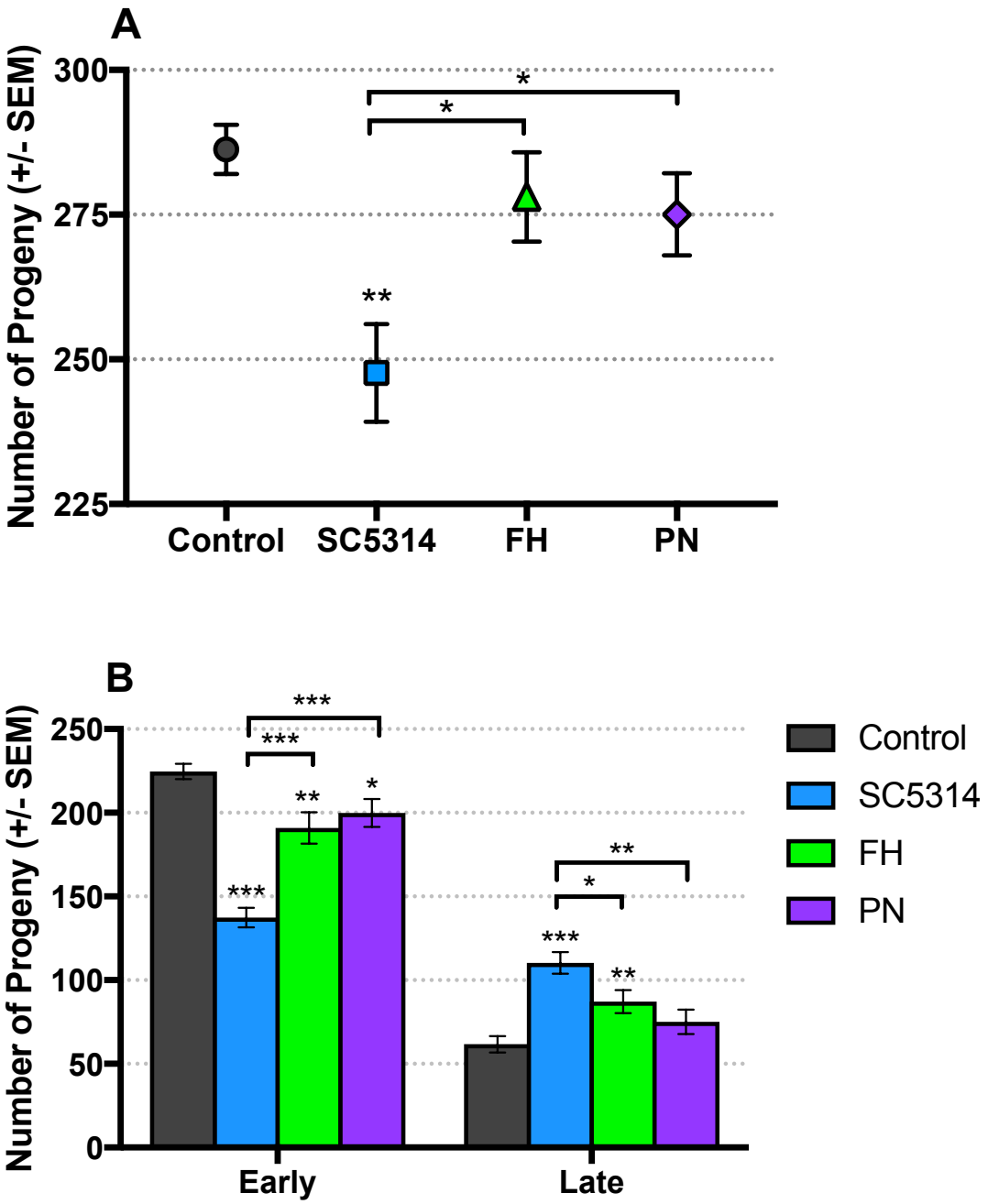

Figure 2. Genetic background of *C. albicans* alters effects on *C. elegans* fecundity. A) Total number of progeny and **B)** progeny produced during early and late periods of reproduction during exposure to control (black) and three different *C. albicans* genetic backgrounds: SC5314 (laboratory reference strain, blue), FH (isolated from bloodstream infection of an HIV-patient, green) or PN (isolated from vaginal and oral cavities of an immune-healthy individual, purple). Data plotted represent the mean (±SE) and * ($P < 0.05$), ** ($P < 0.01$), or *** ($P < 0.001$) indicates a significant difference either relative to the control or between yeast genetic backgrounds.

