

The effect of selection history on extinction risk during severe environmental change

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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. Although 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 towards 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.

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 (Bell & Gonzalez, 2009; Willi & Hoffmann, 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

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increase the supply of variation; by favouring mechanisms that promote gene exchange or recombination such as conjugation, viral infection (Poon & Chao, 2004) and sex (Colegrave, 2002; Lachapelle & Bell, 2012; McDonald *et al.*, 2016); or by changing the type of interactions between genes to promote a more modular genome (Weinreich *et al.*, 2006; Colegrave & Collins, 2008). History can also affect evolvability through differences in the proportion of beneficial mutations that arise because of changes in the distribution of fitness effects of mutations. For example, in rugged landscapes, the probability of jumping from one fitness peak to another decreases as the population climbs a peak because the probability of a mutation with effect size large enough to make the jump decreases (Buckling *et al.*, 2003). Hence, specialization in one environment can reduce the ability to diversify and consequently thrive in other environments.

Evolutionary history may also affect extinction risks if it mediates costs of adaptation through pleiotropy or mutation accumulation. For example, alleles favoured in one environment can have negative impacts on fitness in other environments through antagonistic pleiotropy (MacLean *et al.*, 2004) and therefore lower the probability of survival during environmental change. Similarly, mutations with neutral effects in the current environment but deleterious effects in the new environment can accumulate over time (Kawecki, 1994; Fry, 1996) and lower the probability of survival during environmental change. On the other hand, alleles favoured in one environment can have positive impacts on fitness in other environments through positive pleiotropy, such as when the evolution of resistance to the current stressor indirectly increases resistance to a range of other stressors (Walley *et al.*, 1974; Trindade *et al.*, 2009; Ward *et al.*, 2009; Vogwill *et al.*, 2012; Lagator *et al.*, 2013, 2014a; Rodriguez-Verdugo *et al.*, 2013).

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 whether 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

Selection history

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

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

In 2011, 40 of the LD lines were used to initiate 96 salt lines which were propagated in steadily increasing concentrations of NaCl as described in Lachapelle & Bell (2012) and Lachapelle *et al.* (2015). Ten lines are now surviving in 36 g L⁻¹ NaCl. We refer to these lines as the LDS lines, for light then dark then salt, and they have survived and adapted to two back-to-back stressful environments, first the dark, then a reversion to light with no acetate and added salt (Fig. 1).

Extinction assay

We isolated four spores from each of five lines from each of the three histories. As there are only three ancestral lines for the LD lines, we used the three ancestral lines (i.e. sexual mass-transfer lines) as well as two of the asexual L lines, which have been propagated in parallel. We chose to use the asexual L lines as opposed to the single zygote or unselected lines because

