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Running head: Selection history and extinction risk

Abstract

Environments rarely remain the same over time, and populations are therefore frequently at risk of going extinct when changes are significant enough to reduce fitness. While many studies have investigated what attributes of the new environments and of the populations experiencing these changes will affect their probability of going extinct, limited work has been directed toward determining the role of population history on the probability of going extinct during severe environmental change. Here we compare the extinction risk of populations with a history of selection in a benign environment, to populations with a history of selection in one or two stressful environments. We exposed spores and lines of the green alga *Chlamydomonas reinhardtii* from these three different histories to a range of severe environmental changes. We found that the extinction risk was higher for populations with a history of selection in stressful environments compared to populations with a history of selection in a benign environment. This effect was not due to differences in initial population sizes. Finally, the rates of extinction were highly repeatable within histories, indicating strong historical contingency of extinction risk. Hence, information on the selection history of a population can be used to predict their probability of going extinct during environmental change.

Keywords: Evolutionary rescue, historical contingency, stressor, repeatability, *Chlamydomonas reinhardtii*

Introduction

Determining what factors favour survival is critical for predicting the outcome of severe environmental changes. We know from experiments that the probability of survival is higher in larger populations (Willi & Hoffmann, 2009; Bell & Gonzalez, 2009), with higher amounts of genetic variation (Agashe *et al.*, 2011; Lachapelle & Bell, 2012), immigration (Bell & Gonzalez, 2011; Lagator *et al.*, 2014b), and lower rates of environmental change (Perron *et al.*, 2008; Bell & Gonzalez, 2011; Lindsey *et al.*, 2013). However, lineages also differ in the number and type of environmental changes they have survived in the past. We tested whether a history of selection in stressful environments, compared to selection in a benign environment, affects extinction risks during further environmental change.

In the context of this report, a stressful environment is one that severely reduces fitness to the point of population decline and possibly extinction. A benign environment is one where population survival is not at risk. A stressful environment can become benign once a population successfully adapts to it, and similarly a previously benign environment can become a stressful environment after evolution in another environment. A history of selection in stressful environments, compared to selection in a benign environment, might affect extinction risks if it consistently affects evolvability or costs of adaptation (Colegrave & Collins, 2008). For example, history can affect the ability of a population to respond to natural selection by favouring genes that constitutively increase the genomic mutation rate (Shaver *et al.*, 2002) or modulate the mutation rate (Metzgar & Wills, 2000; Erill *et al.*, 2006), and hence increase the supply of variation; by favouring mechanisms

80 that promote gene exchange or recombination such as conjugation, viral infection (Poon
81 & Chao, 2004), and sex (Colegrave, 2002; Lachapelle & Bell, 2012; McDonald *et al.*,
82 2016); or by changing the type of interactions between genes to promote a more modular
83 genome (Weinreich *et al.*, 2006; Colegrave & Collins, 2008). History can also affect
84 evolvability through differences in the proportion of beneficial mutations that arise
85 because of changes in the distribution of fitness effects of mutations. For example, in
86 rugged landscapes, the probability of jumping from one fitness peak to another decreases
87 as the population climbs a peak because the probability of a mutation with effect size
88 large enough to make the jump decreases (Buckling *et al.*, 2003). Hence specialisation in
89 one environment can reduce the ability to diversify and consequently thrive in other
90 environments.
91
92 Evolutionary history may also affect extinction risks if it mediates costs of adaptation
93 through pleiotropy or mutation accumulation. For example, alleles favoured in one
94 environment can have negative impacts on fitness in other environments through
95 antagonistic pleiotropy (MacLean *et al.*, 2004) and therefore lower the probability of
96 survival during environmental change. Similarly, mutations with neutral effects in the
97 current environment but deleterious effects in the new environment can accumulate over
98 time (Kawecki, 1994; Fry, 1996) and lower the probability of survival during
99 environmental change. On the other hand, alleles favoured in one environment can have
100 positive impacts on fitness in other environments through positive pleiotropy, such as
101 when the evolution of resistance to the current stressor indirectly increases resistance to a
102 range of other stressors (Walley *et al.*, 1974; Trindade *et al.*, 2009; Ward *et al.*, 2009;

Vogwill *et al.*, 2012; Lagator *et al.*, 2013; Rodriguez-Verdugo *et al.*, 2013; Lagator *et al.*, 2014a).

It remains unclear whether a history of environmental stress will increase or decrease the probability of extinction during severe environmental change. We make use of a unique set of experimental populations of *C. reinhardtii* that have survived and adapted to two back-to-back stressful environments in the laboratory to study the effect of selection history on extinction risks, and on variance among populations and individuals within these populations in extinction risk. We sampled from different time points in the history of these populations: before exposure to any stressful environments, after survival and adaptation to the first stressful environment (i.e. the dark), and after survival and adaptation to the second stressful environment (i.e. high salt). We exposed the populations from each time point to each of the three selection environments, as well as to a range of different novel environments. We compared population density and extinction rates across and within time points to determine if selection history affects the overall response to environmental change as well as the variability in responses. In our experiment, previous selection shapes the amount of standing genetic variation and its relevance to survival after any possible change in the environment. Hence, evolutionary rescue (i.e. survival) occurs not as direct result of evolution in the novel environments, but as a correlated response to selection in the previous environment.

Materials and Methods

126 Selection history

127 The selection history of the lineages used in this experiment is depicted in Figure 1. In
128 1997, experimental lines of the unicellular green alga *Chlamydomonas reinhardtii* were
129 set-up using spores from a cross among standard laboratory strains (CC-124 x [CC-1952
130 x (CC-1952 x CC-2343)]). Four types of lines were set-up as described in Bell (2005):
131 sexual mass-transfer (obligately sexual propagated by many zygotes); sexual single-
132 zygote (obligately sexual propagated by single zygote); unselected (sexual lines where
133 unmated cells are not killed at transfer); and asexual (obligately asexual lines propagated
134 en masse). These lines were propagated on Bold's minimal medium solidified with agar,
135 phototrophically in the light. We refer to them as the light lines or L. They have been
136 evolving in a benign environment in one of our laboratories for about 20 years.

137

138 A decade later, three of the sexual mass-transfer L lines were used to initiate 2880 lines
139 which were propagated in the dark in Bold's minimal medium supplemented with 1.2 gL^{-1}
140 sodium acetate as described in Bell (2012). Only 241 lines (8.4%) survived. We refer to
141 these lines as the LD lines, for light then dark, and they have survived and adapted to one
142 stressful environment.