Figure 3

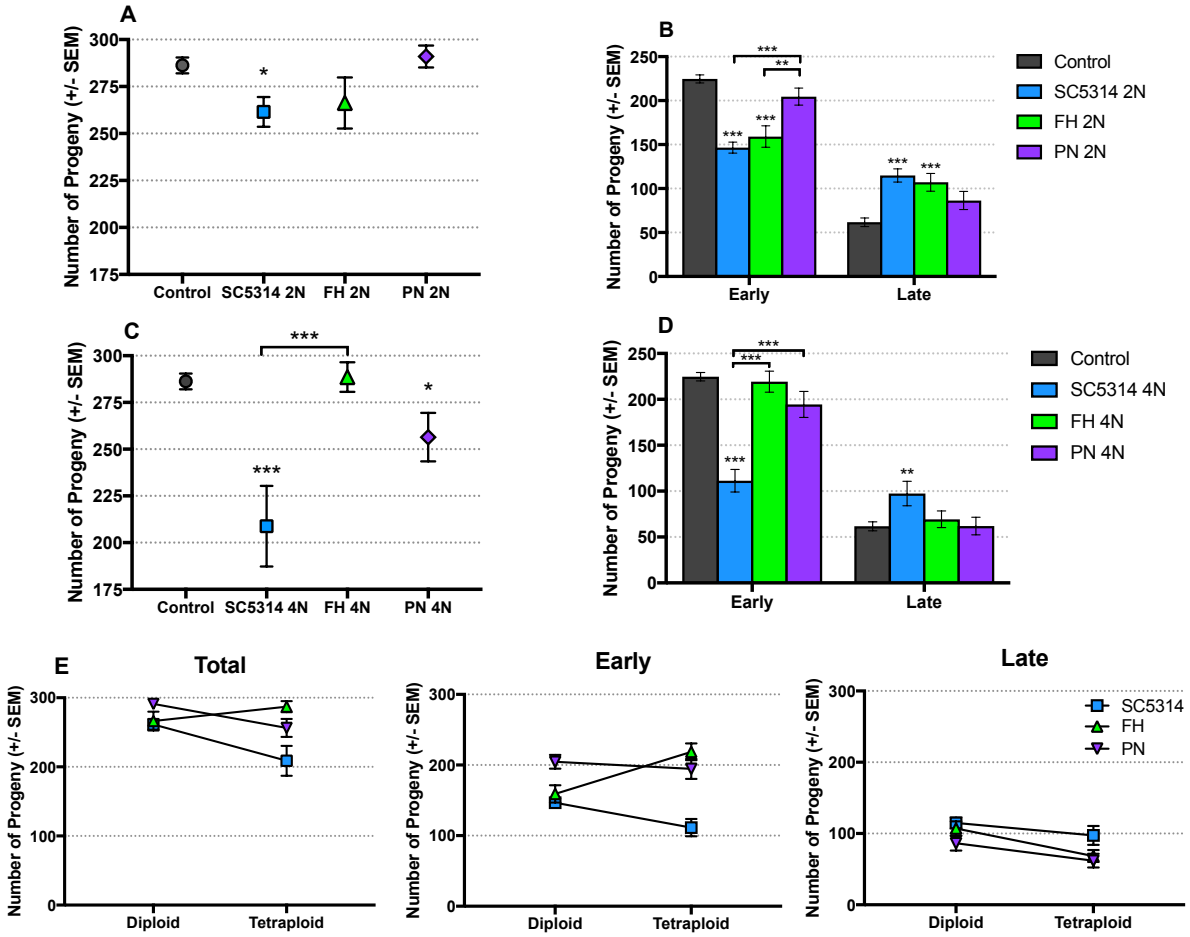

Figure 3. Interactions between *C. albicans* ploidy and genetic background impact virulence properties. Progeny produced by *C. elegans* following exposure to control and diploid *C. albicans* strains shown as **A)** total number of progeny and **B)** number of progeny produced during early and late periods of reproduction. C) Total number of progeny **D)** and progeny produced during early and late reproduction during exposure to tetraploid *C. albicans.* **E-G)** Interaction plots between diploid and tetraploid strains for **E)** total progeny, **F)** early, and **G)** late progeny production. Data plotted represent the mean (±SE) and * ($P < 0.05$), ** ($P < 0.01$), or *** ($P < 0.001$) indicates a significant difference either relative to the control or between yeast genetic backgrounds.