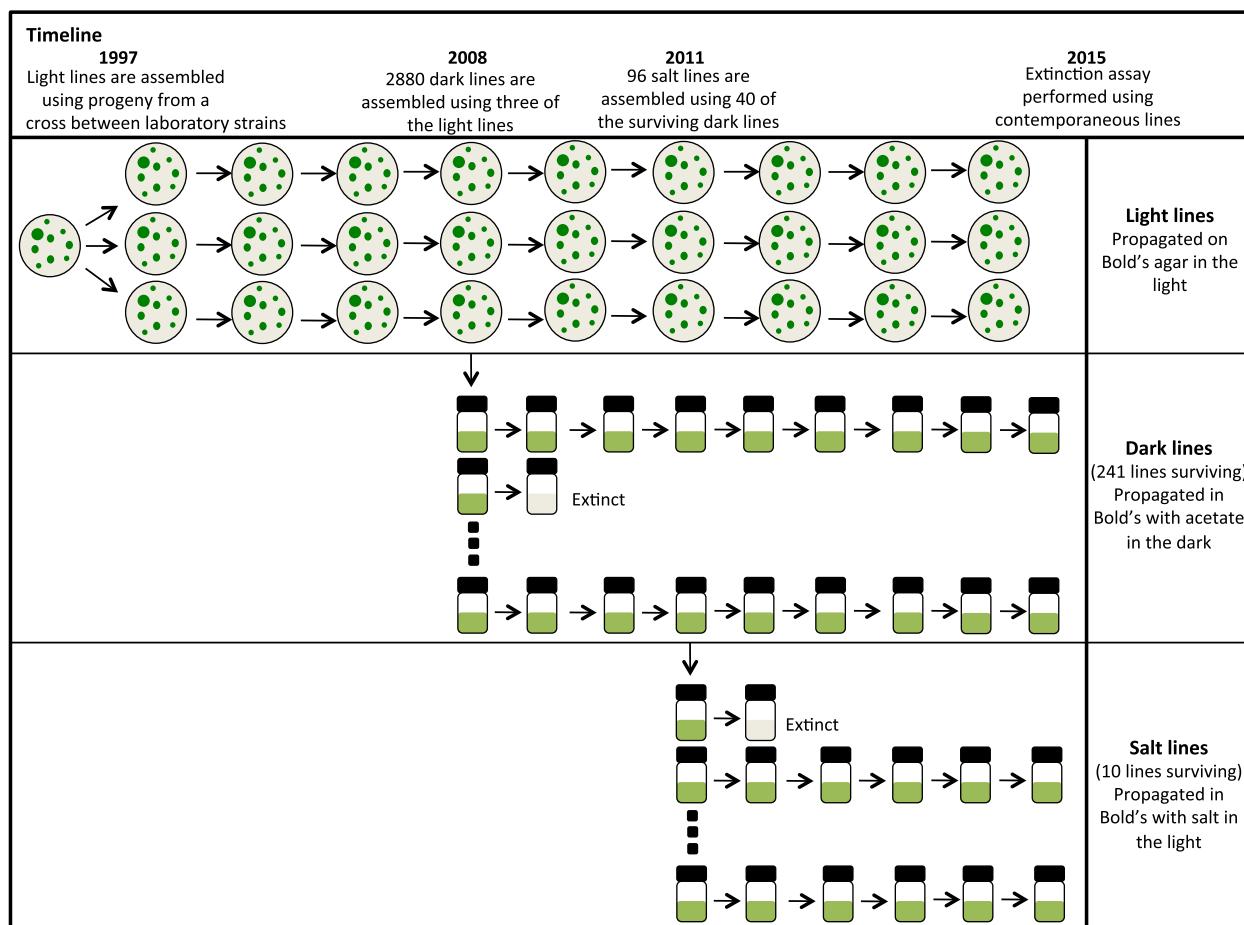


Fig. 1 Schematic of the selection history of the L, LD and LDS lines.

the asexuals have been propagated en masse like the sexual mass-transfer lines, and to avoid the ambiguity of the unselected lines, which by being facultative sexuals, have an unclear history in terms of how much of the progeny is recombinant and how much is clonal. Each spore was assayed three times, in each of six environments for a total of 1080 cultures. To determine whether there has been a direct response to selection, that is if spores from a given selection history have a lower probability of going extinct and a higher yield in their selection environment than spores from other selection histories, we assayed the spores in the three selection environments, that is Bold's minimal liquid media (referred to as 'Bolds'; Harris, 2009); Bold's supplemented with 1.2 gL^{-1} sodium acetate and maintained in the dark (referred to as 'Dark'); and Bold's supplemented with 20 gL^{-1} NaCl (referred to as 'NaCl'). The growth of the L and LD lines in NaCl does not itself represent a direct response to selection, as they have not been selected in NaCl. The direct response is usually determined by comparing the fitness of evolved lines to the fitness of their ancestors. Here,

the L and LD lines therefore serve as the ancestors to which to compare the fitness of the LDS lines in NaCl. To determine the indirect response to selection, that is the 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, that is Bold's media supplemented with 0.4 M atrazine, a herbicide (referred to as 'Atrazine'); Bold's supplemented with $0.1 \mu\text{M}$ CuSO₄ (referred to as CuSO₄); and Bold's buffered to pH4 with a phosphate solution (0.43 gL^{-1} Na₂HPO₄ + 3.36 gL^{-1} KH₂PO₄; 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, CuSO₄ and pH4 were determined by running preliminary growth assays with six wild-type strains (CC-1690, CC-1952, CC-2342, CC-2344, CC-2931 and 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, CuSO₄ 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 Bolds, 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 that of the others. The populations were therefore isogenic at the start of the assays except for any mutation that would have arisen during the growth of the single colony into a population (about four generations). After one cycle of growth, the spores were transferred to all six assay environments. Cultures were then serially transferred once every 7 days by diluting 10 µL of culture into 190 µL fresh media in 96-well plates. To maintain a constant size, a population therefore needs to undergo about 4.3 divisions over a week. The cultures were incubated at 26 °C, 60% air humidity and 7150 Lux constant light intensity.

