



Host Defense Mechanisms Induce Genome Instability Leading to Rapid Evolution in an Opportunistic Fungal Pathogen

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ABSTRACT The ability to generate genetic variation facilitates rapid adaptation in stressful environments. The opportunistic fungal pathogen *Candida albicans* frequently undergoes large-scale genomic changes, including aneuploidy and loss of heterozygosity (LOH), following exposure to host environments. However, the specific host factors inducing *C. albicans* genome instability remain largely unknown. Here, we leveraged the genetic tractability of nematode hosts to investigate whether innate immune components, including antimicrobial peptides (AMPs) and reactive oxygen species (ROS), induced host-associated *C. albicans* genome instability. *C. albicans* associated with immunocompetent hosts carried multiple large-scale genomic changes, including LOH and whole-chromosomal and segmental aneuploidies. In contrast, *C. albicans* associated with immunocompromised hosts deficient in AMPs or ROS production had reduced LOH frequencies and fewer, if any, additional genomic changes. To evaluate whether extensive host-induced genomic changes had long-term consequences for *C. albicans* adaptation, we experimentally evolved *C. albicans* in either immunocompetent or immunocompromised hosts and selected for increased virulence. *C. albicans* evolved in immunocompetent hosts rapidly increased virulence, but *C. albicans* evolved in immunocompromised hosts did not. Taken together, this work suggests that host-produced ROS and AMPs induces genotypic plasticity in *C. albicans* which facilitates rapid evolution.

KEYWORDS *Candida albicans*, *Caenorhabditis elegans*, fungal genomics, innate immunity, reactive oxygen species, genetics

The opportunistic fungal pathogen *Candida albicans* is typically commensal and a component of the human microbiome (1). However, *C. albicans* is a leading cause of fungal bloodstream infections and 40% of these infections result in mortality (2). In addition to bloodstream infections, *C. albicans* causes nonlethal mucosal infections, including vaginal and oral candidiasis (2). *C. albicans* infection is highly dependent on the host context, including high estrogen levels (3), chronic stress (4), antibiotic use (5–7), uncontrolled diabetes (8, 9), and immunosuppression (10, 11). In the absence of proper immune recognition, fungal proliferation is uncontrolled, leading to infection. However, healthy individuals, despite having a fully functioning immune system, also experience *C. albicans* infections. Infections primarily result from the commensal isolates becoming pathogenic rather than an infection acquired from outside sources (12). The transition from commensal to pathogenic may be facilitated by *C. albicans* phenotypic and genotypic heterogeneity. *C. albicans* genetic diversity within and among individuals is very high and often includes numerous single-nucleotide polymorphisms (SNPs) and loss of heterozygosity (LOH) events (13). This genetic variation may be a direct consequence of the stressors *C. albicans* encounters in the host, which include immune stressors and other microbes. Recent work has demonstrated that the host environment elevates *C. albicans* genome instability similarly to *in vitro* stressors

Editor Mairi C. Noverr, Tulane School of Medicine

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The authors declare no conflict of interest.

Received 9 June 2021

Returned for modification 26 July 2021

Accepted 9 December 2021

Accepted manuscript posted online

13 December 2021

Published 17 February 2022

(14–18). However, the specific host components that generate this instability are largely unknown.

Genomic variation is quite common in clinical *C. albicans* isolates (13, 19) and includes polymorphisms, copy number variation, loss of heterozygosity (LOH), and partial or whole-chromosomal aneuploidies (19). This genomic variation indicates that host environments either induce or maintain genetic variation in *C. albicans*. Studies of murine infection models have found that when exposed to different host niches, *C. albicans* has a greater-than-10-fold increase in LOH and frequent aneuploidy compared to that *in vitro* (14–16). Following host exposure, genomic changes generated in the murine environment often resulted in a more commensal-like phenotype and higher fitness inside the host (15, 16, 18, 20). However, the long-term genotypic and phenotypic consequences have not yet been extensively studied. *Caenorhabditis elegans* has also been used as a host model to assess *C. albicans* genome stability. *C. albicans* genome instability following *C. elegans* host association was detected across multiple *C. albicans* strains, and it resulted in changes to virulence (17). Together, murine and *C. elegans* models clearly demonstrate that host environments drive genetic diversity in *C. albicans*, which causes phenotypic changes. However, it remains unclear which specific host components contribute to *C. albicans* genome instability and how host-induced genome instability contributes to the long-term adaptability of *C. albicans*.

C. albicans encounters many different stressors within the host environment, including the immune system, which is designed to control and remove pathogens. The immune system recognizes *C. albicans* and other pathogens through pathogen recognition receptors (PRRs) that detect specific microbial chemical signatures called pathogen-associated molecular patterns (PAMPs) (21). Recognition of pathogens like *C. albicans* triggers production of antimicrobial peptides (AMPs) which recruit phagocytic immune cells to the site of infection. Phagocytic immune cells produce reactive oxygen species (ROS) and reactive nitrogen species to control pathogen proliferation (22). Similar to humans, *C. elegans* produces ROS and AMPs in response to pathogens. AMPs inhibit microbial growth through a variety of methods, including disrupting the cell membrane and halting DNA, RNA, and protein synthesis (23). *C. elegans* AMP production is activated through a mitogen-activated protein kinase (MAPK) signaling cascade (24, 25) and includes SEK-1 (MAPKK), which is homologous to the mammalian MKK3/6 and MKK4 MAPKKs (26). Mutations in *C. elegans* SEK-1 increase host susceptibility to *C. albicans* and other microbial pathogens (26–29). Another conserved host defense mechanism is ROS production. Mammals produce ROS via five NADPH oxidases and two dual oxidases (30, 31) while *C. elegans* ROS production is mediated by a single dual oxidase BLI-3 in response to bacterial and fungal pathogens (29, 32–34). BLI-3 mutant hosts are more susceptible to *C. albicans* infection (35). ROS causes cellular toxicity through structural changes to the DNA (36) and generates double-strand breaks in *C. albicans* (30). Together, host-produced AMPs and ROS act in various ways in order to inhibit *C. albicans* growth.

