Virulence phenotypes result from an interaction between pathogen ploidy and genetic background

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Running title: Pathogen ploidy and genetic background interact to impact fungal virulence

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Abstract

Studying fungal virulence is often challenging and frequently depends on many contexts, including host immune status and pathogen genetic background. However, ploidy has often been overlooked when studying virulence in eukaryotic pathogens. Since fungal pathogens, including the human opportunistic pathogen *Candida albicans*, can display extensive ploidy variation, assessing how ploidy impacts virulence has important clinical relevance. Here, we assessed how *C. albicans* ploidy and genetic background impact virulence phenotypes in both healthy and immunocompromised nematode hosts. In addition to reducing overall host survival, *Candida* negatively impacted host reproduction, which allowed us to survey lethal and non-lethal virulence phenotypes. While we did not detect any global differences in virulence between diploid and tetraploid pathogens, there were significant interactions between ploidy and *C. albicans* genetic background, regardless of host immune function.

Introduction

Virulence is measured by the reduction of host fitness resulting from a host-pathogen interaction[1-3]. Therefore, virulence is not solely the property of the pathogen, but rather the product of the interaction between a host and its pathogen[4,5]. While many biotic and abiotic factors contribute to virulence[6,7], the genotype-by-genotype interaction between hosts and pathogens is a primary determinant of whether a host gets infected and the resulting level of virulence[8,9]. One important, yet understudied, element of an organism's genotype is its ploidy level. Theoretical studies demonstrate that host-pathogens co-evolution should drive hosts towards diploidy and pathogens towards haploidy[10]. However, polyploidy may have an important role in host-pathogen dynamics. For example, polyploidy of some host species is associated with elevated immune response[11,12]. Furthermore, polyploidy and aneuploidy are well documented in fungal pathogens that infect plant and/or animal hosts[13-15]. However there have only been a limited number of studies that investigate whether pathogen ploidy impacts virulence phenotypes and the few that have, often result in contradictory findings[13,16-19].

One potential source of these contradictory results regarding ploidy is the genetic background or allelic composition of the pathogen. Allelic composition refers to not just the specific alleles present in a genome, but the amount of heterozygosity throughout the genome. Pathogen virulence depends on the pathogen's specific allelic combination[20] and phenotypic analysis of diverse clinical isolates within pathogenic species clearly demonstrate that pathogen genetic background contributes to its virulence[21,22]. Ploidy intrinsically impacts allelic composition – haploids contain a single set of gene alleles, whereas diploids and polyploids can

68 either be homozygous or heterozygous for any given locus, and dominance can mask recessive
69 alleles. Allelic composition in polyploids is further complicated by multiple allelic ratios, in which
70 one to four alleles may be present, depending on the mechanism and age of the polyploidization
71 event. Thus, a major challenge in determining the specific role ploidy has on pathogen virulence
72 is disentangling it from allelic composition.
73 The opportunistic fungal pathogen *Candida albicans*, while typically a highly
74 heterozygous diploid[23-25], shows tremendous ploidy variation, ranging from haploid to
75 polyploid[26-29] and is highly tolerant of aneuploidy[26,30-34]. *C. albicans* is a commensal in the
76 human gastrointestinal microbiota and various other niches[35]. Despite its commensal existence,
77 *C. albicans* causes a range of infection, including superficial mucosal infections to life
78 threatening systemic infections[36]. The severity of fungal infection is closely linked to the immune
79 status of the host, with superficial mucosal infections occurring in healthy individuals and
80 serious bloodstream infections occurring almost exclusively in immunocompromised hosts, the
81 latter of which has a mortality of up to 50%[36]. Extensive allelic and ploidy variation, including
82 loss-of-heterozygosity, aneuploidy and polyploidy, are well-documented in laboratory and
83 clinical *C. albicans* isolates[21-25,37,38]. However, these large-scale genomic perturbations are often
84 identified and studied in the context of antifungal drug resistance[22,31,32], and only limited studies
85 have specifically investigated how *C. albicans* ploidy impacts virulence phenotypes[16-18].
86 In this study we sought to identify how *C. albicans* ploidy impacts its virulence by using
87 four diploid-tetraploid pairs of strains, with each pair representing a distinct genetic background.
88 We assessed virulence by monitoring four measures of host fitness using healthy and
89 immunocompromised *C. elegans* hosts[39]. While we find almost no overall relationship between
90 ploidy and virulence, there are detectable differences among *C. albicans* genetic backgrounds
91 and clear interactions between *C. albicans* genetic background and its ploidy state on virulence
92 phenotypes. We also observe these interactions in immunocompromised hosts, however, in
93 some cases, the ploidy-specific pattern and/or the degree of virulence severity is different
94 between healthy and immunocompromised hosts. Taken together, these results emphasize the
95 importance of genotypic interactions on virulence phenotypes, in which genotype includes ploidy
96 state.
97
98 **Results**
99 *Interactions between pathogen ploidy and genetic background impact virulence phenotypes in*
100 *healthy hosts*

101 First, we wanted to investigate whether *C. albicans* genetic background differentially
102 impacted host fitness. To do this we infected *C. elegans* hosts with four genetically distinct
103 strain backgrounds of *C. albicans.* Two of these genetic backgrounds are laboratory strains: a
104 'laboratory heterozygous,' which consists of the SC5314 reference strain and a 'laboratory
105 homozygous,' a derivative of SC5314 in which the genome is completely homozygous. The
106 other two genetic backgrounds are clinical strains: a 'bloodstream,' isolated from a candidemia
107 infection of a male immunosuppressed patient and an 'oral/vaginal,' a pair of clinical strains
108 isolated from a single immune-competent female patient with vulvovaginal candidiasis. For each
109 *C. albicans* genetic background, we assessed four measures of host fitness: host survival (Fig.
110 1A), host lineage growth (Fig. 1B), host brood size (Fig. 1C), and host reproductive timing (Fig.
111 1D). For survival and lineage growth, all *C. albicans* genetic backgrounds were virulent, with
112 significant differences observed between uninfected and infected treatments, and the only
113 statistical difference between *C. albicans* genetic backgrounds was between the laboratory
114 heterozygous and oral/vaginal strains for host survival. For brood size, only the laboratory
115 homozygous genetic background was virulent and resulted in significantly smaller host brood
116 sizes compared to the two clinical *C. albicans* backgrounds. The impact on host reproductive
117 timing also depended on pathogen genetic background: the laboratory heterozygous and the
118 clinical bloodstream strains were virulent (i.e. reduced amount of normal reproductive timing)
119 whereas the other two *C. albicans* genetic backgrounds were not. The laboratory heterozygous
120 strain impacted host reproductive timing significantly more than the three other pathogen
121 genetic backgrounds. Together, these results suggest that while pathogen genetic background
122 may not obviously contribute to host survival phenotypes, it may be important for other
123 measures of fungal infection that are less lethal.
124 One factor that may dampen any differences in virulence among *C. albicans* genetic
125 backgrounds is ploidy. For each genetic background, we used a related diploid strain and a
126 tetraploid strain (Table S1). The tetraploids for both the 'laboratory' strains were produced via
127 mating in the laboratory. The 'bloodstream' pair consisted of a diploid strain isolated early in the
128 infection and its corresponding tetraploid strain was isolated mid- to late- infection following
129 antifungal treatment. The 'oral/vaginal' pair consisted of a diploid strain isolated from the oral
130 cavity and its corresponding tetraploid was isolated from the urogenital tract following antifungal
131 treatment for vulvovaginal candidiasis. There have been limited and conflicted reports on the
132 role of tetraploid in fungal virulence[16,17]. To assess how pathogen ploidy impacts virulence, we
133 compared all the diploid strains to the tetraploid strains for host survival (Fig. 1E), lineage
134 growth (Fig. 1F), host brood size (Fig. 1G), and host reproductive timing (Fig. 1H) in wildtype