At the end of each growth cycle, every culture was inspected using an inverted microscope to record the presence or absence of living cells. A culture was deemed extinct if the absence of living cells was recorded for two cycles in a row. The cell density was also estimated at the end of each growth cycle by measuring the optical density at 750 nm with a spectrophotometer. The assay was terminated after 11 cycles (about 55 generations) or later in the case of some environments, whenever the number of extinctions had stabilized for two cycles and none of the cultures were on the brink of extinction.

Statistical analyses

All analyses were performed in R version 3.2.1 (R Core Team, 2017). We examined the effect of selection history on extinction in two different ways. First, the extinction dynamics, that is the proportion of lines alive over time, were analysed by performing survival analyses using Cox proportional hazards with mixed effects, which assume Gaussian random effects, with the 'coxme' R package (Therneau, 2015). In all models, we included a 'Censor' variable for spores that had not gone extinct by the end of the assay. Second, the extinction risk, that is the proportion of lines extinct by the end of the experiment, was analysed by computing two-tailed Fisher's exact tests for independence of number of extinction events and selection history in a contingency table. We report both survival analyses and

Fisher's exact test results except in assay environments where the survival analysis could not be fitted, that is in cases where extinctions did not occur in all selection histories. This is because proper model fitting requires at least one event to have occurred in each level of the fixed factor. In those cases, we report only the extinction risk.

Yield of surviving spores at the end of the assay was analysed by fitting mixed-effects models using the lmer function in the R package 'lme4' (Bates *et al.*, 2015). Our estimate of yield is the optical density at the end of the extinction assay (cycle 11) when populations had stabilized. Although the assay lasted more than 11 cycles in some environments, we decided to use the yield at the end of cycle 11 to be consistent across all environments. *P* values were obtained using the R package 'lmerTest' (Kuznetsova *et al.*, 2016) with type III sum of squares in an analysis of variance and Satterthwaite approximation for degrees of freedom by using the normal approximation.

More precisely, we divided our analyses into two sections: the direct response to selection and the indirect response to selection. First, to determine whether in a given environment, there are fewer extinctions in the selection history most recently selected in 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. Bolds, Dark and 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 whether 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, to determine whether 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, CuSO₄ and 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 are 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 [$1 - (\text{proportion of LD lines alive in Bolds at time } t - \text{proportion of LD lines alive in novel environment } \times \text{at time } t)$]. From this corrected proportion of lines alive, we back calculated the corrected time of extinction. That is, we multiplied the corrected proportion of lines alive by 20 (total number of cultures) to get n , the corrected absolute number of lines alive at each time point. We created a new data set with n rows for lines alive followed by $(20 - n)$ rows for lines extinct. We assigned a number from 1 to 20 to each row. For each line number, we counted the number of time points where the line was alive, and used that number as the corrected time of extinction. Finally, given that the order in which lines go extinct after correction is the same as before correction because the correction is simply a subtraction, we matched the initial and corrected data sets after ordering them by time of extinction to obtain the actual line and replicate number. We report the corrected extinction risk as the general extinction risk in the analyses of the extinction risk in the novel environments.

To determine whether yield of surviving spores in novel environments differs between selection histories, we fitted a mixed-effects model for each novel environment with selection history as a fixed factor, and line and spore within line as random factors.

Finally, to estimate variance in the dynamics of extinction in novel environments, we fitted a coxme survival model for each selection history with line, spore within line, environment (including only the novel environments Atrazine, CuSO₄ and pH4), the combination of line and environment, the combination of spore and environment, as random factors. Note that the coxme function does not accept interaction terms for the random factors, and therefore, we created two new variables by pasting line and environment or spore and environment together. Similarly, variance in yield of surviving lines in novel environments was compared among selection histories using a lmer model with assay environment (including only the novel environments Atrazine, CuSO₄, 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 (Figs 2 and 3; 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, whereas 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, whereas 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 Bolds environment, which is the benign environment, none of the L lines and none of the LDS lines go extinct, whereas 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 ($t_{12} = 0.14$, $P = 0.89$).