143

144 In 2011, forty of the LD lines were used to initiate 96 salt lines which were propagated in
145 steadily increasing concentrations of NaCl as described in Lachapelle and Bell (2012)
146 and Lachapelle *et al.* (2015). Ten lines are now surviving in 36 gL^{-1} NaCl. We refer to
147 these lines as the LDS lines, for light then dark then salt, and they have survived and

148 adapted to two back-to-back stressful environments, first the dark, then a reversion to
149 light with no acetate and added salt (Figure 1).
150
151 Extinction assay
152 We isolated four spores from each of five lines from each of the three histories. Since
153 there are only three ancestral lines for the LD lines, we used the three ancestral lines (i.e.
154 sexual mass-transfer lines) as well as two of the asexual L lines, which have been
155 propagated in parallel. We chose to use the asexual L lines as opposed to the single
156 zygote or unselected lines because the asexuals have been propagated en masse like the
157 sexual mass-transfer lines, and to avoid the ambiguity of the unselected lines, which by
158 being facultative sexuals, have an unclear history in terms of how much of the progeny is
159 recombinant and how much clonal. Each spore was assayed three times, in each of six
160 environments for a total of 1080 cultures. To determine if there has been a direct response
161 to selection, that is if spores from a given selection history have a lower probability of
162 going extinct and a higher yield in their selection environment than spores from other
163 selection histories, we assayed the spores in the three selection environments, i.e. Bold's
164 minimal liquid media (referred to as 'Bolds'; (Harris, 2009); Bold's supplemented with
165 1.2 gL^{-1} sodium acetate and maintained in the dark (referred to as 'Dark'); Bold's
166 supplemented with 20 gL^{-1} NaCl (referred to as 'NaCl'). The growth of the L and LD
167 lines in NaCl does not itself represent a direct response to selection, as they have not been
168 selected in NaCl. The direct response is usually determined by comparing the fitness of
169 evolved lines to the fitness of their ancestors. Here the L and LD lines therefore serve as
170 the ancestors to which to compare the fitness of the LDS lines. To determine the indirect

response to selection, that is consequence of selection in one environment on the probability of going extinct and the yield in other environments, we assayed the spores in three novel environments, i.e. Bold's media supplemented with 0.4M Atrazine, a herbicide (referred to as 'Atrazine'); Bold's supplemented with 0.1 μM $CuSO_4$ (referred to as $CuSO_4$); and Bold's buffered to pH4 with a phosphate solution (0.43 gL^{-1} Na_2HPO_4 + 3.36 gL^{-1} KH_2PO_4; referred to as pH4). All cultures were grown phototrophically in the light, except in the Dark environment where all growth had to be heterotrophic.

The concentrations used for the three novel environments Atrazine, $CuSO_4$, and pH4 were determined by running preliminary growth assays with six wild-type strains (CC-1690, CC-1952, CC-2342, CC-2344, CC-2931, CC-2937). The use of wild-type strains in these preliminary assays ensured that the choice of concentration was independent of the biological material used in the extinction assay. The wild-type strains were grown in a range of different concentrations of Atrazine, $CuSO_4$ and pH, and the concentration that reduced cell densities to just above the detection limit of the spectrophotometer after two growth cycles was chosen. This ensured that the concentration was severe enough to reduce growth, but would not lead to immediate extinctions (which would limit our ability to detect variance in extinction risk).

To start the extinction assay each spore was grown from a single colony into a population in its home environment (i.e. L lines in Bold's, LD lines in Dark, LDS lines in NaCl). We chose to grow the spores into different environments because we could find no single common environment that would not severely disfavour the growth of one history over

194 that of the others. The populations were therefore isogenic at the start of the assays except
195 for any mutation that would have arisen during the growth of the single colony into a
196 population (about four generations). After one cycle of growth, the spores were
197 transferred to all six assay environments. Cultures were then serially transferred once
198 every 7 days by diluting 10 μL of culture into 190 μL fresh media in 96-well plates. To
199 maintain a constant size a population therefore needs to undergo about 4.3 divisions over
200 a week. The cultures were incubated at 26 degrees Celsius, 60% air humidity, and 7150
201 Lux constant light intensity.
202
203 At the end of each growth cycle, every culture was inspected using an inverted
204 microscope to record the presence or absence of living cells. A culture was deemed
205 extinct if the absence of living cells was recorded for two cycles in a row. The cell
206 density was also estimated at the end of each growth cycle by measuring the optical
207 density at 750 nm with a spectrophotometer. The assay was terminated after 11 cycles
208 (about 55 generations) or later in the case of some environments, whenever the number of
209 extinctions had stabilised for two cycles and none of the cultures were on the brink of
210 extinction.
211
212 Statistical analyses
213 All analyses were done in R version 3.2.1. We examined the effect of selection history on
214 extinction in two different ways. First, the extinction dynamics, i.e. the proportion of
215 lines alive over time, were analysed by performing survival analyses using Cox
216 proportional hazards with mixed effects, which assume Gaussian random effects, with the

217 'coxme' R package (Therneau, 2015). In all models we included a 'Censor' variable for
218 spores that had not gone extinct by the end of the assay. Second, the extinction risk, i.e.
219 the proportion of lines extinct by the end of the experiment, was analysed by computing
220 two-tailed Fisher's exact tests for independence of number of extinction events and
221 selection history in a contingency table. We report both survival analyses and Fisher's
222 exact test results except in assay environments where the survival analysis could not be
223 fitted, i.e. in cases where extinctions did not occur in all selection histories. This is
224 because proper model fitting requires at least one event to have occurred in each level of
225 the fixed factor. In those cases, we report only the extinction risk.
226
227 Yield of surviving spores at the end of the assay was analysed by fitting mixed effect
228 models using the lmer function in the R package 'lme4' (Bates *et al.*, 2015). Our estimate
229 of yield is the optical density at the end of the extinction assay (cycle 11) when
230 populations had stabilised. While the assay lasted more than 11 cycles in some
231 environments, we decided to use the yield at the end of cycle 11 to be consistent across
232 all environments. P values were obtained using the R package 'lmerTest' (Kuznetsova *et*
233 *al.*, 2014) with type III sum of squares in an analysis of variance and Sattertwhaite
234 approximation for degrees of freedom by using the normal approximation.
235
236 More precisely, we divided our analyses into two sections: the direct response to
237 selection and the indirect response to selection. First, to determine if in a given
238 environment, there are fewer extinctions in the selection history most recently selected in
239 that environment than in the other selection histories, we compared the extinction risk

and extinction dynamics of the three selection histories in each selection environment (i.e. Bold's, Dark, NaCl). That is we fitted a coxme survival model with selection history as a fixed factor, and line and spore within line as random factors. The model was applied to each environment individually. To determine if in a given environment, yield is higher for the selection history most recently selected in that environment than for the other selection histories, we fitted a mixed effects model with selection history as a fixed factor, and line and spore within line as random factors.

Second, the determine if past selection in a stressful environment affects the extinction risk and the dynamics of extinction in novel environments compared to selection in a benign environment, we computed Fisher's tests and fitted a coxme survival model with selection history as a fixed factor, and assay environment, line, and spore within line as random factors. Only the three novel environments (Atrazine, $CuSO_4$, pH4) are included in this model. All the novel environments we used had constant lighting and no acetate. Therefore, unlike the L lines and LDS lines, the extinction risk of the LD lines will not only include the general extinction risk due to selection in a stressful environment, but also a special risk associated with the presence of light and lack of acetate. To estimate the general extinction risk of the LD lines we assumed that the effects of novel stressful compounds is additive to the effects of constant light and no acetate (i.e. measured risk = general risk + special risk), which has been shown to be a reasonable assumption in the case of NaCl (Lachapelle *et al.*, 2015). More precisely, we calculated [1 – (proportion of LD lines alive in Bold's at time *t* – proportion of LD lines alive in novel environment *x* at time *t*)]. From this corrected proportion of lines alive, back calculated the corrected time