135 hosts. We did not observe a significant difference between these two ploidy states for any of the
136 host fitness measures tested, suggesting that ploidy may not impact virulence phenotypes.
137 However, when we account for *C. albicans* strain background, differences between pathogen
138 ploidy emerge but depend on strain genetic background and thus, we detect a significant
139 interaction between genetic background and ploidy for host lineage growth ('interaction'
140 $p=0.0354$, two-way ANOVA; Fig. S1B), brood size ('interaction' $p<0.0001$, two-way ANOVA; Fig.
141 S1B), and reproductive timing ('interaction' $p=0.0283$, two-way ANOVA; Fig. S1B). While we
142 cannot directly test for an interaction using host survival curves, we do detect differences
143 between diploids and tetraploids for each *C. albicans* genetic background (Fig. S1 and Table
144 S2). Taken together, these results suggest that there is no global pattern in ploidy state and
145 virulence, but ploidy in combination with genetic background does significantly contribute to *C.*
146 *albicans* virulence phenotypes.
147 When we look at the specific diploid-tetraploid pairs, representing different *C. albicans*
148 genetic backgrounds, we see significant differences in virulence between the diploid and
149 tetraploid for at least one fitness measure, for every *C. albicans* genetic background (Fig. 2). For
150 two genetic backgrounds, the laboratory homozygous and clinical bloodstream strains, the
151 diploid strain was more virulent than its tetraploid counterpart. For the laboratory heterozygous
152 and clinical oral/vaginal strains, the tetraploid strain was more virulent than its diploid
153 counterpart, when differences between ploidy states were detected. Furthermore, these genetic
154 background specific ploidy patterns are generally consistent across host fitness measures. For
155 example, the laboratory heterozygous and clinical oral/vaginal tetraploids are also more virulent
156 than their diploid counterparts for host brood size (Fig 2C), and the clinical bloodstream diploid
157 was more virulent than its tetraploid counterpart for lineage growth and delayed host
158 reproduction (Fig. 2B and D). We performed every pairwise comparison between treatments for
159 host survival (Table S2), lineage growth (Table S3), brood size (Table S4), and reproductive
160 timing (Table S5) and find significant differences in virulence between different *C. albicans*
161 genetic backgrounds and ploidy states for most host fitness measures, except host lineage
162 growth, were very few differences between *C. albicans* strains were detected. Taken together,
163 these data support that *C. albicans* ploidy does contribute to its virulence phenotypes, but
164 whether it attenuates or enhances virulence depends on its genetic background.
165
166 *Host immune status and pathogen genetic background contribute to virulence phenotypes*
167 We have previously established that *C. albicans* and other non-*albicans Candida*
168 species cause more severe infections in an immunocompromised *C. elegans* hosts compared to

169 healthy *C. elegans* hosts [39]. Given that we see pathogen ploidy patterns depend on genetic
170 background for virulence phenotypes in wildtype healthy hosts, we next wanted to assess if we
171 could detect comparable patterns in immunocompromised hosts. For each *C. albicans* strain,
172 we assessed four measures of host fitness and compared *C. albicans* virulence in
173 immunocompromised *sek-1 C. elegans* hosts: host survival (Fig. 3A), host lineage growth (Fig.
174 3B), host brood size (Fig. 3C), and host reproductive timing (Fig. 3D). Nearly all the *C. albicans*
175 genetic backgrounds significantly reduced host fitness compared to uninfected controls, the only
176 exception was the laboratory homozygous strains did not significantly delay host reproduction.
177 The laboratory homozygous background is also less virulent than the clinical oral/vaginal
178 genetic background for host. Together, these results suggest that global differences in pathogen
179 genetic background are revealed in hosts with compromised immune function for both lethal and
180 non-lethal measures of host fitness.
181 To assess how pathogen ploidy impacts virulence in immunocompromised hosts, we
182 compared all the diploid strains to the tetraploid strains for host survival (Fig. 3E), host lineage
183 growth (Fig. 3F), host brood size (Fig. 3G), and host reproductive timing (Fig. 3H). Similar to
184 healthy wildtype hosts, we did not observe a significant difference between these two ploidy
185 states for and of the host fitness measures. However, when we account for *C. albicans* strain
186 background, differences between pathogen ploidy emerge but depends on strain genetic
187 background and a significant interaction between genetic background and ploidy is detected for
188 host lineage growth ('interaction' p=0.0012, two-way ANOVA; Fig. S2B), brood size ('interaction'
189 p=0.0090, two-way ANOVA; Fig. S2C), and reproductive timing ('interaction' p=0.0167, two-way
190 ANOVA; Fig. S2D). While we cannot directly test for an interaction using host survival curves,
191 we do detect differences between diploids and tetraploids for each *C. albicans* genetic
192 background (Fig. S2 and Table S2). Taken together, these results further support the
193 observation that there is no global correlation between ploidy state and virulence, but ploidy
194 does have an important contribution to virulence phenotypes within pathogen genetic
195 backgrounds regardless of host immune status.
196 When we look at specific diploid-tetraploid pairs, representing different *C. albicans*
197 genetic background, we see significant differences in virulence in immunocompromised hosts
198 between the diploid and tetraploid state for at least one fitness measure, for all *C. albicans*
199 genetic backgrounds except for the laboratory homozygous (Fig. 4). For the laboratory
200 heterozygous genetic background, the tetraploid counterpart was more virulent than its diploid
201 counterpart when differences between ploidy states were detected (lineage growth and brood
202 size), similar to the pattern observed in healthy hosts (Fig. 2). Furthermore, the clinical