Overall extinction risk in novel environments is lowest in the L lines

To determine whether 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 with 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

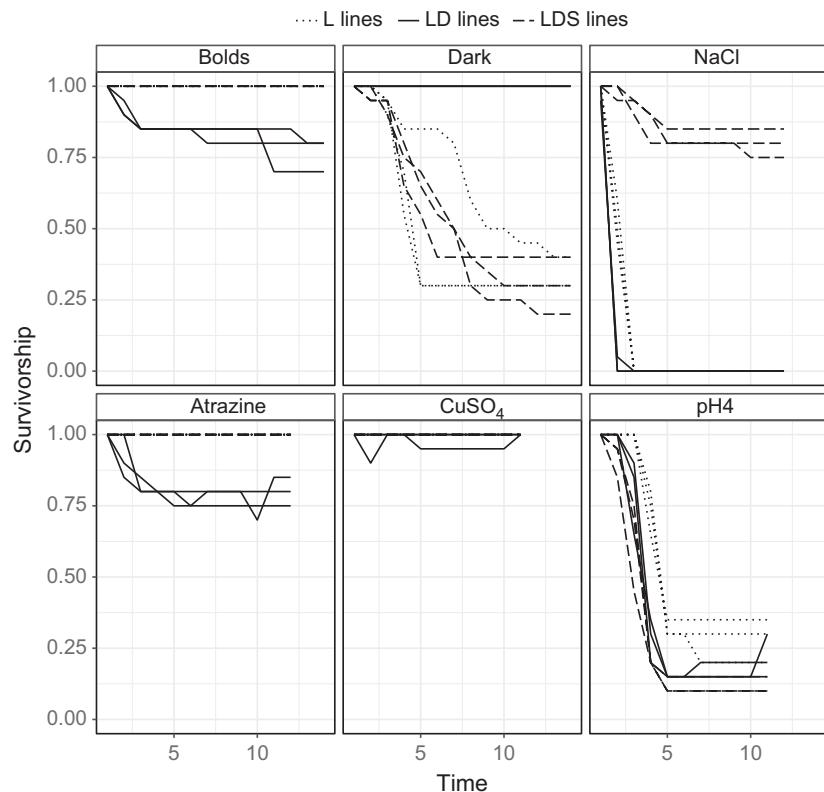


Fig. 2 Extinction dynamics of the different selection histories in each assay environment. Survivorship in the selection environments (i.e. Bolds, Dark, NaCl) corresponds to the proportion of lines and spores alive, whereas survivorship in the novel environments corresponds to the proportion of lines and spores alive corrected by the special risk of constant light and no acetate in the case of the LD lines. The survivorship sometimes increases in the novel environments due to correction. That is, when at a given time point survivorship decreased in Bolds but not in the novel environment, this leads to an increase in survivorship in the novel environment. There are three lines per selection history, one for each of the three replicate assays. In the Bolds, Atrazine and CuSO₄ environments, the extinction dynamics of the L and LDS lines are exactly the same and fall exactly on top of each other at 1. Time corresponds to the growth cycle number.

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 $z = 1.85$, $P = 0.064$). Although 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 L and LDS lines (Fisher's exact test: $P = 1.5 \times 10^{-8}$ for both LD – L and LD – LDS comparisons; L – LDS comparison: $P = 1.0$); in pH4, the LDS and LD lines have a significantly greater extinction risk than the L lines (Fisher's

exact test: LD – L $P = 0.12$; L – LDS $P = 0.038$; LD – LDS $P = 0.79$) and significantly different extinction dynamics (coxme survival model: LD – L comparison: $z = -3.12$, $P = 0.0018$; L – LDS comparison: $z = 1.70$, $P = 0.0073$; LD – LDS comparison: $z = -0.45$, $P = 0.66$). This is with the exception of the CuSO₄ environment where all lines have an equivalent extinction risk (Fisher's exact test: $P = 0.11$ for both LD – L and LD – LDS comparisons).

Yield of surviving lines in novel environment is similar no matter selection history

The surviving lines all reach similar yields in the novel environments (Fig. 3; Atrazine: L – LDS comparison $t_{11} = -1.4$, $P = 0.19$; CuSO₄: LD – L comparison $t_{12} = -1.5$, $P = 0.16$, LDS – L comparison $t_{12} = -0.73$, $P = 0.48$; pH4: LDS – L comparison: $t_{11} = 0.41$, $P = 0.69$), except in Atrazine, where the L lines reach greater yield by cycle 11 than the surviving LD lines ($t_{11} = -2.9$, $P = 0.014$).