Here, we investigated if host-produced AMPs and ROS induce *C. albicans* genome instability, using the model host *C. elegans*. We infected wild-type and two different immunocompromised hosts, with mutations in either *sek-1* (AMP production) or *bli-3* (ROS production), with *C. albicans* and measured the frequency and types of genome changes in *C. albicans*. Wild-type hosts elevate genome instability and generate greater genetic diversity in *C. albicans* compared to immunocompromised hosts. To evaluate the impact of greater genetic diversity in *C. albicans* driven by the host immune response, we evolved *C. albicans* virulence in both immunocompetent and immunocompromised hosts for 10 passages. Within this relatively short *in vivo* experimental evolution, *C. albicans* rapidly increased virulence when evolved in immunocompetent hosts but did not when evolved in immunocompromised hosts. Taken together, our results suggest that host innate immune pathways are a source of genome instability in *C. albicans* and facilitate *C. albicans* adaptation.

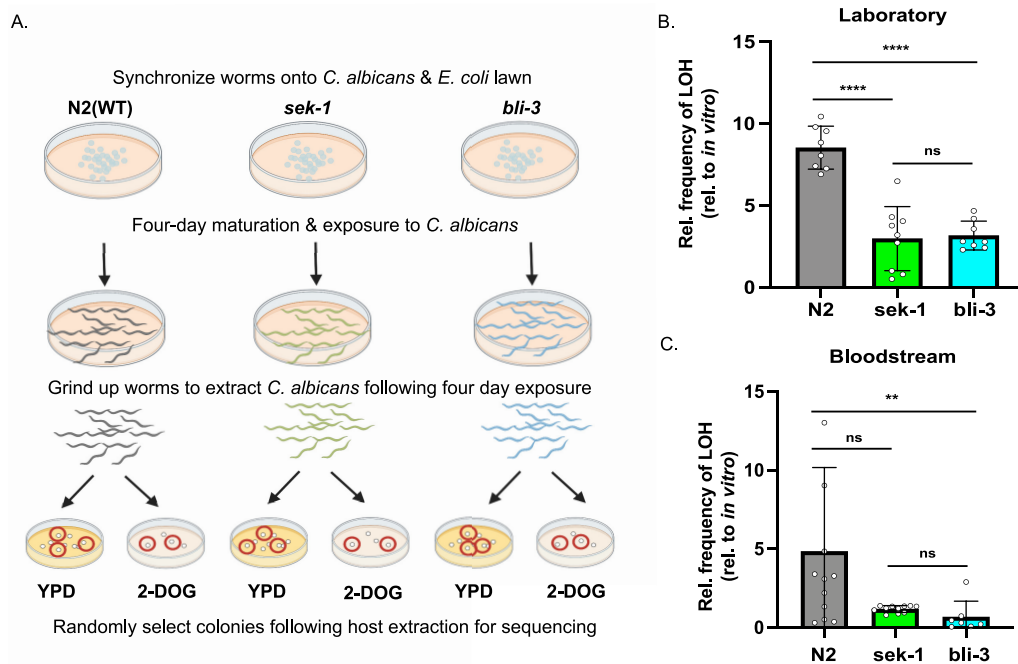


FIG 1 Host immunity impacts *C. albicans* genome stability. (A) Experimental overview of *in vivo* experiments. (B) Laboratory *C. albicans* LOH frequency following host association relative to *C. albicans* no-host control LOH frequency for N2 (wild type, gray, $n = 8$), *sek-1* (green, $n = 9$), and *bli-3* (blue, $n = 8$). (C) Bloodstream *C. albicans* LOH frequency following host association relative to *C. albicans* no-host control LOH frequency for N2 (gray, $n = 11$), *sek-1* (green, $n = 11$), and *bli-3* (blue, $n = 8$). Means and standard deviation (SD) are plotted. Asterisks indicate significant differences (****, $P < 0.0001$; **, $P < 0.01$; ns, not significant; Kruskal-Wallis with *post hoc* Dunn's multiple test).

RESULTS

Host defense pathways elevate *C. albicans* genome instability. We and others have shown that nematode and murine host environments induce *C. albicans* genome instability compared to *in vitro* conditions (14–18), yet the specific host attributes which drive genome instability have not been elucidated. Here, we tested whether components of host innate immune function drive host-associated genome instability by comparing *C. albicans* LOH between yeast extracted from immunocompetent and immunocompromised hosts (Fig. 1A). We used two different immunocompromised host genotypes: one carried a *sek-1* deletion and could not produce AMPs (31), while the second carried a *bli-3* deletion and could not produce ROS (29, 34). *C. albicans* LOH frequency was significantly reduced in yeast extracted from *sek-1* and *bli-3* hosts compared to that in yeast extracted from wild-type (N2) hosts (Fig. 1B, Fig. S1A and B in the supplemental material) which implicates both the *sek-1* and *bli-3* immune pathways as sources of pathogen genome instability.

Since *C. albicans* genetic background impacts mutation rates (16, 17), we also measured host-induced LOH frequency in a clinical *C. albicans* strain (37). Similar to the laboratory *C. albicans* strain, the clinical strain had a significantly reduced LOH frequency when extracted from *bli-3* hosts than it did when extracted from wild-type hosts (Fig. 1C). Yet, the clinical *C. albicans* LOH frequency extracted from *sek-1* and wild-type hosts was not statistically different. However, *C. albicans* LOH frequency was notably higher in wild-type hosts compared to *sek-1* hosts. Together, these data suggest that the *bli-3* pathway induces *C. albicans* genome instability regardless of pathogen genetic background, and that the *sek-1* pathway may only contribute to genome instability in a strain-dependent manner.