263 of extinction. That is, we multiplied the corrected proportion of lines alive by 20 (total
264 number of cultures) to get *n*, the corrected absolute number of lines alive at each time
265 point. We created a new data set with *n* rows for lines alive followed by (20 – *n*) rows for
266 lines extinct. We assigned a number from 1 to 20 to each row. For each line number, we
267 counted the number of time points where the line was alive, and used that number as the
268 corrected time of extinction. Finally, given that the order in which lines go extinct after
269 correction is the same as before correction since the correction is simply a subtraction, we
270 matched the initial and corrected datasets after ordering them by time of extinction to
271 obtain the actual line and replicate number. We report the corrected extinction risk as the
272 general extinction risk in the analyses of the extinction risk in the novel environments.
273
274 To determine if yield of surviving spores in novel environments differs between selection
275 histories, we fitted a mixed effects model for each novel environment with selection
276 history as a fixed factor, and line and spore within line as random factors.
277
278 Finally, to estimate variance in the dynamics of extinction in novel environments, we
279 fitted a coxme survival model for each selection history with line, spore within line,
280 environment (including only the novel environments Atrazine, $CuSO_4$, and pH4), the
281 combination of line and environment, the combination of spore and environment, as
282 random factors. Note that the coxme function does not accept interaction terms for the
283 random factors, and therefore we created two new variables by pasting line and
284 environment or spore and environment together. Similarly, variance in yield of surviving
285 lines in novel environments was compared among selection histories using a lmer model

with assay environment (including only the novel environments Atrazine, $CuSO_4$, pH4), line, spore within line, the interaction between line and assay environment, and the interaction between spore and environment as random factors. The significance of the differences in variance between selection histories was determined using F ratios. The degrees of freedom were calculated based on an analysis of variance model.

Results

Selection reduces extinction risk in most recent environment

To measure the direct response to selection we did a reciprocal transplant, growing the three selection histories in all three selection environments (Figure 2; Figure 4; Table 1). A direct response is detected if spores from a given selection history have a lower extinction risk and higher yield in their selection environment than spores from other selection histories.

In the Dark environment, none of the LD lines go extinct, while on average 67% and 70% of L lines and LDS lines, respectively, go extinct. As such, selection in the Dark has significantly lowered extinction risk (LD line to L line comparison using Fisher's exact test: $P = 7.3 \times 10^{-17}$; LD line to LDS line comparison using Fisher's exact test: $P = 4.4 \times 10^{-18}$). The extinction risk of the LDS lines is no different from that of the L lines ($P = 0.84$). Also, the LD lines reach higher yield than the surviving L lines ($t_{12} = -2.9$, $P = 0.012$) and the surviving LDS lines ($t_{12} = -3.2$, $P = 0.0079$) by cycle 11. Hence, long-term

selection in the Dark increased the capacity for heterotrophic growth that arises spontaneously in unselected populations.

In the NaCl environment, all L lines and all LD lines go extinct, while only 20% of LDS lines on average go extinct. As such, selection in NaCl has significantly lowered the extinction risk (LDS line to LD line and LDS line to L line comparison using Fisher's exact test: $P = 3.2 \times 10^{-22}$). The extinction risk of the LD lines is no different from that of the L lines ($P = 1.00$), although the LD lines go extinct more rapidly than the L lines (coxme survival model: $z = -2.71$, $P = 0.0067$). None of the LD lines or L lines survive to cycle 11, such that we cannot compare their yield to that of the LDS lines.

Finally, in the Bold's environment, which is the benign environment, none of the L lines and none of the LDS lines go extinct, while 25% of the LD lines on average go extinct. The extinction risk of the L lines and LDS lines is significantly lower than that of the LD lines (Fisher's exact test: $P = 5.6 \times 10^{-8}$). The yield of surviving LD lines is no different from that of L lines ($t_{12} = 1.2$, $P = 0.24$) and no different from that of LDS lines ($t_{12} = 0.14$, $P = 0.89$).

<u>Overall extinction risk in novel environments is lowest in the L lines</u>

To determine if the risk of extinction in novel environments is lower for populations with a history of selection in stressful environments than for populations with a history of selection in a benign environment, we compared the general extinction risk (see Methods) of the LD lines and the LDS lines to that of the L lines.

We find that adaptation to a stressful environment increases the extinction risk in a novel environment in comparison to adaptation to a benign environment. That is, over all novel environments, the LD lines and LDS lines, with 39% and 29% of spores extinct on average respectively, have a higher general extinction risk than the L lines with 24% of spores extinct on average over all novel environments (Fisher's exact test: LD – L comparison: $P = 0.0031$; LDS - L comparison: $P = 0.28$). Although the LDS lines do not have a significantly higher probability of extinction than the L lines, they do go extinct at a significantly faster rate (coxme survival model: L – LDS comparison $z = 1.98$, $P = 0.048$; L – LD comparison $z = 1.85$, $P = 0.064$;). While the LDS lines have a lower extinction risk than the LD lines, this difference is not statistically significant (Fisher's exact test: $P = 0.075$) nor are the extinction dynamics significantly different (coxme survival model $z = 0.14$; $P = 0.89$). The difference in extinction dynamics between the selection histories cannot be explained by differences in population size at the start of the assays (coxme survival analysis using yield at the end of cycle 1 in the home environments as a proxy for population size at the start of the assay, and assay environment, line, and spore as explanatory variables: $z = -1.16$, $P = 0.25$).

Examination of the general extinction risk in each novel environment reveals the same overall pattern of higher extinction risk in lines with prior selection in stressful environments: in Atrazine the LD lines have a significantly greater extinction risk than the light and LDS lines (Fisher's exact test: $P = 1.5 \times 10^{-8}$ for both LD - L and LD – LDS comparisons; L – LDS comparison: $P = 1.0$); and in pH4, the LDS and LD lines have a

354 significantly greater extinction risk than the L lines (Fisher's exact test: LD - L P = 0.12;
355 L - LDS P = 0.038; LD - LDS P = 0.79) and significantly different extinction dynamics
356 (coxme survival model: LD - L comparison: z = -3.12, P = 0.0018; L - LDS comparison:
357 z = 1.70, P = 0.0073; LD -LDS comparison: z = -0.45, P = 0.66). This is with the
358 exception of the $CuSO_4$ environment where all lines have an equivalent extinction risk
359 (Fisher's exact test: P = 0.11 for both LD - L and LD - LDS comparisons).
360
361 Yield of surviving lines in novel environment is similar no matter selection history
362 The surviving lines all reach similar yields in the novel environments (Figure 4; Atrazine:
363 L - LDS comparison $t_{11} = -1.4$, P = 0.19; $CuSO_4$: LD - L comparison $t_{12} = -1.5$, P = 0.16,
364 LDS - L comparison $t_{12} = -0.73$, P = 0.48; pH4: LDS - L comparison: $t_{11} = 0.41$, P =
365 0.69), except in Atrazine, where the L lines reach greater yield by cycle 11 than the
366 surviving LD lines ($t_{11} = -2.9$, P = 0.014).
367
368 Repeatability of extinction
369 The amount of variance in the extinction dynamics provides an estimate of the
370 repeatability of extinction. That is, if all populations from a given history go extinct at the
371 same rate or all survive, variance in extinction will be low and repeatability high. High
372 repeatability is an indication that history plays an important role in extinction. If
373 populations from a given history respond in different ways to environmental change,
374 variance in extinction will be high, and repeatability of extinction low. Low repeatability
375 is an indication that chance plays an important role in extinction.
376

By estimating variance among lines within selection histories, among spores within lines, and among novel environments, we found that the repeatability of extinction is highest in the LD lines, and lowest in the salt and L lines (Table 2, Figure 3). Both the LDS and L lines are very sensitive to different environments, having either very high or very low extinction rates depending on the environment, and thus a high amount of variance across environments. The LD lines on the other hand tend to have more similar and intermediate rates of extinction across all environments, and hence much lower environmental variance. On the other hand, genetic variance is higher in the LD lines, as seen by the significantly higher variance among lines, and in the spore by environment interaction. This result is driven mainly by one of the five LD lines consistently having higher extinction rates than the other four lines. Hence, the repeatability of extinction is higher in the LD lines because of a more consistent albeit poor ability to survive in a range of novel environments.