Repeatability of extinction

The amount of variance in the extinction dynamics provides an estimate of the repeatability of extinction. That is, if all populations from a given history go extinct at the same rate or all survive, variance in extinction will

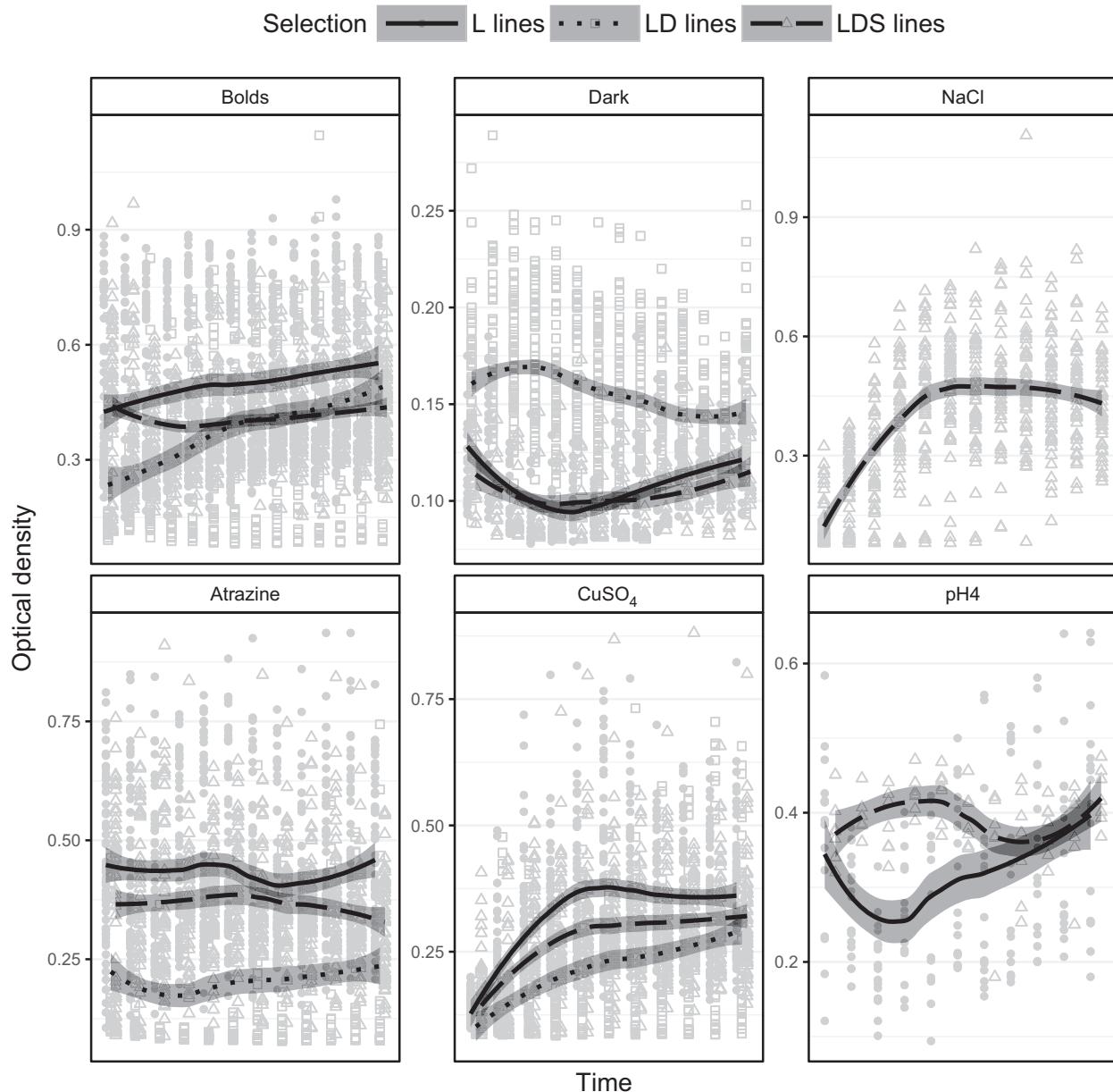


Fig. 3 Yield over time of the L, LD and LDS spores and lines that survived to the end of the assay in each of the three historical environments and the three novel environments. Each point represents one replicate (total of three replicates per spore per line). Curves are smoothed trend lines fitted using loess, with 95% confidence interval shading. Time corresponds to the growth cycle number.

be low and repeatability high. High repeatability is an indication that history plays an important role in extinction. If populations from a given history respond in different ways to environmental change, variance in extinction will be high, and repeatability of extinction low. Low repeatability is an indication that chance plays an important role in extinction.