***In vitro* and *in vivo* ROS elevate genome instability in *cap1* Δ/Δ *C. albicans*.** In response to bacterial and fungal pathogens, *C. elegans* produces ROS via the BLI-3 dual oxidase (35). However, *C. albicans* has several mechanisms to combat ROS, including

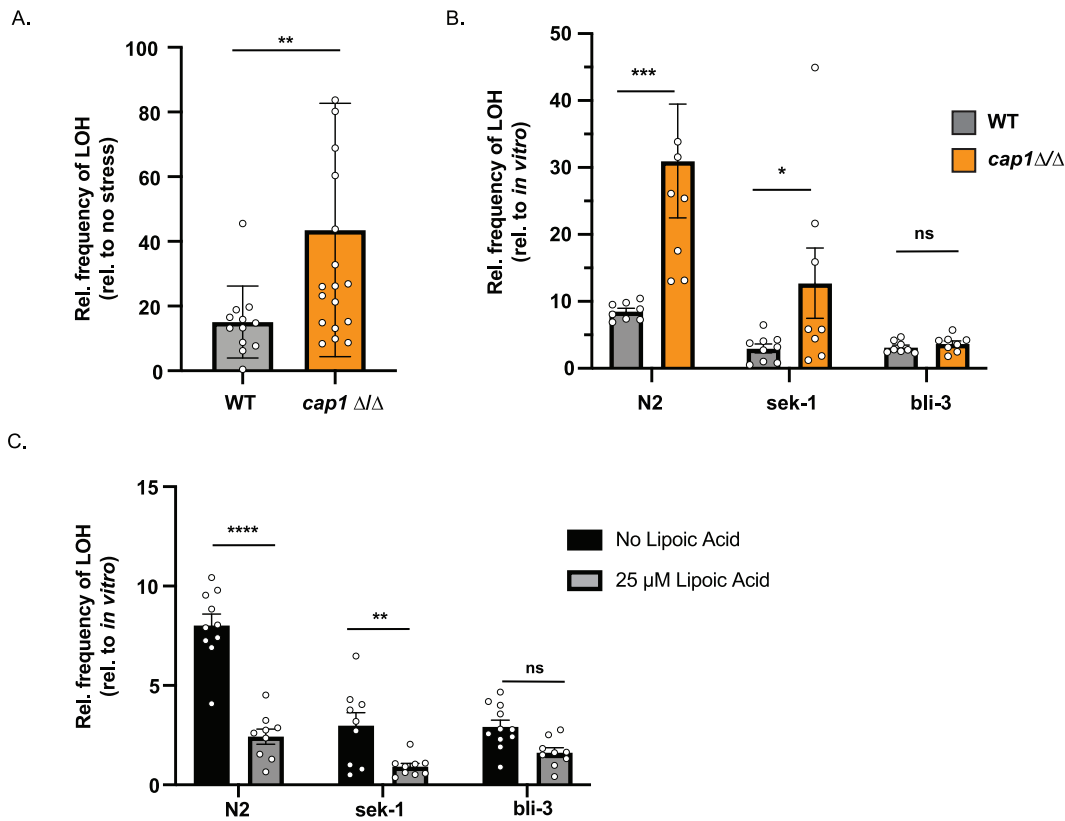


FIG 2 *C. albicans cap1* Δ/Δ strain is more susceptible to *in vitro* and *in vivo* ROS. (A) LOH frequencies of *C. albicans* exposed to 5 mM H_2O_2 for 24 h relative to the frequencies of LOH of *C. albicans* without stress exposure. Means and SD for both wild-type (WT) (gray, $n = 12$) and *cap1* Δ/Δ (orange, $n = 19$) are plotted. Each data point represents an individual measurement. (B) LOH frequencies of *C. albicans* exposed to each host environment relative to the no-host frequency of LOH. Means and SD are plotted. (C) *C. albicans* LOH frequency following host exposure relative to no-host LOH frequency, with (gray: N2, $n = 9$; *sek-1*, $n = 9$; *bli-3*, $n = 9$) and without (black: N2, $n = 10$; *sek-1*, $n = 9$; *bli-3*, $n = 11$) 25 μ M α -lipoic acid. Each data point represents an individual measurement. Means and SD are plotted. Asterisks indicate significant differences (****, $P < 0.0001$; ***, $P < 0.005$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; Mann-Whitney U test).

the activation of antioxidant genes that detoxify ROS, whose expression is regulated by the Cap1p transcription factor (38, 39). To directly test whether CAP1 protects *C. albicans* from ROS-induced genome instability, we compared *in vitro* LOH frequencies of wild-type and *cap1* Δ/Δ *C. albicans* strains exposed to 5 mM H_2O_2 . Exposure to H_2O_2 elevated the LOH frequency in both wild-type and *cap1* Δ/Δ *C. albicans* strains. However, the increase in LOH was significantly higher in *cap1* Δ/Δ , which had a 40-fold increase, compared to the wild type which had a 15-fold increase (Fig. 2A and Fig. S2A). To assess whether CAP1 mitigates *C. albicans* genome instability from host-produced ROS, we compared LOH frequencies between wild-type and *cap1* Δ/Δ *C. albicans* associated with immunocompetent and immunocompromised hosts. If host-produced ROS induces *C. albicans* genome instability, then *cap1* Δ/Δ *C. albicans* will have increased LOH in wild-type and *sek-1* hosts which can produce ROS, but not in *bli-3* hosts which cannot. Compared to *in vitro*, all host environments increased *cap1* Δ/Δ LOH (Fig. S2B). Host-extracted *cap1* Δ/Δ LOH frequencies were higher than those of wild-type *C. albicans* for both wild-type and *sek-1* hosts, but not for *bli-3* hosts (Fig. 2B). Together, these data demonstrate that host-produced ROS elevates *C. albicans* genome instability. Additionally, Cap1p-mediated ROS detoxification is important for mitigating ROS-induced *C. albicans* genome instability both *in vitro* and *in vivo*.

Endogenous and exogenous antioxidants break down ROS through a variety of mechanisms. We next wanted to determine whether antioxidants mitigate the *C. albicans* genome instability which results from host-produced ROS. We compared host-associated

C. albicans LOH in the presence or absence of 25 μ M lipoic acid, an antioxidant involved in the breakdown of ROS (34). In the presence of lipoic acid, host-associated *C. albicans* LOH was significantly reduced when extracted from wild-type and *sek-1* hosts (Fig. 2C and Fig. S2C). In contrast, lipoic acid did not decrease *C. albicans* LOH when extracted from *bli-3* hosts, (Fig. 2C and Fig. S2C). This suggests that antioxidants are effective in reducing *C. albicans* genome instability in ROS-producing hosts. Together, these data suggest that both *in vitro* and *in vivo* ROS elevate genome instability in a *cap1* Δ/Δ mutant, and that *C. albicans* genome instability caused by host-produced ROS can be alleviated with the addition of antioxidants.