Variance in yield of surviving populations

The amount of variance in proportion to mean yield, i.e. the variance-to-mean ratio, can provide an estimate of the ability of populations to respond to natural selection, with larger ratios predicted to increase rates of adaptation, and lower ratios predicted to slow or even prevent adaptation. Hence the variance-to-mean ratio is an indication of the evolvability of populations (Houle, 2002). We estimated the variance-to-mean ratio among lines, among spores (i.e. within lines), among environments, and among line by environment and spore by environment interactions. The total ratio is the sum of all these ratios. The total amount of variation in yield is highest in the surviving LD lines, with

400 close to two times more variation than in the surviving L lines, and more than three times
401 more variation than in the surviving LDS lines (Table 3, Figure 5). We obtain the same
402 qualitative results when using variance instead of the variance-to-mean ratio.
403
404 Contrary to variance in extinction which is driven mainly by variance among
405 environments, we find that variation in yield is driven mainly by genetic and gene by
406 environment variation. The L lines have high line-by-environment and spore-by-
407 environment variation, indicating that the surviving spores and lines from the light history
408 respond differently to different environments. The LD lines have the highest amount of
409 line-by-environment variation, and almost no other sources of variation, indicating
410 limited variation within lines, but high variability among lines in their response to
411 different environments. Finally, the LDS lines have the highest amount of variation
412 among lines, indicating significant differences among lines that are independent of the
413 environment of assay.
414
415
416 **Discussion**
417 We made use of lineages that have undergone two back-to-back events of selection in
418 stressful environments to test for a role of selection history on extinction risk in novel
419 environments. Survival in this case occurs as a correlated response to selection in the
420 previous environment. We exposed four spores from each of five lines from before any
421 selection in stressful environments (L lines), after selection in one stressful environment
422 (LD lines), and after selection in two stressful environments (LDS lines) to a range of

423 novel and severe environmental changes. The general extinction risk in a novel
424 environment tended to be higher for lines with a history of selection in stressful
425 environments than for lines with a history of selection in a benign environment.
426
427 Our main finding of greater extinction risk after selection in stressful environments is in
428 agreement with what Samani and Bell (2016) found in yeast populations, where
429 populations that had been exposed to long-term starvation had a higher probability of
430 going extinct after exposure to a novel stressor than populations selected in conditions of
431 plenitude. It is also in part in agreement with findings by Gonzalez and Bell (2013) who
432 selected replicate populations of two species of yeast, *Saccharomyces cerevisiae* and *S.*
433 *paradoxus* in different concentrations of salt before exposing all surviving populations to
434 an initially lethal concentration of 150 gL^{-1} NaCl. In accordance with our results, in *S.*
435 *cerevisiae*, selection in stressful salt concentrations increased the extinction risk.
436 However, the opposite was found in *S. paradoxus*, where selection in stressful salt
437 concentrations reduced the extinction risk. Hence, while there is evidence that selection
438 in stressful environments increases extinction risks during environmental change, other
439 factors, such as species identity, can mediate the effect of selection history.
440
441 Extinction risk depends on latest stress encountered
442 Given that our experimental lines have survived two back-to-back stressful environments,
443 it gives us the opportunity to ask whether the number of past stressful environments itself,
444 i.e. one or two, affects the extinction risk. If stressful environments select for greater
445 evolvability or positive genetic correlations for fitness among environments, selection in

446 two back-to-back stressful environments should lead to even lower extinction risks than
447 after selection in one stressful environment. We found that there was no general trend of
448 increasing or decreasing extinction risk with number of stressful environments survived
449 in the past. How much of this result is down to the history of stress per se, and how much
450 down to the specific stresses that these populations have encountered is impossible to say
451 from this data. Replication of this study using different selection histories would be
452 needed to determine the generality of the results with regards to the effect of the number
453 of events of evolutionary recue on extinction risk. The lack of general trend in extinction
454 risk with number of stressful environment survived in the past could be because it is only
455 the latest stressful environment that determines evolvability and/or costs of adaptation
456 (i.e. there is no accumulation of effects from multiple stressful environments), or
457 although additive, the effects of different stressful environments can be opposite in
458 direction and/or magnitude and thus can lead to a reduction in extinction risk over
459 sequential selection in stressful environments.
460
461 The fact that the LDS lines have the same extinction risk in the Dark environment as the
462 L lines, and that LDS lines have significantly different patterns of variance in extinction
463 risk and yield in novel environments than the LD lines, suggests that selection in salt
464 erased the prior signature of selection in the dark. Hence, our results suggest that the
465 latest stressful environment to have survived is more important than the accumulation of
466 evolutionary rescue events. This is in agreement with findings by Lagator *et al.* (2014a)
467 who selected replicate populations of the green alga *Chlamydomonas reinhardtii* in one
468 of three herbicides before exposing all surviving populations to the two other herbicides

469 sequentially. Survivability during exposure to the second and third herbicides was either
470 increased, decreased, or not affected, depending on what herbicide in particular was used
471 for the initial selection phase.
472
473 The importance of the particular stressor experienced is also indicated by the different
474 results in different novel environment. The $CuSO_4$ environment was not stressful enough
475 and barely any populations went extinct in it. It was therefore not very informative for
476 distinguishing extinction risks between selection histories. As for the other two novel
477 environments, in Atrazine, it is the LD lines that have the highest extinction risk and rate
478 of extinction, whereas in pH4 it is the LDS lines that have the highest extinction risk and
479 both LD and LDS have the highest rate of extinction. Hence, selection history in stressful
480 environments leads to higher extinction risks and rates overall, but this effect does vary
481 between novel environments depending on the identity of the previous stressor.
482
483 Factors other than the stress per se can also affect extinction risks and evolutionary
484 responses. For example, differences in the severity of the stress can affect population
485 sizes and the fraction of beneficial mutations available (Gonzalez & Bell, 2013; Samani
486 & Bell, 2016); differences in the genetic basis of adaptation to different stresses, such as
487 the presence and amplitude of antagonistic epistasis, can lead to differences in how much
488 of a reduction there is in the fitness costs of resistance mutations (Lagator *et al.*, 2014a);
489 and finally, the tempo of environmental change, such as a gradual increase in the stressor
490 or a sudden exposure to high levels of the stressor, can lead to differences in the
491 magnitude of costs of adaptation (Collins & De Meaux, 2009; Lindsey *et al.*, 2013). We