203 bloodstream diploid was more virulent than its tetraploid counterpart for immunocompromised
204 host survival and brood size (Fig. 4A and C), similar to the pattern observed in healthy hosts.
205 However, the oral/vaginal strain had significant differences between its diploid and tetraploid
206 counterparts for host survival and reproductive timing (Fig. 4A and D), however the tetraploid
207 was more virulent in the former, while the diploid was more virulent in the latter. We also
208 performed every pairwise comparison between treatments for host survival (Table S2), lineage
209 growth (Table S3), brood size (Table S4), and reproductive timing (Table S5) and find significant
210 differences in virulence between different *C. albicans* genetic backgrounds and ploidy states for
211 most host fitness measures, except host reproductive timing, were very few differences between
212 *C. albicans* strains were detected. These results emphasize that while global differences
213 between *C. albicans* genetic backgrounds or ploidy states may be difficult to identify in
214 immunocompromised hosts (Fig. 3), ploidy-specific and genetic background differences in
215 virulence are detectable across multiple host fitness measures.
216
217 *Ploidy-specific interactions between host and pathogen genotypes.*
218 Finally, we were curious if host immune status impacted the virulence relationship
219 between each diploid-tetraploid pair of strains. Since there are significant differences for most
220 host fitness measures between healthy and immunocompromised hosts even when uninfected
221 (Table S6), we normalized each *C. albicans*-infected host fitness metric to that of the uninfected
222 control to directly compare the severity of *C. albicans* infection between host genotypes. This
223 analysis shows that immunocompromised hosts are more significantly more susceptible to *C.*
224 *albicans* infection and show larger reductions in survival, brood size and lineage growth
225 compared to those observed in healthy hosts, while the amount of delayed reproduction caused
226 by *C. albicans* infection is similar (Fig. 5A-D). We also detect significant interactions between *C.*
227 *albicans* strain and host immune status for lineage growth ('interaction' p=0.0004, two-way
228 ANOVA), brood size ('interaction' p=0.0042, two-way ANOVA; Fig. S2C), and reproductive
229 timing ('interaction' p=0.0001, two-way ANOVA).
230 We next examined whether the relationship between ploidy-specific virulence differences
231 changed depending on host immune status. To do this, we plotted the relative impact of the
232 diploid (solid lines) and tetraploid (dotted lines) in healthy (N2) and immunocompromised (*sek-*
233 *1*) host backgrounds for all four *C. albicans* genetic backgrounds (Fig 5E-H). In general, there
234 was a high degree of similarity in the relationship between diploid and tetraploids across for both
235 host genotypes, as indicated by a non-significant interaction term measured by two-way
236 ANOVA. However, there were a couple of notable exceptions, particularly in the bloodstream

237 pair of strains. In healthy hosts, we observed the diploid displaying more severe virulence
238 phenotypes than its tetraploid counterpart for lineage growth and reproductive timing, yet these
239 differences are diminished in immunocompromised hosts. Thus, we detect a significant
240 interactions between host immune status and *C. albicans* ploidy for the bloodstream genetic
241 background. Strikingly, we detect the reverse pattern for host survival with the *C. albicans*
242 bloodstream diploid and tetraploid strains, in which no detectable differences are observed in
243 healthy hosts, but the diploid is significantly more virulent than the tetraploid in
244 immunocompromised hosts, yet regardless of the host context or specific fitness measure, the
245 diploid is more virulent than its tetraploid counterpart. From these results, we conclude that
246 interactions between host immune status, pathogen genetic background and ploidy state
247 determine the severity of virulence phenotypes in *C. albicans*.
248
249 **Discussion**
250 Here, we sought to understand how the genetic background and ploidy of the human
251 fungal opportunistic pathogen *Candida albicans* contributes to virulence phenotypes in the
252 nematode host *C. elegans*. Overall, we found that most of the strains we tested were virulent for
253 at least one measure of host fitness, but cannot generalize any over-arching patterns in how
254 ploidy or genetic background contribute to virulence phenotypes. Rather, we have found that
255 there is a significant interaction between *C. albicans* ploidy and its genetic background. We
256 detect this interaction in both healthy and immunocompromised host contexts. However,
257 immunocompromised hosts display more severe fungal infections compared to healthy hosts,
258 regardless of *C. albicans* ploidy and/or genetic background.
259 *C. albicans* was considered an 'obligate' heterozygous diploid for many years [40-43], and
260 its diploid-tetraploid parasexual cycle only discovered and characterized in the last two
261 decades[15,44]. Much of the research regarding *C. albicans* ploidy states have focused on the
262 mechanisms involved with the parasexual cycle and how the ploidy reduction process that
263 tetraploids undergo promote cellular heterogeneity and genetic variation[26,45-49]. There are only a
264 small number of studies investigating if different ploidy states impact its virulence. These earlier
265 studies investigating whether *C. albicans* ploidy impacts virulence had contradictory results –
266 Hubbard et al demonstrated that derivative tetraploid had similar results in a murine tail vein
267 infection model as its diploid progenitor[17], whereas Ibrahim et al demonstrate that tetraploids are
268 less virulent than diploids[16]. Given that tetraploid *C. albicans* have been identified in clinical
269 settings[37] and that other fungal pathogens display extensive ploidy variation[14,15], assessing how
270 ploidy impacts virulence has newfound clinical relevance. We think our systematic approach of

271 comparing paired diploid and tetraploid strain across genetic backgrounds and host contexts
272 provides a more comprehensive analysis of ploidy and virulence. Furthermore, our results
273 support the contradictory findings reported earlier, as we observe that sometimes certain
274 tetraploids are less virulent than diploids, sometimes there are no differences between ploidy
275 states, and sometimes certain tetraploids are more virulent than diploids (Tables S2-S5).
276 Importantly, not only does our analysis account for differences in *C. albicans* genetic
277 background, but, by utilizing *C. elegans* as an infection system we can assess multiple
278 measures of host fitness beyond host survival. As an opportunistic pathogen, *C. albicans*
279 causes a wide-range of infections with the most severe infections occurring in
280 immunocompromised individuals and only a fraction of these severe infections result in patient
281 death. As such, we need to broaden our scope to investigate fungal virulence beyond a lethal
282 phenotype. We have previously demonstrated that *C. albicans* infection of *C. elegans* not only
283 results in reduced survival, but also has reduced fecundity and delays in reproduction[39]. Here,
284 we have leveraged this infection system to screen for differences in virulence between *C.*
285 *albicans* ploidy and genetic backgrounds. While we did not find any overall pattern in virulence
286 between diploids and tetraploids, we did identify ploidy-specific differences depending on
287 genetic background (Figs. 2 and 4). Importantly, these specific patterns were consistent across
288 multiple host fitness measures and host immune status (Fig. 5). Furthermore, we found that
289 immunocompromised hosts had significantly more severe infections than immune competent
290 hosts (Fig. 5), similar to what is observed clinically.
291 It is important to note that the clinical *C. albicans* genetic backgrounds, bloodstream and
292 oral/vaginal, are not completely isogenic between the diploid and tetraploid strains, and that
293 some allelic variation exists in addition to their differences in ploidy[37]. It is feasible that some of
294 the differences in virulence that we observe between the diploid and tetraploids isolates in these
295 two clinical *C. albicans* backgrounds (i.e. the bloodstream diploid is more virulent than its
296 tetraploid, whereas the oral/vaginal tetraploid is more virulent than its diploid) is due to these
297 allelic differences. By also measuring virulence phenotypes in laboratory-derived genomes that
298 only differ in the number of chromosome sets they contain, we can directly assess the impact
299 ploidy has on virulence. Here, we still observe different patterns of ploidy-specific virulence,
300 depending on genetic background. In the laboratory heterozygous genome, we consistently
301 observe the tetraploid as more virulent than its diploid in both healthy (Fig. 2) and
302 immunocompromised (Fig. 4) host contexts. However, in the laboratory homozygous genome,
303 we frequently failed to find any significant differences between diploid and tetraploid for any of
304 the host fitness measures, the only exception being healthy host survival, in which the tetraploid