Figure 4

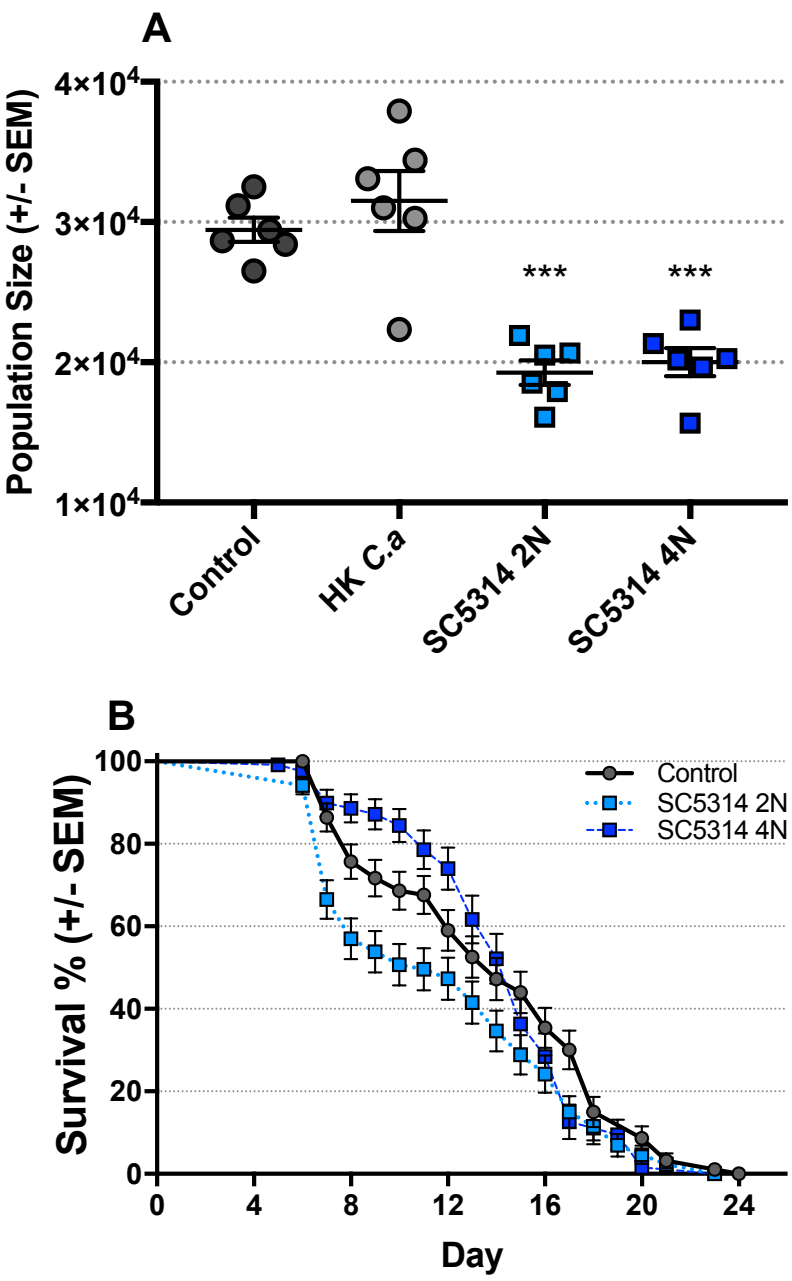

Figure 4. *C. albicans* mediated decreases in fecundity result in smaller *C. elegans* population sizes. **A)** Total *C. elegans* population size of six independent populations each after seven days of exposure to control (black circles), HK *C.a* (grey circles), diploid SC5314 (blue squares) or tetraploid SC5314 (dark blue squares). Each data point represents the mean of a single population randomly sampled six times. The mean for each treatment is indicated by a black horizontal line (±SE) and *** ($P < 0.001$) indicates a significant difference relative to the control. **B)** Survival curve of *C. elegans* exposed to *E. coli* control (black circles), diploid SC5314 (2N, blue squares), or tetraploid SC5314 (4N, dark blue squares) treatments. Data plotted represent the mean (±SE) of three replicates.

References

Abbey, D. A., J. Funt, M. N. Lurie-Weinberger, D. A. Thompson, A. Regev, C. L. Myers, and J. Berman. 2014. YMAP: a pipeline for visualization of copy number variation and loss of heterozygosity in eukaryotic pathogens. Genome Medicine 6:1-15.

Casadevall, A. and L. Pirofski. 2001. Host-Pathogen Interactions: The Attributes of Virulence. The Journal of Infectious Diseases 184:337-344.

Cressler, C. E., L. D. Mc, C. Rozins, V. D. H. J, and T. Day. 2016. The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. Parasitology 143:915-930.

Hickman, M. A., G. Zeng, A. Forche, M. P. Hirakawa, D. Abbey, B. D. Harrison, Y. M. Wang, C. H. Su, R. J. Bennett, Y. Wang, and J. Berman. 2013. The 'obligate diploid' Candida albicans forms mating-competent haploids. Nature 494:55-59.