Host-produced AMPs and ROS cause aneuploidy and abundant LOH events in *C. albicans*. LOH assays are an easy, useful, and established way to measure *C. albicans* genome instability (40, 41), however, they are limited to measuring a heterozygous marker at a single genomic location. To characterize whether host immunity drives other genomic alterations, we performed whole-genome sequencing of single colony isolates associated with wild-type, *sek-1*, and *bli-3* hosts that were isolated either on media that selected for LOH events (2-DOG; Fig. 3) or on media that did not select for LOH events (YPD; Fig. S3). Among LOH-selected isolates extracted from wild-type hosts, *GAL1* LOH on Chr1 was mediated via break-induced recombination (4/6) or via whole chromosome loss and reduplication (1/6). For the break-induced recombination LOH events, there was no evidence for a common breakpoint or recombination hot spot mediating host-induced *GAL1* LOH. Additional large-tract LOH events (42, 43), homozygous from the site of the DNA break to the end of the chromosome, and whole-chromosomal and segmental aneuploidies were identified in 5/6 LOH-selected isolates from wild-type hosts (Fig. 3). However, no aneuploidy was detected in *C. albicans* from immunocompromised host backgrounds (Fig. 3). Four isolates carried a long-tract LOH or segmental aneuploidy on Chr2 with breakpoints ranging between positions 1,795,723 and 2,155,170; four carried a 170 kb LOH tract or segmental aneuploidy on Chr6, whose breakpoint corresponds with position 861,044, the beginning of a major repeat sequence (MRS). One isolate was trisomic for Chr7 and tetrasomic for Chr6. Even among non-LOH selected isolates associated with wild-type hosts, 50% had undergone whole-chromosome LOH on Chr2 (Fig. S3). However, no large-scale genomic changes were detected in non-LOH selected isolates associated with *sek-1* hosts (Fig. S3, second row). Thus, immune components in wild-type hosts induced large-scale genomic changes in *C. albicans*.

***C. albicans* evolves rapidly in immunocompetent hosts, but not in immunocompromised hosts.** Thus far, our results support a model wherein innate immunity generates *C. albicans* genetic variation by inducing genome instability. Because genetic variation is a fundamental requirement for a response to natural selection, we hypothesized that *C. albicans* would evolve more rapidly in immunocompetent hosts than in immunocompromised hosts. To test this hypothesis, we performed *in vivo* experimental evolution utilizing immunocompetent and immunocompromised hosts and selected for increased *C. albicans* virulence. Given that exposure to both *bli-3* and *sek-1* hosts reduced genome-wide genetic diversity in *C. albicans* (Fig. 3), we selected AU-37 (*glp-4; sek-1*) as our immunocompromised hosts for logistical purposes (see Materials and Methods). We infected six parallel populations of 50 hosts for both the immunocompetent and immunocompromised host groups. When a host population reached 50% mortality, we extracted *C. albicans* from the dead hosts to infect a new host population, selecting for *C. albicans* associated with early host death (Fig. 4A). We evolved *C. albicans* in this manner for a total of 10 passages (Fig. 4A). After five passages in immunocompetent hosts, the average time to 50% mortality was \sim 8 days, which was significantly lower than the initial passage with an average of 12 days (Fig. 4B), indicating increased virulence in *C. albicans*. This increased virulence was subsequently maintained throughout the remainder of the evolution experiment (Fig. 4B). In contrast, there was no change in the average time to 50% mortality in immunocompromised hosts throughout 10 passages, despite them being more susceptible initially to *C. albicans* infection compared to the immunocompetent hosts (Fig. 4C). Therefore, *C. albicans* did not respond to selection for increased virulence in