305 was avirulent and the diploid was virulent (Fig. 2A). This result is consistent with previous
306 findings that *C. albicans* homozygous genomes do not show significant growth differences *in*
307 *vitro* or *in vivo* between ploidy states[27,46].
308 In this work we found that ploidy and genetic background interact to contribute to *C.*
309 *albicans* virulence (Figs. S1 and S2). We also observe significant interactions between *C.*
310 *albicans* strains and host immune status (Fig. 5). These results indicate that virulence is not
311 simply a binary 'avirulent/virulent' classification, but rather a complex trait and we need to start
312 dissecting fungal virulence from this perspective. Recently, genome analysis of clinical isolates
313 revealed genomic features that differ between clinical isolates and SC5314, the laboratory
314 reference strain of *C. albicans,* and/or identify genetic determinants of antifungal drug
315 resistance[22,38,50-53]. Only a small number have attempted to identify the genetic loci underpinning
316 virulence and only two genes were identified and validated, *EFG1*[21,51] and *PHO100*[46].
317 Importantly, these approaches may overlook other factors and contexts, such as ploidy or host
318 immune status, that contribute to virulence. While it is clear that there variation in virulence
319 occurs across *C. albicans* genetic backgrounds, there is still much work to do in elucidating the
320 drivers of virulence.
321
322 **Material and Methods**
323 *Strains and media*
324 For this study, the *C. albicans* strains used are described in Table S1. *C. elegans* N2
325 Bristol (WT) and *sek-1* mutant[54] were used to test host survival, fecundity and population
326 growth in healthy and immunocompromised hosts, respectively. *C. elegans* populations were
327 maintained at 20°C on lite nematode growth medium (NGM, US Biological) with *E. coli* OP50 as
328 a food source and were transferred to a newly seeded *E. coli* plate every 3-4 days. For survival,
329 fecundity and lineage growth assays, NGM was supplemented with 0.08 g/L uridine, 0.08 g/L
330 histidine, and 0.04 g/L adenine to facilitate growth of auxotrophic *C. albicans* strains and 0.2 g/L
331 streptomycin sulfate to inhibit *E. coli* overgrowth so *C. albicans* strains could proliferate.
332
333 *Survival and Fecundity Assays*
334 Seeding NGM plates and *C. elegans* egg preparation for survival and fecundity assays
335 were performed as previously described[39]. Briefly, *C. albicans* strains and *E. coli* OP50 were
336 inoculated in 3 mL of YPD or 5 mL of LB, respectively, and incubated at 30°C for 1-2 days. *C.*
337 *albicans* cultures were diluted to a final volume of 3.0 OD_{600} per mL and *E. coli* was pelleted and
338 washed twice with 1 mL of ddH_2O. The washed pellet was centrifuged for 60 sec at maximum

339 and any excess liquid removed. The pellet was weighed and suspended with ddH_2O to a final
340 volume of 200 mg/mL. For uninfected treatments, 6.25 μL *E. coli* was mixed with 43.75 μL
341 ddH_2O. For *C. albicans* treatments, 1.25 μL *C. albicans* was mixed with 6.25 μL *E. coli* and
342 brought to a final volume of 50 μL with ddH_2O. All 50 μL was spotted onto the center of a 35mm
343 supplemented-NGM Lite agar plate and incubated at room temperature overnight before
344 addition of nematode eggs or transferred nematode.
345 To synchronize host populations, *C. elegans* populations (~100 nematodes) were
346 washed off NGM plates with M9 buffer and transferred to a 15 mL conical tube and pelleted by
347 centrifugation for 2 min at 1200 rpm. The pellet was re-suspended in a 5.25% bleach solution,
348 transferred to a microcentrifuge tube and inverted for 90-120 sec and subsequently centrifuged
349 for 30 sec at 1500 rpm. The pellet was washed thrice with 1 mL M9 buffer and resuspended in
350 500 μL M9 buffer. ~100 eggs were added to uninfected or *C. albicans* treatment plates. 48 h
351 later, a single L4 nematode (x10 per treatment) was randomly selected and transferred to an
352 uninfected or *C. albicans* treatment plate and incubated at 20°C. Each founder nematode was
353 transferred to new treatment plates every 24 h for seven consecutive days. For survival
354 analysis, we documented whether the founder was alive, dead, or censored (i.e. crawled off the
355 plate or were otherwise unaccounted). For fecundity analysis, any eggs laid for each 24-hour
356 interval remained undisturbed on the plate and incubated at 20°C for an additional 24 h and the
357 number of viable progeny produced per day was scored. Total brood size is the sum of viable
358 progeny produced over seven days. Delayed reproduction is calculated by the number of
359 progeny produced on Day 4 or later divided by the total progeny produced for each founder
360 nematode. All experiments were performed in triplicate or more.
361
362 *Lineage Growth Assays*
363 Lineage growth assays were performed as previously described[39]. In brief, a single L4
364 founder nematode (x6 founders per treatment) was randomly selected from a synchronized
365 population and transferred to a 100mm treatment plate that contained a 300 μl seed of *C.*
366 *albicans* and/or *E. coli* and incubated at 20°C for five days in which the founder produces F1
367 and F2 progeny. The entire population derived from the single founder were washed with M9
368 buffer and transferred to 15mL conical tubes and brought to a final volume of 10mL. Tubes were
369 placed at 4°C for 1h to allow the nematodes to settle at the bottom. 20 μL samples were taken
370 from each population and counted six independent times to determine the final population size
371 for each founder nematode. All experiments were performed at least in duplicate.