492 therefore cannot exclude the possibility that the greater extinction risk of the LD lines is
493 due, for example, to the fact that survival in the LD lines occurred after a sudden change,
494 which has been shown to involve greater costs than adaptation to gradually changing
495 environments such as in the LDS lines (Collins & De Meaux, 2009; Lindsey *et al.*, 2013).
496
497 The role of plasticity in extinction in novel environments
498 The spores that survived in the novel environments follow a diverse range of dynamics in
499 yield over time, from constant, to steady increase, steady increase followed by a plateau,
500 and U-shaped dynamics (Figure 4). All populations were initiated from a single spore.
501 The only genetic variation present at the time of environmental change was therefore
502 limited to novel mutations generated during the four generations of growth prior to the
503 assay. Population decline upon environmental change would have also reduced the
504 supply of mutations and reduced the probability of fixation. Changes in yield over time
505 are therefore unlikely to be due to genetic changes given the absence of standing genetic
506 variation, and the short evolutionary timescale of the experiment. They are more likely to
507 be due to physiological acclimation or positive growth rates in initially bottlenecked
508 populations. Given that most of the spores that go extinct do so within the first five cycles
509 (about 25 generations) in the new environment, survival during severe environmental
510 change will depend almost entirely on the presence of spores in the population that can
511 either plastically respond or constitutively withstand the novel stressor enough to prevent
512 population extinction. Significant differences in the magnitude of the plastic response to
513 novel stressors have been found in yeast populations with different selection histories
514 (Samani & Bell, 2016). Hence prior selection regimes can affect the probability of

515 survival in novel environments by favouring or hindering the evolution of plastic
516 responses (Lande, 2009) or by altering the health of the population and therefore its
517 ability to physiologically respond to stressors.
518
519 Within and among line variance in extinction risk
520 By characterizing the rates of extinction of different spores within lines, of different
521 independent lines within selection histories, and of different selection histories, in
522 multiple novel environments, we are able to quantify precisely the repeatability of
523 extinction across a whole range of environments. History played an important role in
524 driving the repeatability of extinction, as lines and spores from each given history tended
525 to go extinct at a similar rate in a given novel environment. Almost all variation in
526 extinction rates arose from differences among novel environments, as histories tended to
527 go extinct at different rates in different novel environments. This is with the exception of
528 the LD lines, which showed even greater levels of repeatability than the L and LDS lines,
529 by having similar rates of extinction in all novel environments.
530
531 Repeatability in yield differed significantly from repeatability of extinction in terms of
532 what is the source of variation. The environment appears to be the most important
533 determinant of the probability of extinction given it is the largest source of variation in
534 extinction, whereas genetic and gene by environment interactions appear to be the most
535 important determinants of yield. This suggests that chance plays an important role in
536 yield and contributes to low repeatability of yield. The difference between extinction and
537 yield in the main source of variation could be due to extinction being a binary trait (rather

than a continuous trait like yield), meaning that subtler genetic differences are not detected; it could be due to the fact that variation in yield was calculated for surviving populations, thus eliminating all the values of zero and leading to a much reduced environmental variance; or it could be due to differences in the genetic underpinning of extinction risk and yield. It is interesting to note that although the extinction risk was overall highest for the LD lines, the LD lines had the highest overall variance in yield amongst surviving populations. Hence, surviving LD lines have the highest potential evolvability in spite of sustaining the highest rate of extinction.

To conclude, selection in stressful environments tends to increase the risk of extinction in novel environments compared to selection in benign conditions. We also found that back-to-back episodes of selection in stressful environments did not increase or decrease that risk further, suggesting that effects of selection in stressful environments do not accumulate over time. Rather, our results suggest that it is the latest environment of selection that determines the evolvability of the population and the magnitude of costs of adaptation. By examining not only averages but also the amount variation in extinction risk and yield, we found that rates of extinction were highly repeatable within selection histories, despite there being significant amounts of genetic and gene by environment variation in yield within histories. Hence, lineages from the same selection history will have a similar probability of going extinction during environmental change, and this probability will be higher if the last selection environment was stressful.

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651

652

653 ## Tables

654 Table 1. Proportion of spores extinct per line per selection history, in each of the assay
655 environments. The proportions for the LD lines in novel assay environments (i.e.
656 Atrazine, $CuSO_4$, and pH4) are corrected proportions (see Methods). The proportions
657 represent the number of spores over three assays that were extinct by the end of the
658 assay (4 spores x 3 replicate assays = 12 total spores), such that a number of 1 means

659 that all 12 spores went extinct. Each row represents one of five lines.

	Selection history		
Assay environment	L	LD	LDS
Bold's	0	0.75	0
	0	0	0
	0	0	0
	0	0	0
	0	0.42	0
Dark	0.75	0	0.33
	0.58	0	0.67
	0.83	0	0.83
	0.75	0	1
	0.42	0	0.67
NaCl	1	1	0.08
	1	1	0
	1	1	0.08
	1	1	0.08
	1	1	0.75
Atrazine	0	1	0
	0	0	0
	0	0	0
	0	0	0
	0	0.33	0
$CuSO_4$	0	0.25	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0
pH4	0.33	1	1
	1	1	0.42
	0.67	0.67	1
	1	0.92	1
	0.58	0.67	1

660

661 Table 2. Significance of differences in variance in extinction dynamics in novel
662 environments between selection histories. Only data from the three novel environments
663 (i.e. Atrazine, $CuSO_4$, pH4) are included in the model.

Source	Selection histories	Df (numerator, denominator)	F ratio	P value
Line	LD - LDS	1, 1	7.86×10^{3}	7.18×10^{-3}
	LD - L	1, 1	7.90×10^{3}	7.16×10^{-3}
	LDS - L	1, 1	1.00	0.499
Line : Environment	LD - L	1, 1	1.87	0.402
	LD - LDS	1, 1	1.91	0.399
	L - LDS	1, 1	1.02	0.497

Spore	LDS - LD	1, 1	1.40	0.446
	LDS - L	1, 1	82.3	0.0699
	LD - L	1, 1	58.7	0.0826
Spore : Environment	LD - LDS	2, 2	22.4	0.0427
	LD - L	2, 2	1.87×10^{3}	5.35×10^{-4}
	LDS - L	2, 2	83.3	0.0119
Environment	L - LDS	2, 2	1.24	0.446
	L - LD	2, 2	31.8	0.0305
	LDS - LD	2, 2	25.6	0.0376
Total	L - LDS	7, 7	1.24	0.392
	L - LD	7, 7	13.3	1.47×10^{-3}
	LDS - LD	7, 7	1.07	2.83×10^{-3}

664

665

666 Table 3. Significance of differences in variance-to-mean ratios in optical density between
667 selection histories when cultured in all three novel environments (i.e. Atrazine, $CuSO_4$,
668 pH4).