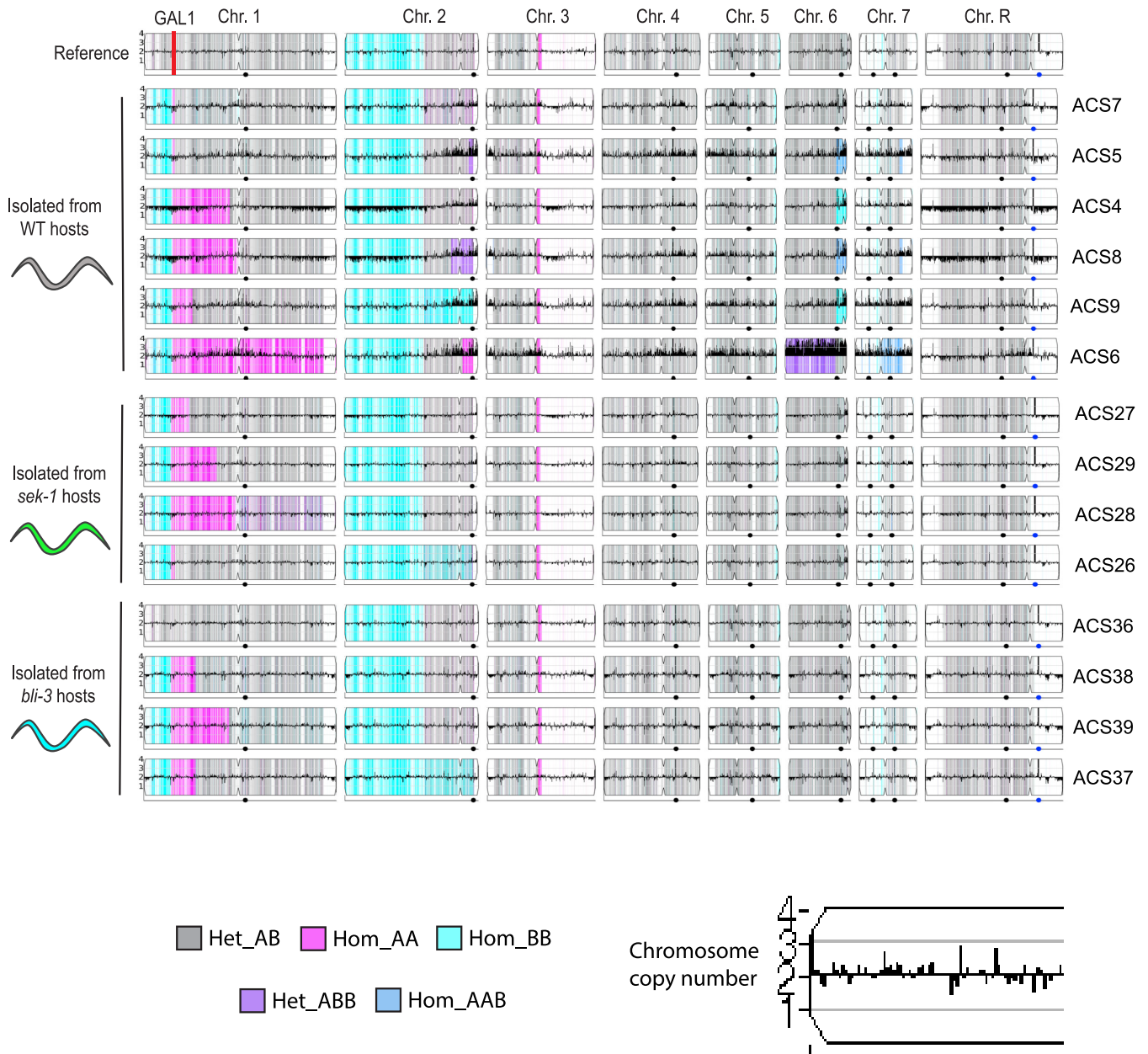


FIG 3 Genome-wide changes following host association. YMAPs of *C. albicans* following 4-day exposure to N2 (WT), *sek-1*, and *bli-3* hosts with *GAL1* LOH selection. Reference strain with the location of *GAL1* on chromosome 1 (indicated with a red line) is displayed at the top. The y axis on each chromosome indicates the chromosomal copy number. Chromosomal color indicates allelic ratio (gray = heterozygous, cyan/pink = homozygous, darker blue/purple = heterozygous with >2 alleles).

the immunocompromised hosts within 10 passages. Together, these results support our hypothesis that host immunity-induced genomic changes generated in *C. albicans* populations facilitated a strong response to this selection regime.

To validate that the increased virulence observed from immunocompetent hosts was a direct result of our selection pressure and not the passaging itself, we passaged *C. albicans* in parallel experimental conditions but in the absence of hosts. We then infected immunocompetent and immunocompromised hosts with the ‘no-host’ evolved (gray, P10) *C. albicans* and found that ‘no-host’ evolved *C. albicans* did not exhibit changes in virulence relative to the ancestral (P0) state, regardless of host immune status (Fig. 4D). Thus, selection for virulence was responsible for the increase in virulence in *C. albicans* evolved in immunocompetent hosts.