Statistical Analyses

All statistical analyses were performed with GraphPad Prism. Survival curves were tested for significant differences using log-rank (Mantel Cox) tests. Lineage growth, total brood size and delayed reproduction data sets were tested for normality using the D'Agostino & Pearson omnibus normality test. For comparisons across genetic backgrounds, Kruskal-Wallis and Dunn's multiple comparison tests were performed. For pairwise comparisons between ploidy or host context, Mann Whitney tests were performed. Two-way ANOVAs were performed to test for interactions between *C. albicans* genetic background and ploidy or between *C. albicans* ploidy and host context.

Author contributions

DFJ and MAH devised the experiments. DFJ and RE performed the experiments. DJF and MAH analyzed the data. MAH wrote the manuscript.

Competing interests

The author(s) declare no competing interests.

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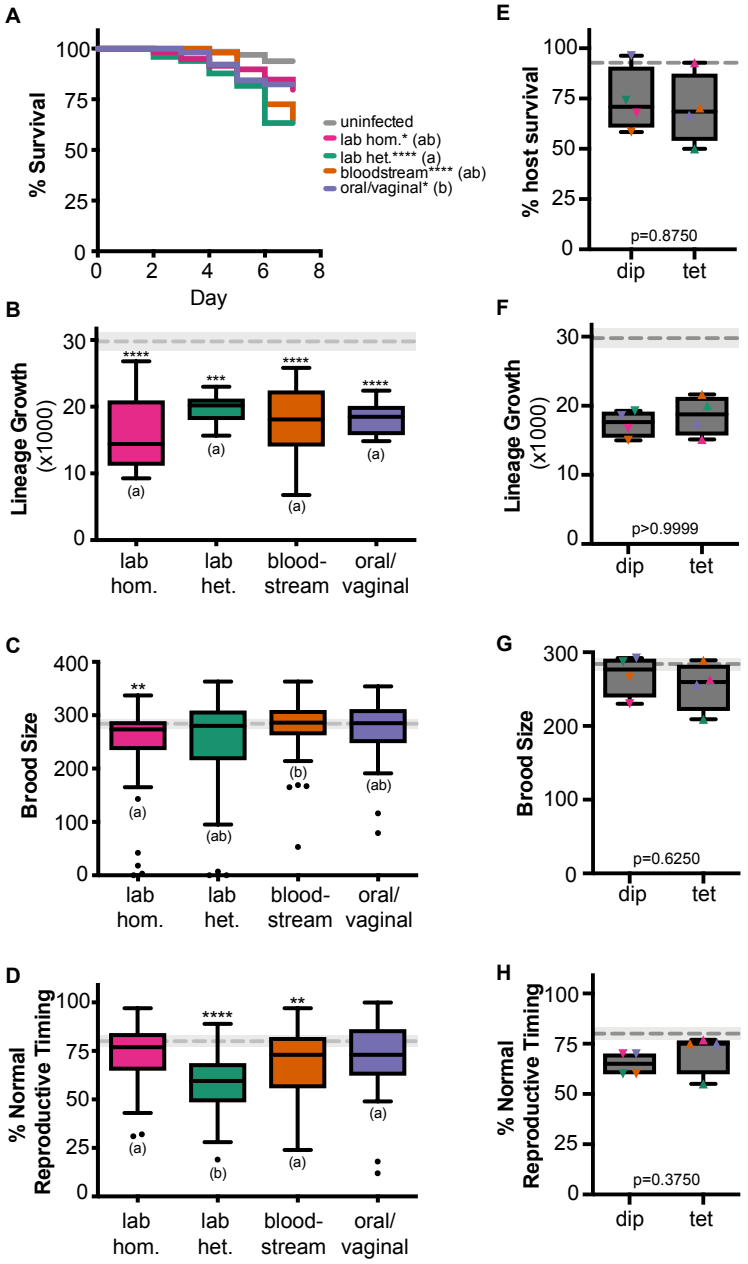

Figure 1: Different *C. albicans* genetic backgrounds reduce multiple measures of wildtype *C. elegans* host fitness.

A) Survival curves for *C. elegans* populations that are either uninfected (exposed to just an E. coli food source, grey) or when infected with different *C. albicans* strains (indicated in legend). The number of worms analyzed (n) for each treatment is indicated in Table S1. Statistical significance was tested using pairwise comparisons of survival curves with Log-rank (Mantel-Cox) test. Astrisks denote statistical differences compared to the uninfected control (* indicates p <0.05, **** indicates p < 0.0001). *C. albicans* treatments that share letters are not significantly different, whereas treatments with differing letters are stastically different.

B) Box and whiskers plot of host lineage growth which represents the total population size (representing the number of F1 and F2 progeny) produced within 7 days from a single founder *C. elegans* host infected with *C. albicans*. Boxes indicate the 25-75th quartiles with median indicated. Error bars are the normalized range of the data and circles indicate outliers. The mean and 95% CI of the uninfected control treatment are indicated by the grey dashed line and shaded grey box. Statistical significance was tested using one-way ANOVA. Astrisks denote statistical differences compared to the uninfected control (* indicates p <0.05, *** indicates p < 0.001). C. albicans treatments that share letters are not significantly different, whereas treatments with differing letters are stastically different, post-hoc Dunn's multiple comparison test. **C)** Total brood size and **D)** Average percentage of host progeny produced during days 1-3 of adulthood (normal reproductive timing) of *C. elegans* infected with *C. albicans*. Data and statistical analysis are the same as (B).

E) % host survival on Day 7 for diploid (dip) and tetraploid (tet) *C. albicans* strains (colored symbols indicate specific *C. albicans* genetic background). Statistical significance was tested using Wilcoxon matched-pairs signed rank test and p values are indicated.

F) Lineage growth, **G)** Brood size, and **H)** Reproductive timing of hosts infected with *C. albicans* diploid and tetraploid strains. Data and statistical analysis are the same as for (E).