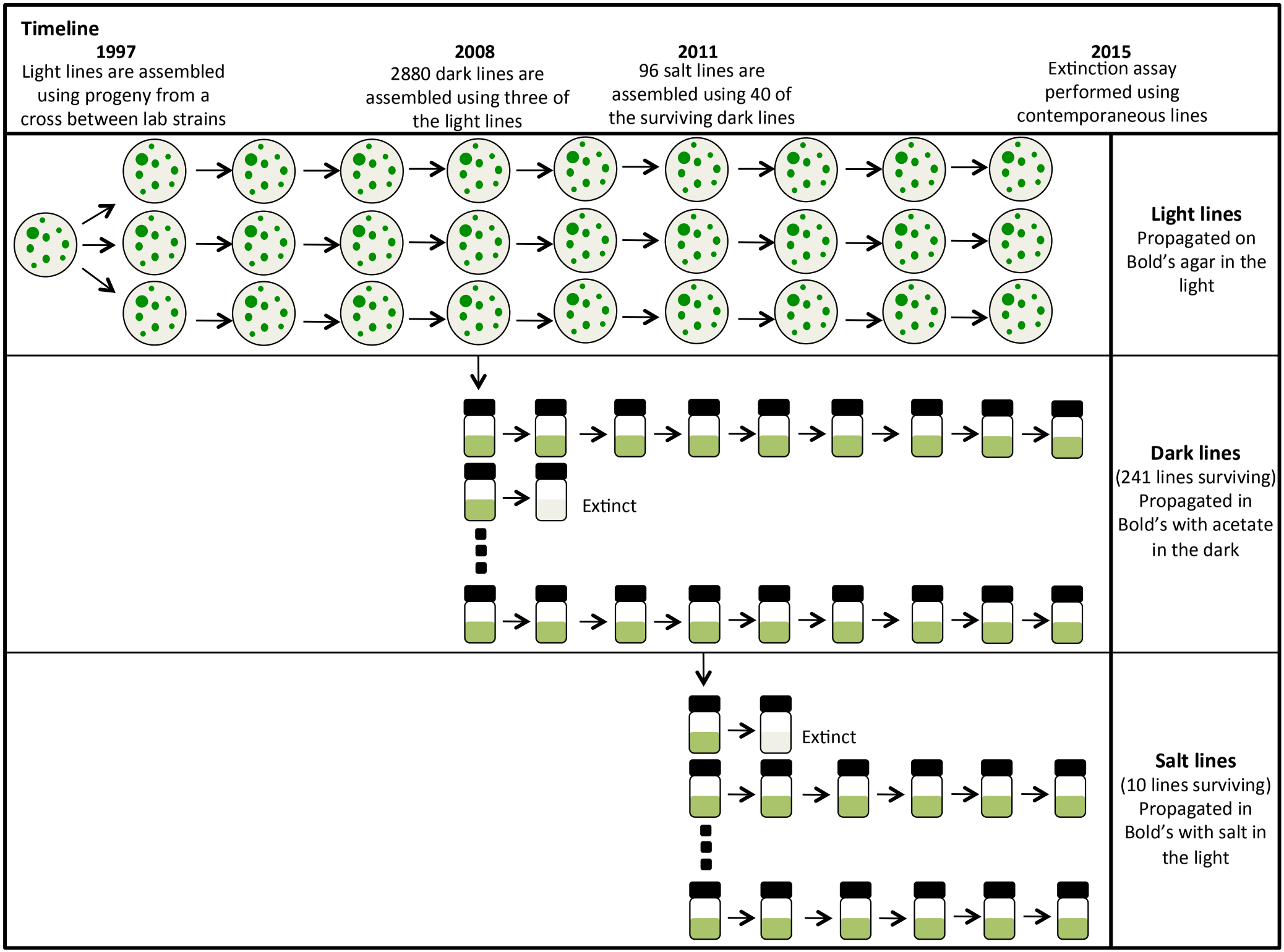
Source	Selection histories	Df (numerator, denominator)	F ratio	P value
Line	L - LD	4, 4	Inf	0.00
	LDS - L	4, 4	4.20	0.0969
	LDS - LD	4, 4	Inf	0.00
Line : Environment	L - LDS	6, 4	Inf	0.00
	LD - L	3, 6	3.97	0.0710
	LD - LDS	3, 4	Inf	0.00
Spore	L - LD	15, 12	4.90	4.23×10^{-3}
	L - LDS	15, 15	4.05	5.13×10^{-3}
	LDS - LD	15, 12	1.21	0.374
Spore : Environment	L - LD	22, 9	27.7	8.48×10^{-6}
	L - LDS	22, 17	Inf	0.00
	LD - LDS	9, 17	Inf	0.00
Environment	LD - L	1, 2	Inf	0.00
	LDS - L	2, 2	Inf	0.00
	LDS - LD	2, 1	8.72×10^{13}	7.57×10^{-8}
Total	L - LDS	49, 42	2.88	3.28×10^{-4}
	LD - L	29, 49	1.92	0.0218
	LD - LDS	29, 42	5.52	3.56×10^{-7}