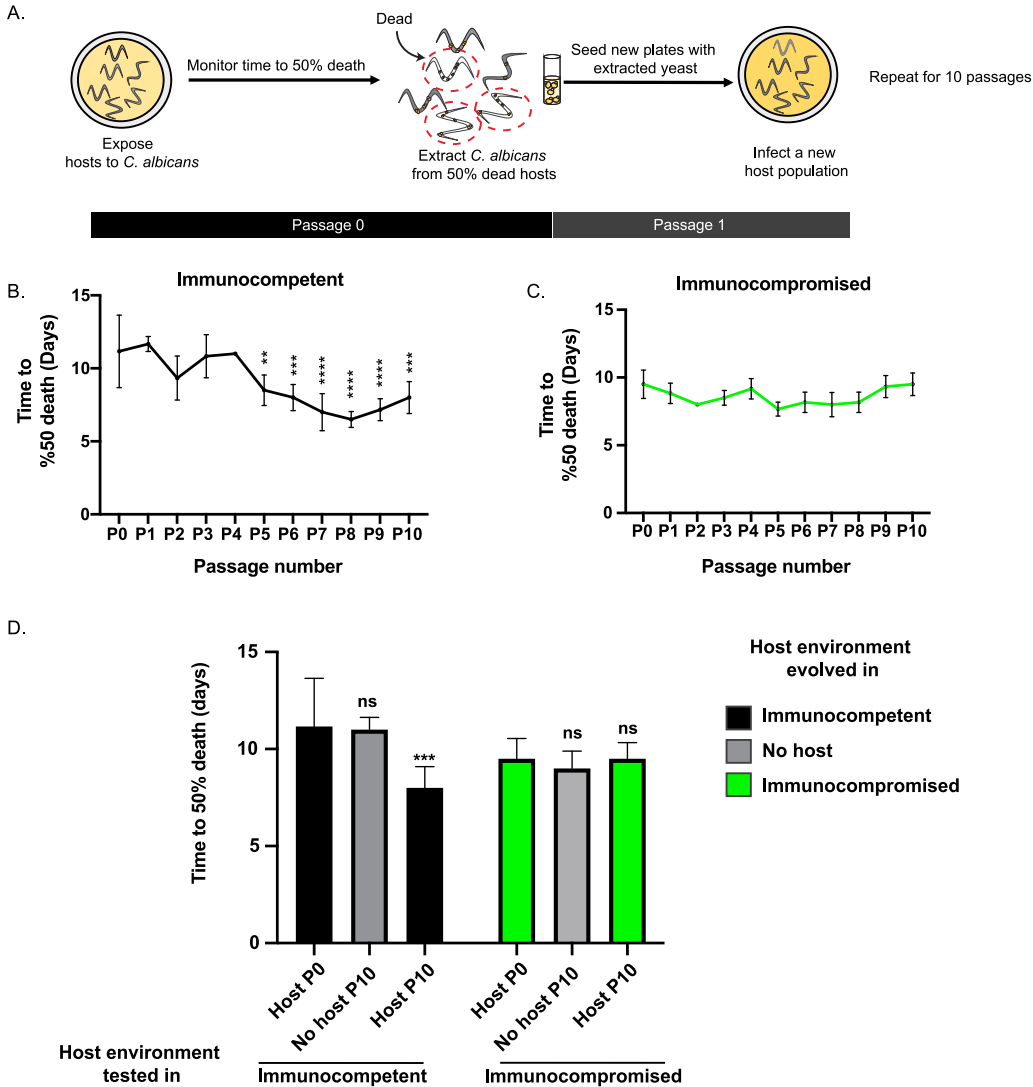


FIG 4 *C. albicans* evolves rapidly in immunocompetent hosts. (A) Experimental schematic. (B) Time to 50% mortality for 10 passages in healthy, immunocompetent (*glp-4*) hosts. Mean ($n = 6$) and SD for each passage infected with *C. albicans* are plotted. (C) Time to 50% mortality for 10 passages in immunocompromised (*glp-4; sek-1*) hosts infected with *C. albicans*. Mean ($n = 6$) and SD for each passage are plotted. Asterisks represent significant differences compared to the initial (P0) time point (****, $P < 0.0001$; *** $P < 0.005$, ** $P < 0.01$; *, $P < 0.05$; Kruskal-Wallis with *post hoc* Dunn's multiple test). (D) Time to 50% mortality for immunocompetent and immunocompromised hosts infected with *C. albicans* from the initial passage (Host P0), *C. albicans* evolved for 10 generations in the absence of hosts (No host P10), and *C. albicans* evolved in the host environment for 10 generations (Host P10). Asterisks represent significant differences compared to the initial (P0) time point (***, $P < 0.005$; ns, not significant; Kruskal-Wallis with *post hoc* Dunn's multiple test).

DISCUSSION

We previously reported that healthy, immunocompetent hosts induce *C. albicans* genome instability. We followed this up by investigating whether host innate immune pathways drive pathogen genome instability (17). By using a wild-type host and two immunocompromised hosts deficient for AMP production (*sek-1*) and ROS production (*bli-3*), we compared the differences in host-associated *C. albicans* genome stability and mutational landscape. *C. albicans* associated with either of the immunocompromised hosts had reduced relative LOH frequencies compared to those associated with wild-type hosts (Fig. 1). Similar to several other host-passaging experiments and whole-genome sequencing results from clinical isolates (14, 16, 19), many of our isolates extracted from wild-type hosts contained large-scale genomic changes, including

whole and segmental chromosomal aneuploidy and/or additional LOH events (Fig. 3). We detected the presence of an extra copy of Chr6 following host exposure, consistent with previous observations of host-induced genomic alterations which used a murine OPC infection model (14). The aforementioned study suggested that Chr6 aneuploidy produces more commensal-like phenotypes inside the host environment. Whether this occurred in our isolate with Chr6 aneuploidy following host exposure has yet to be investigated. We also detected an extra copy of Chr7 in one isolate following wild-type host exposure. In a gastrointestinal (GI) murine model of candidiasis, Chr7 trisomy results in higher *C. albicans* fitness within the GI tract compared to the euploid strain (16). Therefore, specific aneuploidies may enable host adaptation. Following immunocompromised (*sek-1* or *bli-3*) host association, *C. albicans* isolates did not carry any detectable aneuploidy and only a small number (2/8) had an LOH event that was not selected for (Fig. 2). These results suggest that both AMPs and ROS act as stressors on *C. albicans* that enable the generation of genetic variation, which might lead to phenotypic changes that create a more commensal host-pathogen relationship.

The host has a variety of mechanisms capable of controlling microbial growth. Because *C. albicans* is normally commensal, it must strike a delicate balance with the host to evade detection. Overgrowth of *C. albicans* initiates an immune response that initially includes the production of AMPs. AMPs then recruit phagocytes to the site of infection, which produce ROS (22). We found that the removal of host-produced AMPs and ROS decrease LOH frequency and overall genomic changes in *C. albicans* compared to that in *C. albicans* extracted from wild-type hosts (Fig. 1 and 3). We only found a significant decrease in the relative LOH frequency in both the laboratory and blood-stream *C. albicans* extracted from *bli-3* hosts compared to wild-type hosts. However, we did not detect a significant decrease in relative LOH frequency in the bloodstream strain extracted from *sek-1* hosts (Fig. 1B and C). This suggests that host-produced ROS generates genome instability regardless of pathogen background. However, the *sek-1* pathway may only produce AMPs in response to certain strains of *C. albicans*. We propose that the differences observed in genome instability between different strains of *C. albicans* is potentially due to recognition of different PAMPs by host PRR which can trigger different immune responses (44).

Because host-produced ROS represented a conserved source of genome instability across two different strains of *C. albicans*, we further investigated the role of host-produced ROS in generating genome instability. *C. albicans* has several mechanisms to detoxify ROS, one of which is regulated by the transcription factor Cap1p. *Cap1Δ/Δ* mutants are more susceptible to *in vitro* killing by ROS (38, 45). Here, we show that *cap1Δ/Δ C. albicans* genome instability was increased compared to that of wild-type *C. albicans* in hosts capable of producing ROS (N2 and *sek-1*), but not in *bli-3* hosts incapable of producing ROS (Fig. 2B). These findings suggest that host-produced ROS, through the *bli-3* dual-oxidase, induces genome instability in *C. albicans*, and that Cap1p is important for detoxification of host-produced ROS. Recent work in *E. coli* demonstrated that when exposed to low levels of ROS, *E. coli* exhibits a priming response, where evolution in ROS occurs faster and cells develop greater resistance as opposed to non-primed cells (46). Our results suggest that host-produced ROS might be priming *C. albicans*, allowing for tolerance of greater stress.