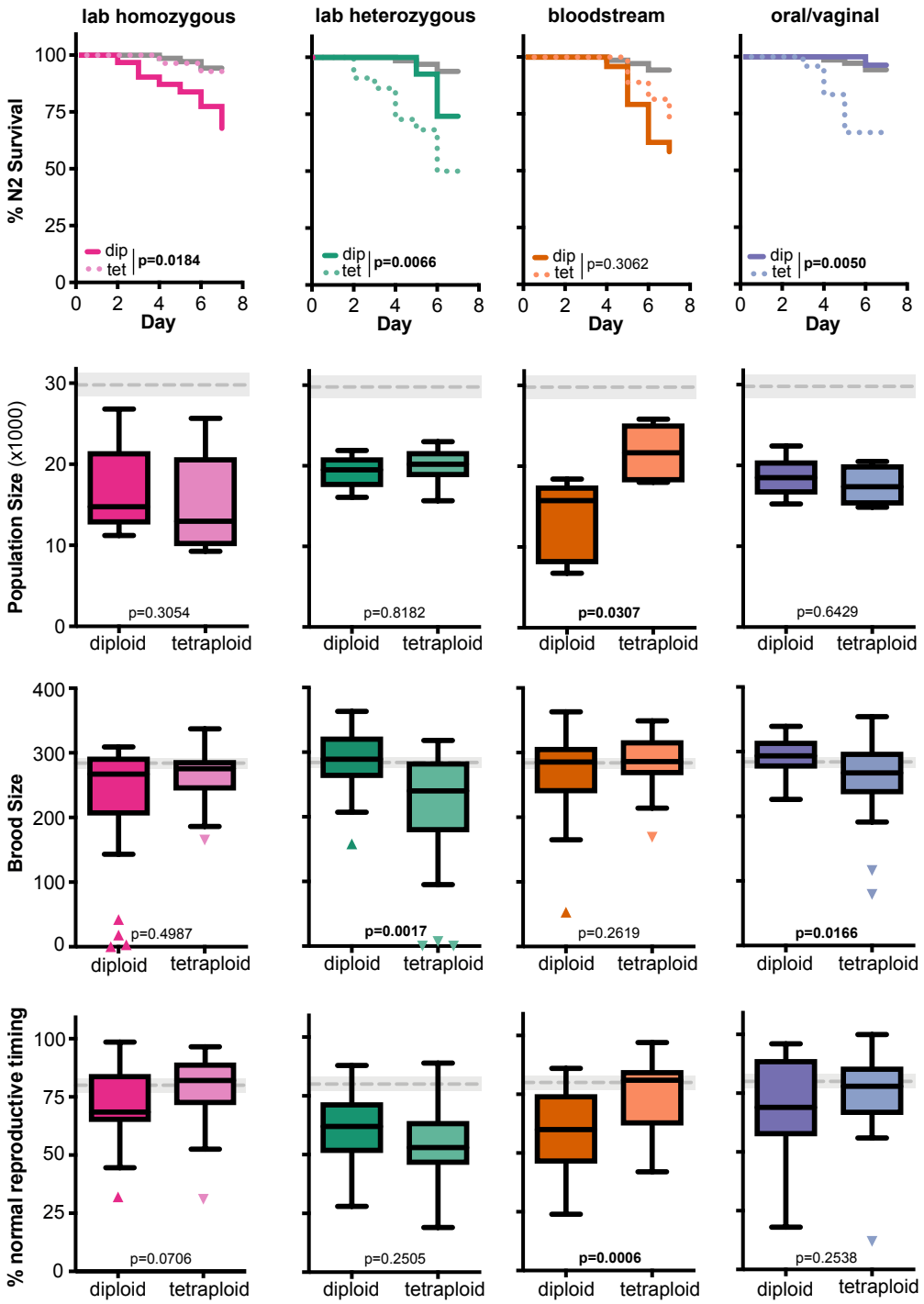

Figure 2: Ploidy-specific differences across *C. albicans* genetic backgrounds.
A) Survival curves for *C. elegans* populations that are either uninfected (exposed to just an E. coli food source, grey) or when infected with diploid or tetraploid *C. albicans* strains from laboratory homozygous (pink), laboratory heterozygous (green), bloodstream (orange), or oral/vaginal (blue) genetic backgrounds. Statistical significance was tested using pairwise comparisons of diploid and tetraploid survival curves with Log-rank (Mantel-Cox) test and p-values are indicated and significant differences are highlighted in bold text.
B) Box and whiskers plot of host lineage growth which represents the total population size (representing the number of F1 and F2 progeny) produced within 7 days from a single founder *C. elegans* host infected with *C. albicans*. Boxes indicate the 25-75th quartiles with median indicated. Error bars are the normalized range of the data and circles indicate outliers. The mean and 95% CI of the uninfected control treatment are indicated by the grey dashed line and shaded grey box. Statistical significance between diploid and tetraploid strains was determined using Mann Whitney test and p-values are indicated and significant differences are highlighted in bold text.
C) Total brood size and **D)** Average percentage of host progeny produced during days 1-3 of adulthood (normal reproductive timing) of *C. elegans* infected with diploid and tetraploid *C. albicans*. Data and statistical analysis are the same as (B).