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 LDS and L lines (Table 2, Fig. 4). 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, that is 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

Table 1 Proportion of spores extinct per line per selection history, in each of the assay environments. The proportions for the LD lines in novel assay environments (i.e. Atrazine, CuSO₄ and pH4) are corrected proportions (see Methods). The proportions represent the number of spores over three assays that were extinct by the end of the assay (4 spores × 3 replicate assays = 12 total spores), such that a number of 1 means that all 12 spores went extinct. Each row represents one of five lines.

Assay environment	Selection history		
	L	LD	LDS
Bolds	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

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 close to two times more variation than in the surviving L lines, and more than three times more variation than in the surviving LDS lines (Table 3, Fig. 5). We obtain the same qualitative results when using variance instead of the variance-to-mean ratio.

Contrary to variance in extinction which is driven mainly by variance among environments, we find that variation in yield is driven mainly by genetic and gene by environment variation. The L lines have high line-by-environment and spore-by-environment variation, indicating that the surviving spores and lines from the L history respond differently to different environments. The LD lines have the highest amount of line-by-environment variation, and almost no other sources of variation, indicating limited variation within lines, but high variability among lines in their response to different environments. Finally, the LDS lines have the highest amount of variation among lines, indicating significant differences among lines that are independent of the environment of assay.

Discussion

We made use of lineages that have undergone two back-to-back events of selection in stressful

Table 2 Significance of differences in variance in extinction dynamics in novel environments between selection histories. Only data from the three novel environments (i.e. Atrazine, CuSO₄ and pH4) are included in the model.

Source	Selection histories	d.f. (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}

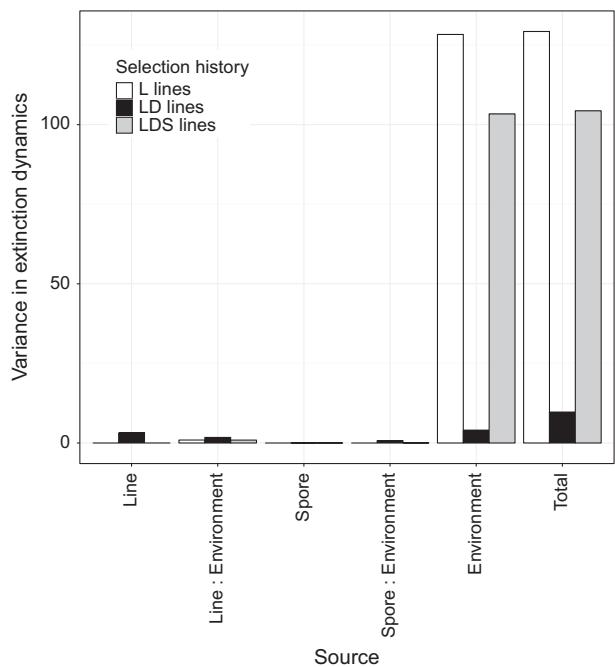


Fig. 4 Variance in extinction dynamics in novel environments depending on selection history.

Table 3 Significance of differences in variance-to-mean ratios in optical density between selection histories when cultured in all three novel environments (i.e. Atrazine, CuSO₄ and pH4).