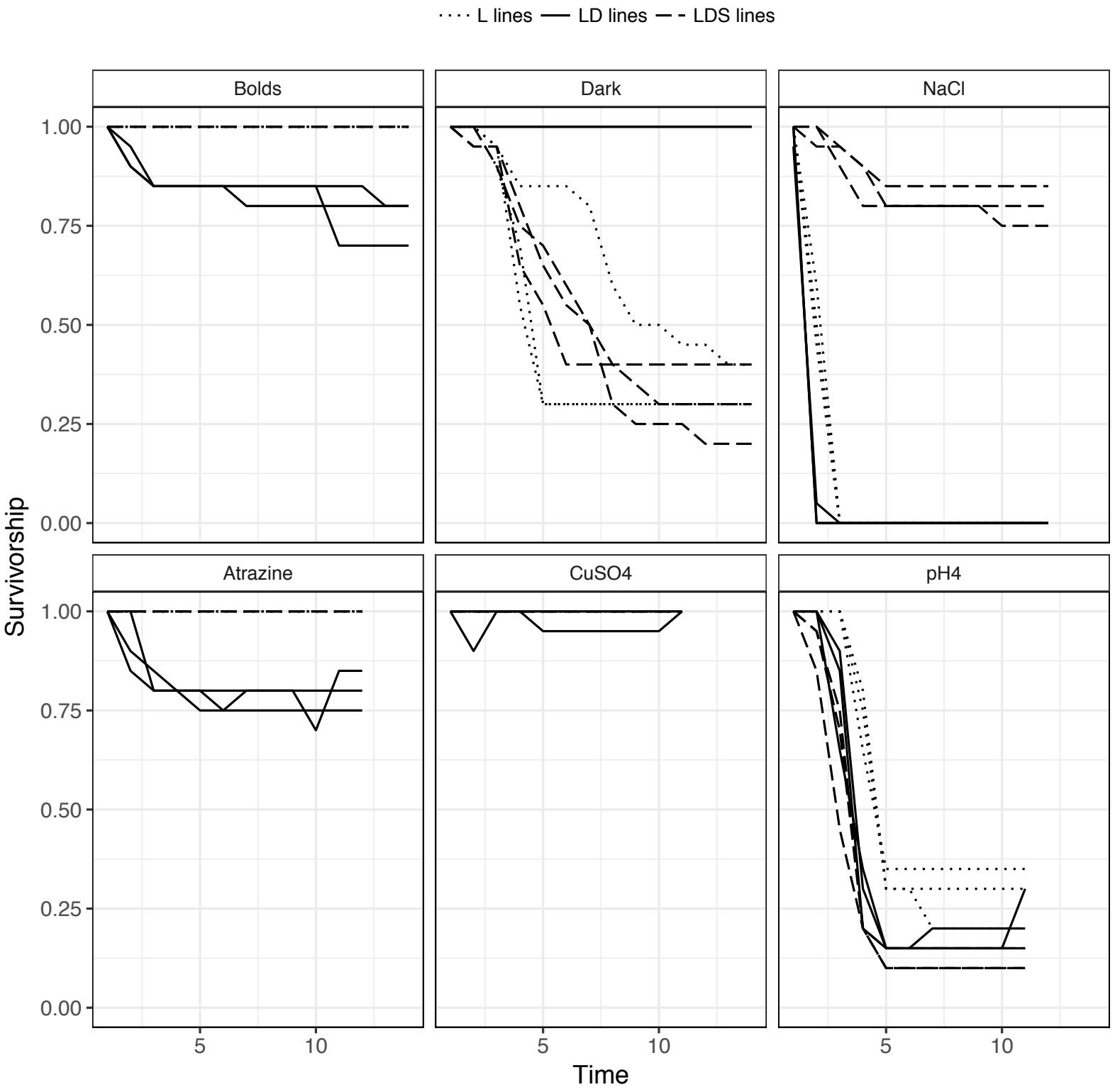
669

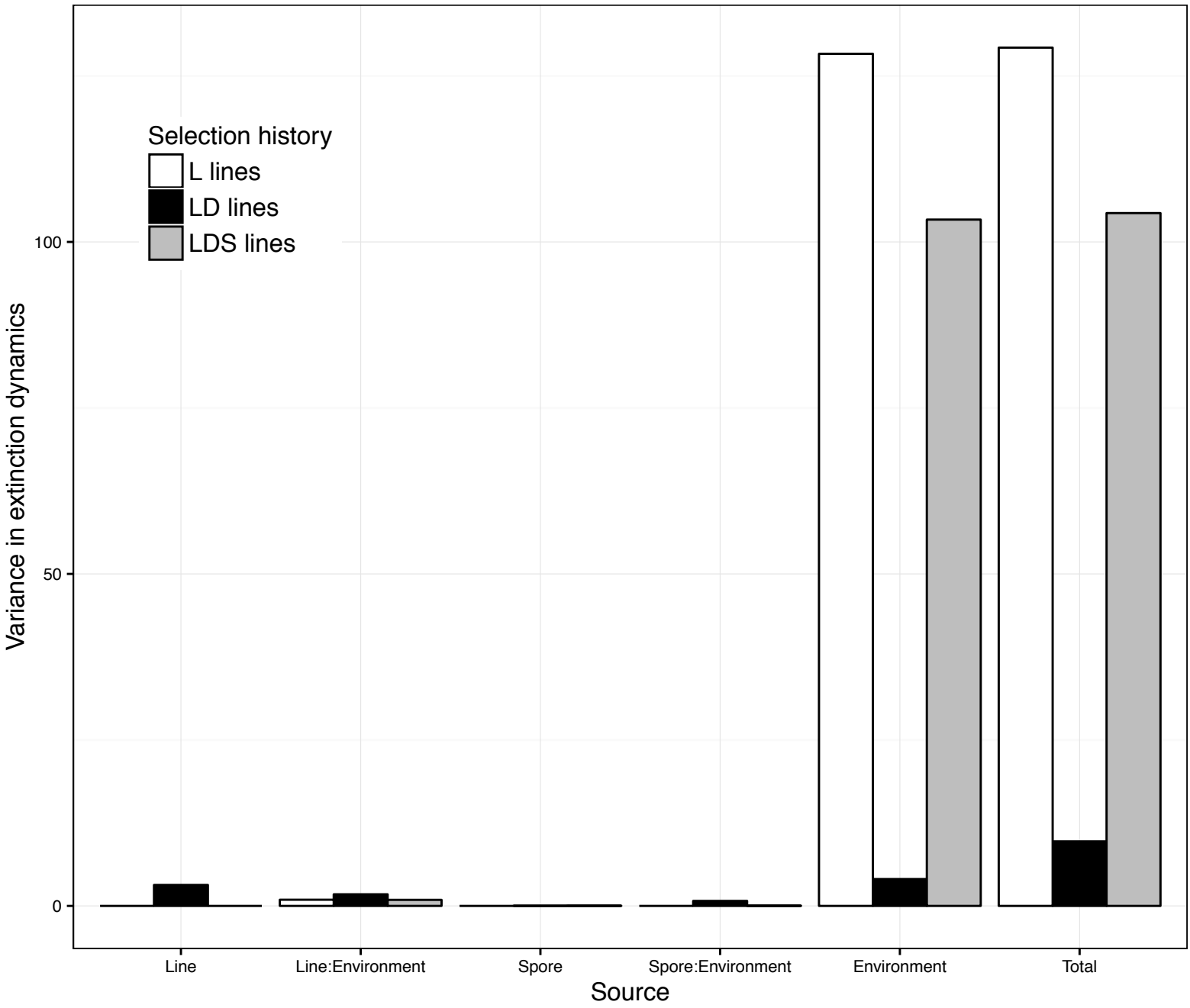
670

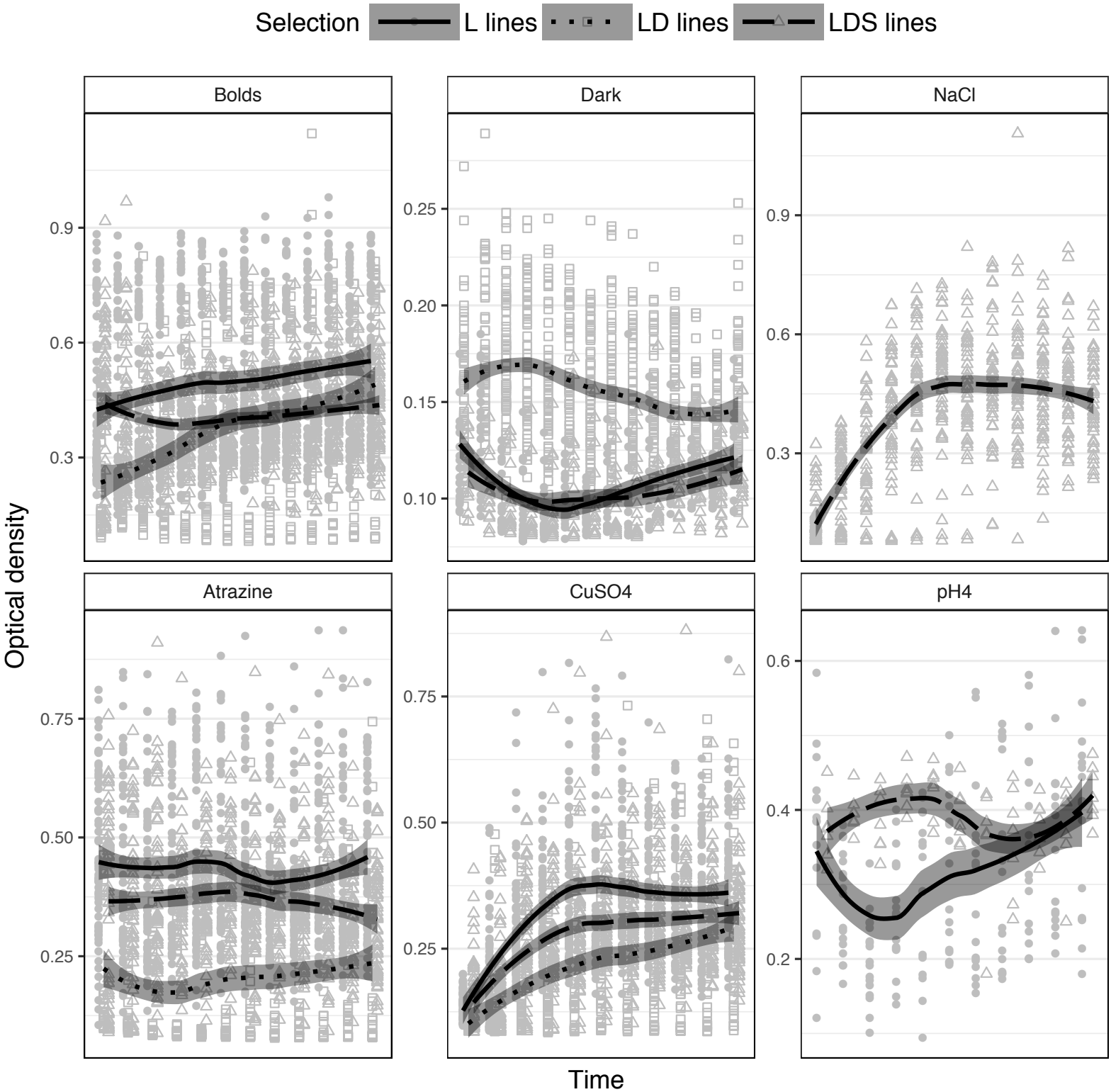
671 **Figure legends**

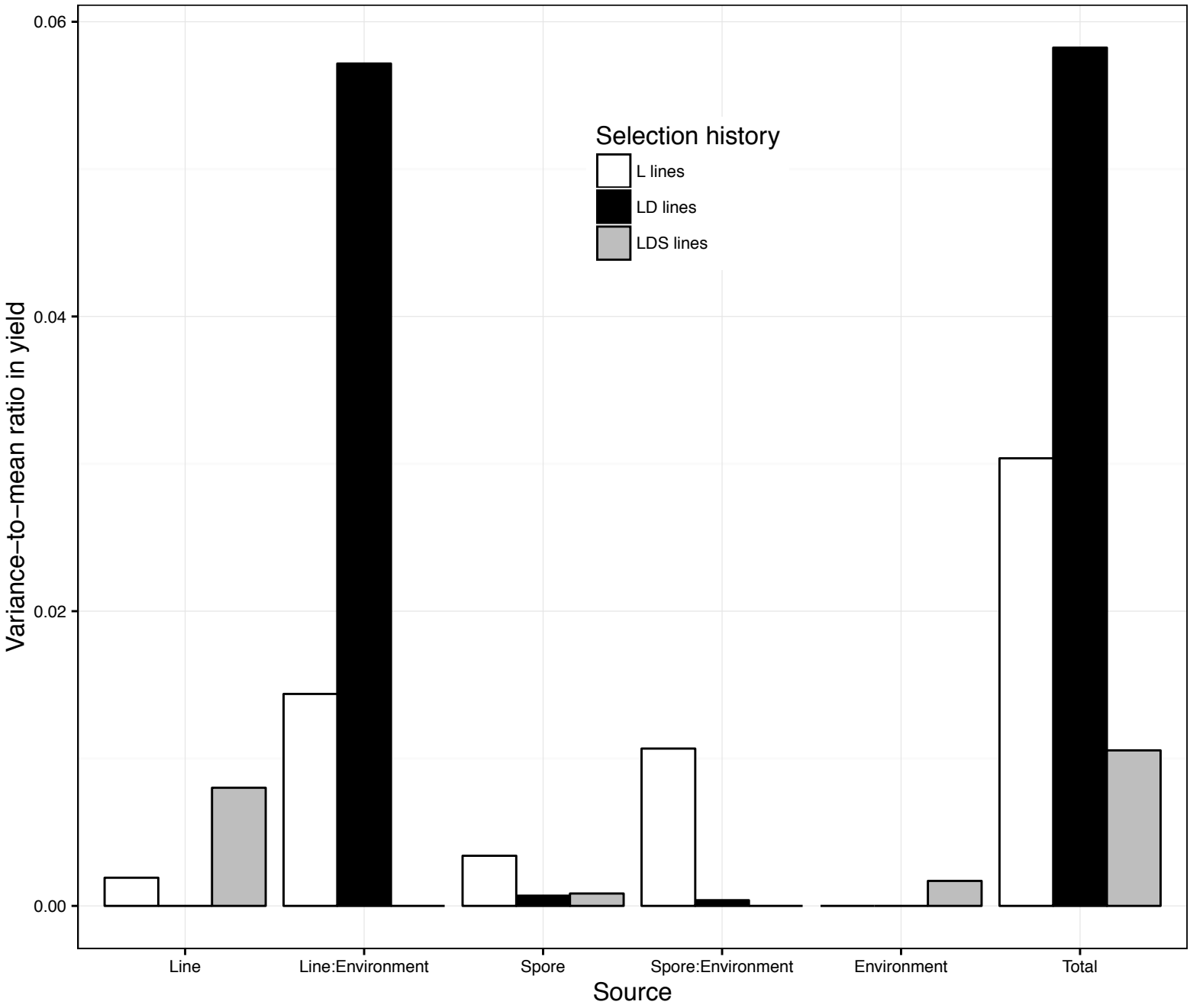
672 Figure 1. Schematic of the selection history of the L, LD, and LDS lines.
673
674 Figure 2. Extinction dynamics of the different selection histories in each assay
675 environment. Survivorship in the selection environments (i.e. Bolds, Dark, NaCl)
676 corresponds to the proportion of lines and spores alive, whereas survivorship in the novel
677 environments corresponds to the proportion of lines and spores alive corrected by the
678 special risk of constant light and no acetate in the case of the LD lines. The survivorship
679 sometimes increases in the novel environments due to correction. That is, when at a given
680 time point survivorship decreased in Bolds but not in the novel environment, this leads to
681 an increase in survivorship in the novel environment. There are three lines per selection
682 history, one for each of the three replicate assays. In the Bolds, Atrazine, and $CuSO_4$
683 environments, the extinction dynamics of the L and LDS lines are exactly the same and
684 fall exactly on top of each other at 1. Time corresponds to the growth cycle number.
685
686 Figure 3. Variance in extinction in novel environments depending on selection history.
687
688 Figure 4. Yield over time of the L, LD, and LDS spores and lines that survived to the end