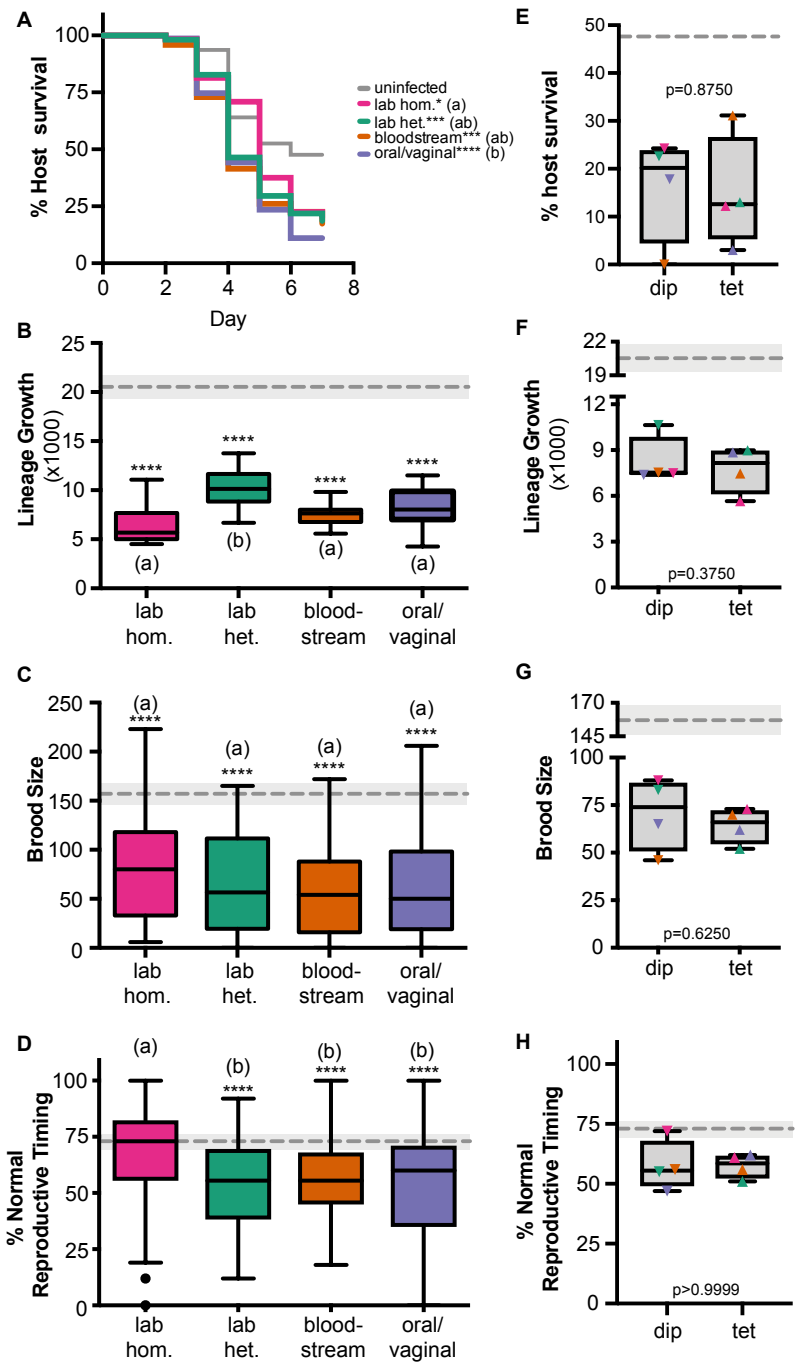

Figure 3: Immunocompromised hosts are highly susceptible to *C. albicans* infection regardless of genetic backgrounds or ploidy.

A) Survival curves for *sek-1 C. elegans* populations that are either uninfected (exposed to just an *E. coli* food source, grey) or when infected with different *C. albicans* strains (indicated in legend). Statistical significance was tested using pairwise comparisons of survival curves with Log-rank (Mantel-Cox) test. Astrisks denote statistical differences compared to the uninfected control (* indicates p <0.05, **** indicates p < 0.0001). *C. albicans* treatments that share letters are not significantly different, whereas treatments with differing letters are stastically different.

B) Box and whiskers plot of host lineage growth which represents the total population size (representing the number of F1 and F2 progeny) produced within 7 days from a single founder *sek-1 C. elegans* host infected with *C. albicans*. Boxes indicate the 25-75th quartiles with median indicated. Error bars are the normalized range of the data and circles indicate outliers. The mean and 95% CI of the uninfected control treatment are indicated by the grey dashed line and shaded grey box. Statistical significance was tested using one-way ANOVA. Astrisks denote statistical differences compared to the uninfected control (* indicates p <0.05, *** indicates p < 0.001). *C. albicans* treatments that share letters are not significantly different, whereas treatments with differing letters are stastically different, post-hoc Dunn's multiple comparison test.

C) Total brood size and **D)** Average percentage of host progeny produced during days 1-3 of adulthood (normal reproductive timing) of *sek-1 C. elegans* infected with *C. albicans*. Data and statistical analysis are the same as (B).

E) % *sek-1* host survival on Day 7 for diploid (dip) and tetraploid (tet) *C. albicans* strains (colored symbols indicate specific *C. albicans* genetic background). Statistical significance between diploid and tetraploids was tested using Wilcoxon matched-pairs signed rank test and p values are indicated.

F) Lineage growth, **G)** Brood size, and **H)** Reproductive timing of *sek-1* hosts infected with *C. albicans* diploid and tetraploid strains. Data and statistical analysis are the same as for (E).