Source	Selection histories	d.f. (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
Spore	LD – LDS	3, 4	Inf	0.00
	L – LD	15, 12	4.90	4.23 × 10 ⁻³
	L – LDS	15, 15	4.05	5.13 × 10 ⁻³
Spore: environment	LDS – LD	15, 12	1.21	0.374
	L – LD	22, 9	27.7	8.48 × 10 ⁻⁶
	L – LDS	22, 17	Inf	0.00
Environment	LD – LDS	9, 17	Inf	0.00
	LD – L	1, 2	Inf	0.00
	LDS – L	2, 2	Inf	0.00
Total	LDS – LD	2, 1	8.72 × 10 ¹³	7.57 × 10 ⁻⁸
	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

environments to test for a role of selection history on extinction risk in novel environments. Survival in this case occurs as a correlated response to selection in the previous environment. We exposed four spores from

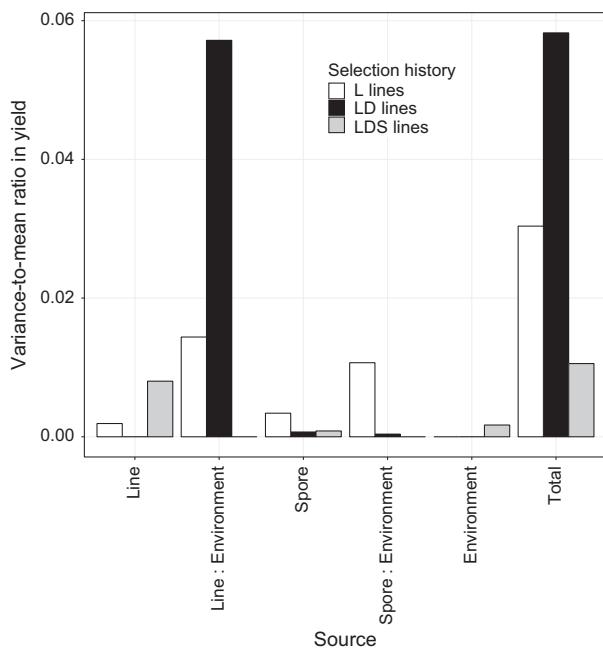


Fig. 5 Variance-to-mean ratio in yield in novel environments at the end of the assay depending on selection history.

each of five lines from before any selection in stressful environments (L lines), after selection in one stressful environment (LD lines) and after selection in two stressful environments (LDS lines) to a range of novel and severe environmental changes. The general extinction risk in a novel environment tended to be higher for lines with a history of selection in stressful environments than for lines with a history of selection in a benign environment.

Our main finding of greater extinction risk after selection in stressful environments is in agreement with what Samani & Bell (2016) found in yeast populations, where populations that had been exposed to long-term starvation had a higher probability of going extinct after exposure to a novel stressor than populations selected in conditions of plenitude. It is also in part in agreement with findings by Gonzalez & Bell (2013) who selected replicate populations of two species of yeast, *Saccharomyces cerevisiae* and *S. paradoxus*, in different concentrations of salt before exposing all surviving populations to an initially lethal concentration of 150 g L⁻¹ NaCl. In accordance with our results, in *S. cerevisiae*, selection in stressful salt concentrations increased the extinction risk. However, the opposite was found in *S. paradoxus*, where selection in stressful salt concentrations reduced the extinction risk. Hence, although there is evidence that selection in stressful environments increases extinction risks during environmental change, other factors, such as species identity, can mediate the effect of selection history.

Extinction risk depends on latest stress encountered

Given that our experimental lines have survived two back-to-back stressful environments, it gives us the opportunity to ask whether the number of past stressful environments itself, that is one or two, affects the extinction risk. If stressful environments select for greater evolvability or positive genetic correlations for fitness among environments, selection in two back-to-back stressful environments should lead to even lower extinction risks than after selection in one stressful environment. We found that there was no general trend of increasing or decreasing extinction risk with the number of stressful environments survived in the past. How much of this result is down to the history of stress *per se*, and how much down to the specific stresses that these populations have encountered is impossible to say from these data. Replication of this study using different selection histories would be needed to determine the generality of the results with regard to the effect of the number of events of evolutionary rescue on extinction risk. The lack of general trend in extinction risk with the number of stressful environment survived in the past could be because it is only the latest stressful environment that determines evolvability and/or costs of adaptation (i.e. there is no accumulation of effects from multiple stressful environments), or although additive, the effects of different stressful environments can be opposite in direction and/or magnitude and thus can lead to a reduction in extinction risk over sequential selection in stressful environments.