689 of the assay in each of the three historical environments and the three novel
690 environments. Each point represents one replicate (total of 3 replicates per spore per line).
691 Curves are smoothed trend lines fitted using loess, with 95% confidence interval shading.
692 Time corresponds to the growth cycle number.
693
694 Figure 5. Variance-to-mean ratio in yield in novel environments at the end of the assay
695 depending on selection history.
696


Timeline
1997
Light lines are assembled using progeny from a cross between lab strains
2008
2880 dark lines are assembled using three of the light lines
2011
96 salt lines are assembled using 40 of the surviving dark lines
2015
Extinction assay performed using contemporaneous lines
Light lines
Propagated on Bold's agar in the light
Extinct
Dark lines
(241 lines surviving)
Propagated in Bold's with acetate in the dark
Extinct
Salt lines
(10 lines surviving)
Propagated in Bold's with salt in the light


L lines
LD lines
LDS lines
Bolds
Dark
NaCl
Atrazine
CuSO4
pH4
Survivorship
1.00
0.75
0.50
0.25
0.00
5
10
Time


Selection history
L lines
LD lines
LDS lines
Variance in extinction dynamics
100
50
0
Line
Line:Environment
Spore
Spore:Environment
Environment
Total
Source


Selection
L lines
LD lines
LDS lines
Bolds
Dark
NaCl
Atrazine
CuSO4
pH4
Optical density
Time
0.9
0.6
0.3
0.25
0.20
0.15
0.10
0.75
0.50
0.25
0.6
0.4
0.2


0.06
0.04
0.02
0.00
Variance−to−mean ratio in yield
Selection history
L lines
LD lines
LDS lines
Line
Line:Environment
Spore
Spore:Environment
Environment
Total
Source