Hirakawa, M. P., D. A. Martinez, S. Sakthikumar, M. Z. Anderson, A. Berlin, S. Gujja, Q. Zeng, E. Zisson, J. M. Wang, J. M. Greenberg, J. Berman, R. J. Bennett, and C. A. Cuomo. 2015. Genetic and phenotypic intra-species variation in Candida albicans. Genome Res 25:413-425.

Ibrahim, A. S., B. B. Magee, D. C. Sheppard, M. Yang, S. Kauffman, J. Becker, J. E. Edwards, Jr., and P. T. Magee. 2005. Effects of ploidy and mating type on virulence of Candida albicans. Infect Immun 73:7366-7374.

Irazoqui, J. E., J. M. Urbach, and F. M. Ausubel. 2010. Evolution of host innate defence: insights from Caenorhabditis elegans and primitive invertebrates. Nat Rev Immunol 10:47-58.

Jain, C., K. Pastor, A. Y. Gonzalez, M. C. Lorenz, and R. P. Rao. 2013. The role of Candida albicans AP-1 protein against host derived ROS in in vivo models of infection. Virulence 4:67-76.

Katsir, L., H. S. Chung, A. J. Koo, and G. A. Howe. 2008. Jasmonate signaling: a conserved mechanism

of hormone sensing. Curr Opin Plant Biol 11:428-435.

Lambrechts, L. 2010. Dissecting the genetic architecture of host-pathogen specificity. PLoS Pathog 6:e1001019.

Lin, X., K. Nielsen, S. Patel, and J. Heitman. 2008. Impact of mating type, serotype, and ploidy on the virulence of Cryptococcus neoformans. Infect Immun 76:2923-2938.

Lipsitch, M., S. Siller, and M. A. Nowak. 1996. The Evolution of Virulence in Pathogens with Vertical and Horizontal Transmission. Evolution 50:1729-1741.