The fact that the LDS lines have the same extinction risk in the Dark environment as the L lines, and that LDS lines have significantly different patterns of variance in extinction risk and yield in novel environments than the LD lines, suggests that selection in salt erased the prior signature of selection in the dark. Hence, our results suggest that the latest stressful environment to have survived is more important than the accumulation of evolutionary rescue events. This is in agreement with findings by Lagator *et al.* (2014a) who selected replicate populations of the green alga *Chlamydomonas reinhardtii* in one of three herbicides before exposing all surviving populations to the two other herbicides sequentially. Survivability during exposure to the second and third herbicides was either increased, decreased or not affected, depending on what herbicide in particular was used for the initial selection phase.

The importance of the particular stressor experienced is also indicated by the different results in different novel environment. The CuSO₄ environment was not stressful enough and barely any populations went extinct in it. It was therefore not very informative for distinguishing extinction risks between selection histories. As for the other two novel environments, in

Atrazine, it is the LD lines that have the highest extinction risk and rate of extinction, whereas in pH4 it is the LDS lines that have the highest extinction risk and both LD and LDS have the highest rate of extinction. Hence, selection history in stressful environments leads to higher extinction risks and rates overall, but this effect does vary between novel environments depending on the identity of the previous stressor.

Factors other than the stress *per se* can also affect extinction risks and evolutionary responses. For example, differences in the severity of the stress can affect population sizes and the fraction of beneficial mutations available (Gonzalez & Bell, 2013; Samani & Bell, 2016); differences in the genetic basis of adaptation to different stresses, such as the presence and amplitude of antagonistic epistasis, can lead to differences in how much of a reduction there is in the fitness costs of resistance mutations (Lagator *et al.*, 2014a); and finally, the tempo of environmental change, such as a gradual increase in the stressor or a sudden exposure to high levels of the stressor, can lead to differences in the magnitude of costs of adaptation (Collins & De Meaux, 2009; Lindsey *et al.*, 2013). We therefore cannot exclude the possibility that the greater extinction risk of the LD lines is due, for example, to the fact that survival in the LD lines occurred after a sudden change, which has been shown to involve greater costs than adaptation to gradually changing environments such as in the LDS lines (Collins & De Meaux, 2009; Lindsey *et al.*, 2013).

The role of plasticity in extinction in novel environments

The spores that survived in the novel environments follow a diverse range of dynamics in yield over time, from constant, to steady increase, steady increase followed by a plateau and U-shaped dynamics (Fig. 3). All populations were initiated from a single spore. The only genetic variation present at the time of environmental change was therefore limited to novel mutations generated during the four generations of growth prior to the assay. Population decline upon environmental change would have also reduced the supply of mutations and reduced the probability of fixation. Changes in yield over time are therefore unlikely to be due to genetic changes given the absence of standing genetic variation, and the short evolutionary timescale of the experiment. They are more likely to be due to physiological acclimation or positive growth rates in initially bottlenecked populations. Given that most of the spores that go extinct do so within the first five cycles (about 25 generations) in the new environment, survival during severe environmental change will depend almost entirely on the presence of spores in the population that can either plastically respond or constitutively withstand the novel stressor

enough to prevent population extinction. Significant differences in the magnitude of the plastic response to novel stressors have been found in yeast populations with different selection histories (Samani & Bell, 2016). Hence, prior selection regimes can affect the probability of survival in novel environments by favouring or hindering the evolution of plastic responses (Lande, 2009) or by altering the health of the population and therefore its ability to physiologically respond to stressors.

Within- and among-line variance in extinction risk

By characterizing the rates of extinction of different spores within lines, of different independent lines within selection histories and of different selection histories, in multiple novel environments, we are able to quantify precisely the repeatability of extinction across a whole range of environments. History played an important role in driving the repeatability of extinction, as lines and spores from each given history tended to go extinct at a similar rate in a given novel environment. Almost all variation in extinction rates arose from differences among novel environments, as histories tended to go extinct at different rates in different novel environments. This is with the exception of the LD lines, which showed even greater levels of repeatability than the L and LDS lines, by having similar rates of extinction in all novel environments.

Repeatability in yield differed significantly from repeatability of extinction in terms of what is the source of variation. The environment appears to be the most important determinant of the probability of extinction given it is the largest source of variation in extinction, whereas genetic and gene by environment interactions appear to be the most important determinants of yield. This suggests that chance plays an important role in yield and contributes to low repeatability of yield. The difference between extinction and 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 among 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 variations 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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