Although counterintuitive, LOH increases genetic diversity by unmasking recessive alleles, leading to phenotypic changes (43). For example, LOH of drug-resistant alleles of *ERG11*, *TAC1*, or *MRR1* increases antifungal drug resistance (47–49). Similarly, aneuploidy offers a short-term solution that organisms use during adaptation (50–52) and have been shown to be advantageous under certain conditions, including in the host environment (14–16). Because we detected large-scale LOH events and aneuploidy in isolates exposed to immunocompetent hosts, we suggest that these genetic changes facilitate rapid adaptation to the host. Through experimental evolution in immunocompetent and immunocompromised *C. elegans* hosts, we found that *C. albicans* evolved increased virulence rapidly in immunocompetent hosts, but not in immunocompromised hosts (Fig. 4). Our work

identifies AMPs and ROS as important conserved innate immune responses that generate genome instability in the fungal pathogen *C. albicans*. We propose that the generation of genetic variation in response to host-produced ROS and AMPs represents a way in which *C. albicans* can quickly respond to host stressors, thus further tolerating these stressors or avoiding further immune attack. Finally, we demonstrate that immunocompetent hosts facilitate rapid evolution under selection for increased virulence. We hypothesize this is a result from the combination of strong selective pressure and the greater genetic variation generated in *C. albicans* by the innate immune response. Therefore, the host environment can significantly alter the evolutionary trajectories of *C. albicans* populations. While many factors may contribute to the shift from commensal to pathogen in *C. albicans* populations, it is clear that the host immune system can determine the level of genetic variation available for a response to selection. Thus, identifying the selective pressures that drive the shift from commensal to pathogen is critical for understanding *C. albicans* evolution within hosts.

MATERIALS AND METHODS

Strains and maintenance. *C. albicans* and *C. elegans* strains for this study are listed in Table S1 in the supplemental material. Yeast strains (53–55) were stored at -80°C and maintained on YPD (yeast peptone dextrose; 1% yeast extract, 2% bactopectone, 2% glucose, 0.004% adenine, 0.008% uridine) at 30°C . Strains were initially struck onto YPD agar plates from frozen glycerol stocks and incubated at 30°C for 48 h, and single colonies were used as the “parental strain” in subsequent *in vivo* experiments. Nematode populations were maintained on plates containing nematode growth media (NGM) with *E. coli* (OP50) for a food source. *C. elegans* were transferred to a new plate containing freshly seeded *E. coli* every 3 to 4 days. For genome stability assays, treatment plates were seeded with both *C. albicans* and *E. coli* and supplemented with 0.2 g/L streptomycin to inhibit overgrowth of *E. coli*. For fecundity and genome stability assays, NGM was supplemented with 0.08g/L of uridine and 0.08g/L of histidine to facilitate growth of auxotrophic *C. albicans* strains.

Host-associated *C. albicans* genome stability. *Host preparation:* NGM plates were seeded with a mixture of *E. coli* and *C. albicans* 24 h prior to host preparation. To seed plates, single colonies of *C. albicans* were inoculated into 3 mL YPD and incubated overnight at 30°C . Cultures were adjusted with double-distilled water (ddH₂O) to a final concentration of 3.0 OD₆₀₀ per mL. Simultaneously, a single colony of *E. coli* was inoculated into 50 mL LB and incubated for 24 to 48 h at 30°C . The *E. coli* culture was pelleted and washed twice with 1 mL ddH₂O. The pellet was weighed and diluted to a final concentration of 200 mg/mL. For *in vitro* treatments, 250 μL *C. albicans* was spread onto NGM + streptomycin agar plates and incubated overnight at 30°C . For *in vivo* treatment plates, 6.25 μL *C. albicans* and 31.25 μL *E. coli* were brought to a final volume of 250 μL with ddH₂O, spread onto NGM + streptomycin agar plates, and incubated overnight at 30°C . For experimental evolution experiments, *C. albicans* treatment plates had 1.25 μL *C. albicans* and 6.25 μL *E. coli*, and were brought to a final volume of 50 μL . The final 50- μL volume was spotted onto the center of a 35-mm-diameter NGM-plus-streptomycin agar plate followed by incubation at room temperature overnight before the addition of eggs or transferred nematodes.

To synchronize *C. elegans* populations, nematodes and eggs were washed off plates with M9 buffer, transferred to 15-mL conical tubes, and pelleted via centrifugation (2 min at 1,200 rpm). The pellet was resuspended in 2 mL of 25% bleach, mixed via inversion for 2 min, and centrifuged for 2 min at 1,200 rpm. The pellet was washed twice with 3 mL ddH₂O and resuspended with 1 mL ddH₂O. To determine the concentration of eggs, 10 μL was pipetted onto a concave slide, eggs were visualized under a microscope and counted, and the suspension was adjusted to a concentration of ~ 100 eggs per 100 μL with M9.

Host-associated yeast extractions: Yeast extractions were performed as described previously (17). Hosts exposed to *C. albicans* were washed from plates with 3 mL M9 and pelleted via centrifugation (2 min at 2,000 RPM). The supernatant was removed, and the pellet was resuspended with 1 mL 3% bleach, transferred to a microcentrifuge tube, incubated for 3 min, and subsequently centrifuged for 1 min at 12,000 rpm. The supernatant was removed, washed with 1 mL of M9, and centrifuged for 1 min at 12,000 rpm. The wash was repeated two more times to ensure all bleach was removed. Next, 100- μL aliquots of nematode suspension were transferred to 0.6-mL clear microtubes for manual disruption with a motorized pestle. After 1 min of manual disruption, the worm intestine solution was then diluted accordingly with an M9 buffer and plated on YPD + 0.034 mg/L chloramphenicol to prevent any bacterial colonies from arising.

GAL1 Loss of Heterozygosity assay. *In vitro:* Single colonies of *C. albicans* were inoculated in 3 mL YPD grown overnight at 30°C and subsequently diluted to 3 OD in ddH₂O. A volume of 250 μL was plated and spread onto NGM + streptomycin plates, incubated overnight at 30°C , and transferred to 20°C for 4 days to mimic the conditions of the *in vivo* LOH assay. On day 4, yeast cells were washed off with ddH₂O, harvested by centrifugation, washed once with ddH₂O, resuspended in 1 mL of ddH₂O and serially diluted for single colony growth. To determine the total cell viability, 100 μL of 10^{-6} dilution was plated onto YPD and grown for 48 h at 30°C . To identify cells that lost *GAL1*, 100 μL of 10^{-2} and 10^{-3} dilution was plated onto 2-deoxygalactose (2-DOG; 0.17% yeast nitrogen base without amino acids, 0.5% ammonium sulfate, 0.0004% uridine, 0.0004% histidine, 0.1% 2-deoxygalactose, 3% glycerol) and

CFU was counted following 72 h incubation at 30°C. LOH assays with α -lipoic acid were performed as described above, but α -lipoic acid (Sigma-Aldrich cat. no. 1077-28-7) was dissolved in 100% ethanol and added to NGM media containing 0.2 g/L streptomycin sulfate to a final concentration of 25 μ M.