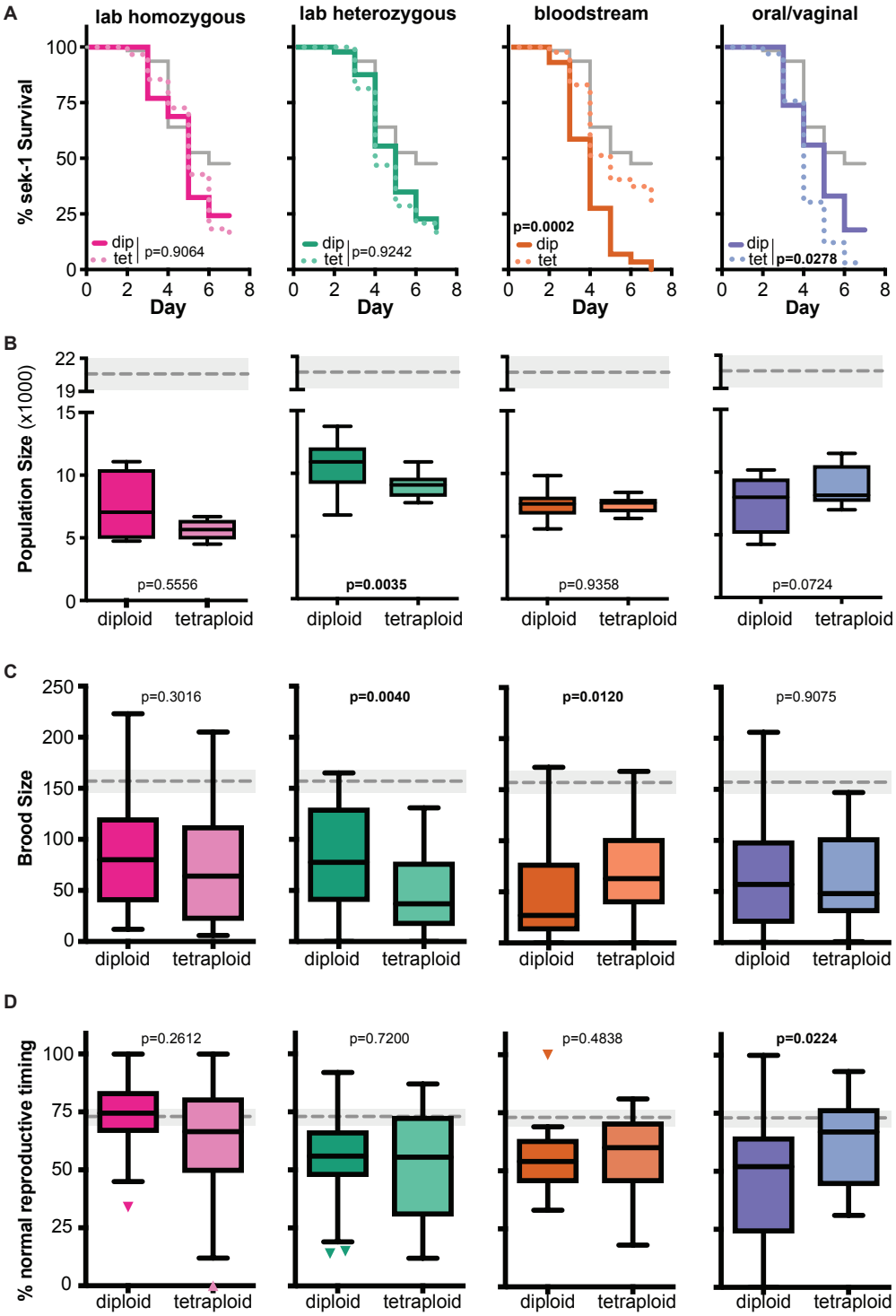

Figure 4: Ploidy-specific differences across *C. albicans* genetic backgrounds in immunocompromised hosts.
A) Survival curves for *sek-1 C. elegans* populations that are either uninfected (exposed to just an E. coli food source, grey) or when infected with diploid or tetraploid *C. albicans* strains from laboratory homozygous (pink), laboratory heterozygous (green), bloodstream (orange), or oral/vaginal (blue) genetic backgrounds. Statistical significance was tested using pairwise comparisons of diploid and tetraploid survival curves with Log-rank (Mantel-Cox) test and p-values are indicated and significant differences are highlighted in bold text.
B) Box and whiskers plot of host lineage growth which represents the total population size (representing the number of F1 and F2 progeny) produced within 7 days from a single founder *sek-1 C. elegans* host infected with *C. albicans*. Boxes indicate the 25-75th quartiles with median indicated. Error bars are the normalized range of the data and circles indicate outliers. The mean and 95% CI of the uninfected control treatment are indicated by the grey dashed line and shaded grey box. Statistical significance between diploid and tetraploid strains was determined using Mann Whitney test and p-values are indicated and significant differences are highlighted in bold text.
C) Total brood size and **D)** Average percentage of host progeny produced during days 1-3 of adulthood (normal reproductive timing) of *sek-1 C. elegans* infected with diploid and tetraploid *C. albicans*. Data and statistical analysis are the same as (B).

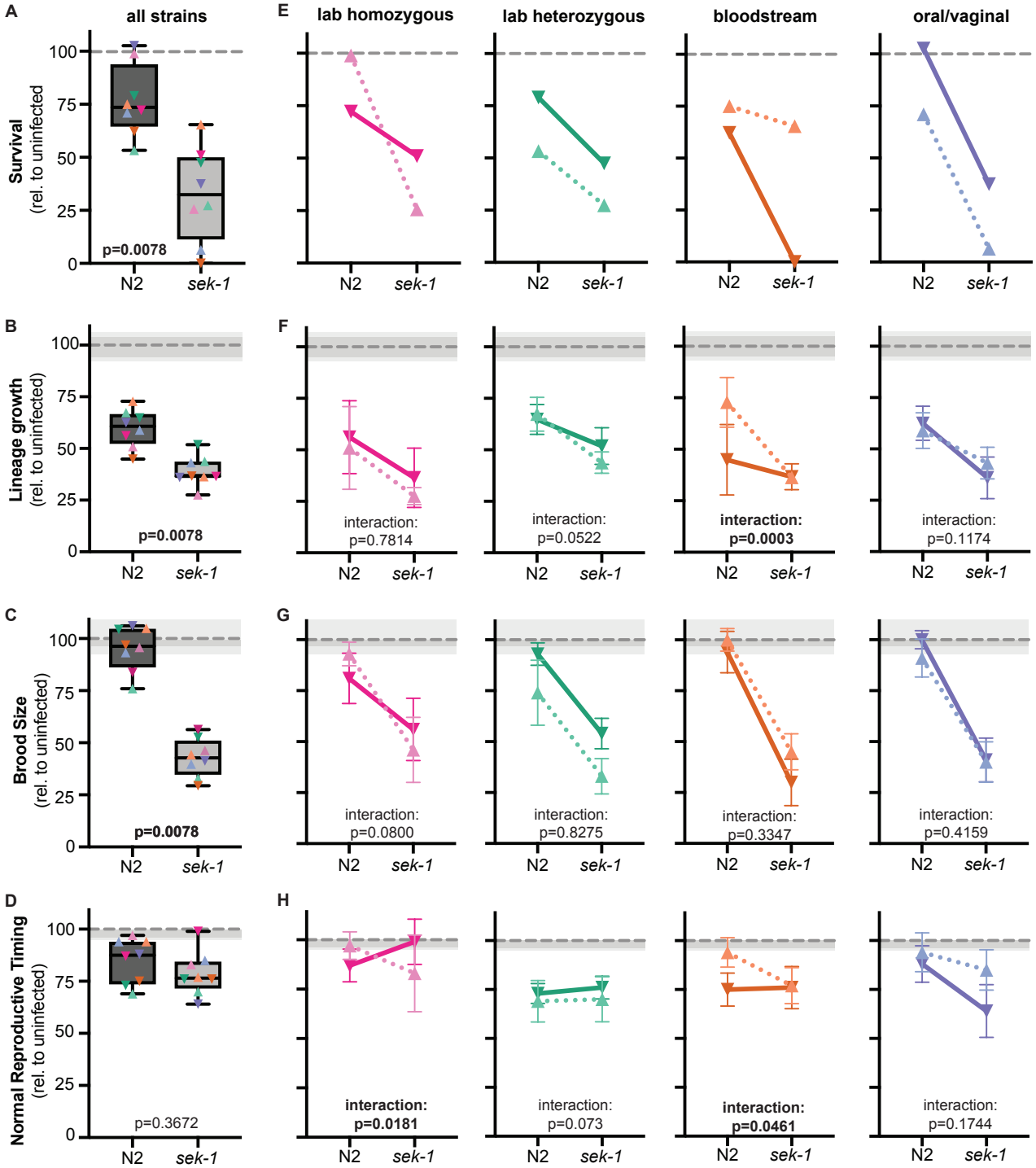

Figure 5: Ploidy-specific interactions between healthy and immunocompromised hosts.
A) Relative impact on host survival from Day 7 survival data (*C. albicans* D7 survival/uninfected D7 survival for each host type) for all *C. albicans* strains (colored symbols indicate specific *C. albicans* genetic background) in healhty (N2) and immunocompromised hosts (*sek-1*). The mean and 95% CI of the uninfected control treatment are indicated by the grey dashed line and shaded box. Statistical significance between host genotypes was tested using Wilcoxon matched-pairs signed rank test and p values are indicated.
B) Box and whiskers plot of relative host lineage growth, **C)** Brood size, and **D)** Reproductive timing between healthy. Data and statistical analysis are the same as (A).
E) Relative impact of *C. albicans* ploidy on host survival. Relative virulence of diploid (solid lines) and tetraploid (dotted lines) *C. albicans* lab homozygous (pink), lab heterozygous (green), bloodstream (orange), and oral/vaginal (blue) genetic backgrounds in healthy (N2) and immunocompromised hosts (sek-1). Y-axis scale bar is the same as in (A).
F) Relative host lineage growth, **G)** brood size, and **H)** reproductive timing between healthy and immunocompromised hosts across *C. albicans* genetic backgrounds. Symbols represent the mean value and error bars +/-SD. Y-axis scale bar is the same as in (B, C and D) respectively. Stastistical significance was tested by two-way ANOVA and 'interaction' p value indicated.