Lockhart, S. R., W. Wu, J. B. Radke, R. Zhao, and D. R. Soll. 2005. Increased virulence and competitive advantage of a/alpha over a/a or alpha/alpha offspring conserves the mating system of Candida albicans. Genetics 169:1883-1890.

Maccallum, D. M. 2012. Hosting infection: experimental models to assay Candida virulence. Int J Microbiol 2012:363764.

Manning, S. D., A. S. Motiwala, A. C. Springman, W. Qi, D. W. Lacher, L. M. Ouellette, J. M. Mladonicky, P. Somsel, J. T. Rudrik, S. E. Dietrich, W. Zhang, B. Swaminathan, D. Alland, and T. S. Whittam. 2008. Variation in virulence among clades of Escherichia coli O157:H7 associated with disease outbreaks. Proc Natl Acad Sci U S A 105:4868-4873.

Marr, K. A., T. C. White, J. H. van Burik, and R. A. Bowden. 1997. Development of Fluconazole Resistance in Candida albicans Causing Disseminated Infection in a Patient Undergoing Marrow Transplantation. Clinical Infectious Diseases 25:908–910.

Mauch-Mani, B. and F. Mauch. 2005. The role of abscisic acid in plant-pathogen interactions. Curr Opin Plant Biol 8:409-414.

McCullough, M. J., B. C. Ross, and P. C. Reade. 1996. Candida albicans: a review of its history, taxonomy, epidemiology, virulence attributes, and methods of strain differentiation. International

Journal of Oral and Maxillofacial Surgery 25:136-144.

Miller, R. A., B. Buehner, Y. Chang, J. M. Harper, R. Sigler, and S. Smith-Wheelock. 2005. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance.

Morrow, C. A. and J. A. Fraser. 2013. Ploidy variation as an adaptive mechanism in human pathogenic fungi. Semin Cell Dev Biol 24:339-346.

Muschiol, D., F. Schroeder, and W. Traunspurger. 2009. Life cycle and population growth rate of Caenorhabditis elegans studied by a new method. BMC Ecol 9:14.

Muzzey, D., K. Schwartz, J. S. Weissman, and G. Sherlock. 2013. Assembly of a phased diploid Candida albicans genome facilitates allele-specific measurements and provides a simple model for repeat and indel structure. Genome Biology 14:1-14.

Odds, Frank C. *Candida and candidosis: a review and bibliography*. Bailliere Tindall, 1988.

Osnas, E. E. and C. M. Lively. 2006. Host ploidy, parasitism and immune defence in a coevolutionary snail-trematode system. J Evol Biol 19:42-48.

Purnell, Dallas M., and George M. Martin. "Heterozygous diploid strains of Aspergillus nidulans: enhanced virulence for mice in comparison to a prototrophic haploid strain." *Mycopathologia* 49.4 (1973): 307-319.

Read, A. F. 1994. The Evolution of Viruence. Trends In Microbiology 2:73-76.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Schulte, R. D., C. Makus, B. Hasert, N. K. Michiels, and H. Schulenburg. 2010. Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. Proc Natl Acad Sci U S A 107:7359-7364.

Sifri, C. D., J. Begun, and F. M. Ausubel. 2005. The worm has turned--microbial virulence modeled in Caenorhabditis elegans. Trends Microbiol 13:119-127.

Therneau, T. M. and Grambsch, P. M. (2000). _Modeling Survival Data: Extending the Cox Model_. Springer, New York. ISBN 0-387-98784-3.

Therneau, T. (2015). _A Package for Survival Analysis in S_. version 2.38, <URL: http://CRAN.R-project.org/package=survival>. Springer, New York. ISBN 0-387-98784-3.

Underhill, D. M. and I. D. Iliev. 2014. The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol 14:405-416.

Yoshiki, A. and K. Moriwaki. 2006. Mouse Phenome Research: Implications of Genetic Background. ILAR 47:94-102.