In vivo: The approach was similar to the *in vitro* LOH assay described above, with the following changes. A population of \sim 100 nematodes was plated on each treatment plate containing both *C. albicans* and *E. coli*. On day 4, yeast was extracted as described in the previous section. A dilution of 10^{-1} and 10^{-2} was plated on YPD + chloramphenicol to enumerate total growth, and undiluted cells were plated on 2-DOG to select for the loss of *GAL1*. Three technical replicates were used for each *C. albicans* strain for both *in vitro* and *in vivo* experiments. At least three biological replicates were used for each genome stability assay.

Hydrogen peroxide exposure and genome stability. Six single colonies of *C. albicans* were inoculated in either 2 mL of YPD or 2 mL of YPD containing 5 mM H₂O₂, and were grown for 20 h at 30°C. Cultures were centrifuged at 2,000 rpm for 2 min. The supernatant was removed, and the pellet was washed once with 1 mL ddH₂O. Cultures were serially diluted for single colony growth. Loss-of-heterozygosity assays were performed to determine the frequency of LOH.

Whole-genome sequencing and analysis. Genomic DNA was isolated with phenol chloroform as described previously (56). Whole-genome sequencing was performed through the Microbial Genome Sequencing Center using a single library preparation method based on the Illumina Nextera kit. Libraries were sequenced using paired-end (2 \times 150 bp) reads on the NextSeq 550 platform. Adaptor sequences and low-quality reads were trimmed using Trimmomatic (v0.39 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36 TOPHRED33) (57). All reads were mapped to the phased *C. albicans* reference genome using the haplotyping python script 'mapping.py'. This tool uses the Burrows-Wheeler Aligner MEM (BWA v0.1.19) algorithm to align the sequencing reads to the reference genome, followed by Samtools (v0.1.19) to sort, mark duplicates, and create a BAM file. The average coverage and read depth for each isolate were calculated using samtools 'coverage' (see Supplemental File 2). Variant files were created using the haplotyping python script 'var_calling.py' using the BCFtools calling method with a minimum coverage of 30. Variants were filtered using the following parameters "DP \leq 30 || QD < 2.0 || MQ < 40.0 || FS > 60.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0". Identification of aneuploidy, CNVs, and LOH were conducted using whole-genome sequencing data and the Yeast Mapping Analysis Pipeline (YMAP). BAM files were uploaded to YMAP and plotted using the *Candida albicans* reference genome (A21-s02smo8-r09) with corrections for chromosome end bias and GC content (58).

Experimental evolution. Wild-type SC5314 *C. albicans* was evolved in immunocompetent (*glp-4*) and immunocompromised (*glp-4; sek-1*) nematode host backgrounds. We chose to use the *glp-4* background because of the temperature sensitive sterility mutation. At 25°C, the *glp-4* nematodes are unable to reproduce, allowing us to track the survival of a single generation of nematodes (59). Additionally, we chose to use the *sek-1* background for our immunocompromised host because of the availability of *sek-1* and *glp-4* mutations in the same background. This allowed for complete control over tracking the same population of 50 worms for each passage, which was required for our selection regime. For each host background, experimental evolution was performed with six replicate evolved lines. NGM plates were seeded with *C. albicans* and *E. coli* 24 h prior to the start of the evolution. On day 1, nematodes were synchronized and incubated at 25°C for 48 h. On day 3, 50 L4 nematodes were transferred to a newly seeded NGM plate containing both *C. albicans* and *E. coli*. Every day the host population was monitored for survival and dead nematodes were transferred to a 1.5-mL microcentrifuge tube containing 500 μ L of M9 buffer. Every other day, the remaining live nematodes were transferred to newly seeded plates to replenish their food supply. Once the host population reached 50% mortality, *C. albicans* was extracted from only the 25 dead nematodes by centrifuging the 1.5-mL microcentrifuge tube for 30 s at maximum speed. The supernatant was removed, 500 μ L of 3% bleach was added for 2 min to kill any microbes on the nematode surfaces, and it was then centrifuged for 30s at maximum speed. The supernatant was removed and the nematode pellet was washed three times with 500 μ L M9. The nematodes were manually disrupted using a motorized pestle for 1 min and all the intestinal extracts were inoculated into 2 mL YPD containing 0.034 mg/L chloramphenicol to prevent any bacterial growth. This inoculum was used to seed a new population of 50 hosts in the subsequent passage.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 8 software. Data sets were tested for normality using the D'Agostino-Pearson omnibus normality test.

Data availability. All supplemental files and relevant data will be posted on the Dryad Digital Repository, pending manuscript acceptance. Raw sequencing reads will be deposited NCBI sequence read archive pending manuscript acceptance.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.6 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.03 MB.

ACKNOWLEDGMENTS/AUTHOR CONTRIBUTIONS

We thank Eduardo Scopel Ferreira Da Costa (University of Georgia), Abdul-Rhanman Adamu Bukari (University of Manitoba), and Venkat Talla (Emory University) for helpful

discussions regarding sequencing analysis. We also thank Ognenka Avramovska for thoughtful conversations and critical reading of the manuscript.

This research is supported by NSF DEB-1943415 (M.A.H.), NSF DEB-1750553 (L.T.M.), and Emory University startup funds (M.A.H.).

A.C.S. and M.A.H. designed the study. A.C.S. conducted all of the experiments. A.C.S. and M.A.H. analyzed the data. A.C.S., L.T.M., and M.A.H. wrote, reviewed, and edited the manuscript.

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