Evolutionary rescue and adaptation to abrupt environmental change depends upon the history of stress

Andrew Gonzalez and Graham Bell

Biology Department, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada H3A 1B1

Whether evolution will be rapid enough to rescue declining populations will depend upon population size, the supply of genetic variation, the degree of maladaptation and the historical direction of selection. We examined whether the level of environmental stress experienced by a population prior to abrupt environmental change affects the probability of evolutionary rescue (ER). Hundreds of populations of two species of yeast, Saccharomyces cerevisiae and Saccharomyces paradoxus were exposed to a range of sublethal concentrations of salt for approximately a hundred generations before transfer to a concentration of salt lethal to the ancestor (150 g l⁻¹ NaCl). The fitness of surviving populations of both species was a quadratic function of yield: fitness was greatest for large populations that had been selected on low salt concentrations (less than 20 g l⁻¹ NaCl) and small populations that had adapted to high salt (more than 80 g l⁻¹ NaCl). However, differences occurred between species in the probability of ER. The frequency of ER was positively correlated with salt concentration for S. cerevisiae, but negatively correlated with salt concentration in S. paradoxus. These results not only demonstrate that past environmental conditions can determine the probability of ER after abrupt environmental change, but also suggest that there may even be differences between closely related species that are worth further exploration.

1. Introduction

Biodiversity loss is projected to increase over the next 50 years as drivers of population extinction increase in rate and magnitude. Predicting the regions where populations are most at risk of extinction owing to environmental change requires a theory of extinction that combines both ecological and evolutionary processes [1–7]. Current theory suggests that evolution may have no effect, enhance, or even hamper long-term population persistence in the face of environmental change [4]. Here, we are concerned with evolutionary rescue (ER), the case where evolution can reverse population decline owing to environmental stress, and prevent otherwise inevitable extirpation.

The experimental basis of this theory is currently in development [8–11]. Outstanding questions include the extent of variation among species in the rate and probability of ER, and the effects of past selective conditions on the evolutionary and demographic response to abrupt environmental change. In particular, can adaptation to past stress increase the probability of ER when abruptly exposed to acute levels of environmental stress? We addressed these questions here through experimental evolution in which the fate of many hundreds of populations were followed across a range of selective conditions prior to and following abrupt environmental change, using robot liquid-handling technology.

The probability of ER after environmental change depends upon the initial population size [8], the rate of decline (the degree of maladaptation) and the supply of useful genetic variation. Differences between populations in these characteristics, and their tolerance to stress, are expected to depend upon the history of prior selective conditions. Population tolerance will have arisen as a combination of physiological acclimation and adaptation by natural selection [12,13]. We expect that the frequency of ER will be increased by prior...
selection in a stressful environment because beneficial mutations conferring tolerance to lethal conditions will spread because of their correlated advantage at sublethal levels of stress. If future environmental change is an extension of stressful conditions found within the current range then a genetic correlation between prevailing and future conditions may be expected to facilitate rapid adaptation and ER.

Given population-to-population variation in the harshness of historical patterns of selection, what is the expectation for the response to selection, and the likelihood of ER when all populations are suddenly exposed to lethal stress? Our previous experiments [9] provide us with an expectation. The rate of adaptation depends upon the supply rate of beneficial mutations, the effect of beneficial mutation on population fitness and the correlation between the effect of a mutation in sublethal and lethal conditions. Bell & Gonzalez [9] hypothesized that the overall supply rate of mutations will be lower in smaller populations, and that the fraction of all mutations that are beneficial for population fitness is likely to be low in populations that have experienced little or no stress, but higher in populations that have experienced stressful conditions [14,15]. The supply of beneficial mutations will also be affected by the prior adaptive state of a strain. A strain which is initially more tolerant of a stress will maintain a larger population size in the presence of severe stress and will have a shorter adaptive walk in order to adapt to that stress, relative to a strain that is initially less tolerant. Given these two arguments, fitness will be a quadratic function of stress, with populations subjected to little or no stress adapting slowly, populations at intermediate levels of stress failing to adapt at all, whereas those subjected to severe stress will adapt more rapidly. Bell & Gonzalez [9] provide evidence for this quadratic response in metapopulations of Saccharomyces cerevisiae under gradual and rapidly increasing salt stress.

We report here a test of three aspects of this theory: (i) that historical selection can establish a genetic correlation for fitness in past and future environmental conditions that will increase the probability of ER; (ii) that strains of species with lower tolerance to salt stress will have a lower probability of ER at lethal salt concentrations; (iii) the response to selection under harsh environmental conditions will be a quadratic function of the population yield. We used two species of yeast, S. cerevisiae and Saccharomyces paradoxus, grown at high concentrations of salt (NaCl) as a model system [16]. We use methods in experimental evolution, and a robotic liquid-handling system to attain high levels of population replication required to measure changes in population fitness (as measured by the change in population yield) and estimate extinction probability as a function of increasing salt concentration. Our previous work with this system has shown that the probability of ER following abrupt severe stress is directly related to population size [8,10] and dispersal pattern in metapopulations of yeast [8,10]. Here, we report the effect of historical environmental stress on population fitness and the probability of ER following abrupt environment change.

2. Methods

We cultured the haploid wild-type strains of two yeast species: S. cerevisiae, Meyen, BY4741 and S. paradoxus BY432. Reproduction was asexual during these experiments because there was only a single mating type in the populations. Populations of both species were started from a single colony, so genetic variation was initially low. Salt causes osmotic and ionic stress that reduces growth [17,18]; concentrations in excess of 10 g l⁻¹ inhibit growth rate and yield and a concentration of 125 g l⁻¹ or more was lethal to both of our wild-type strains [8,10].

(a) Experiment

The central 60-wells of two sets of nine 96-well plates were each inoculated with 10 μl of a 24 h culture of S. cerevisiae and S. paradoxus, in yeast proteose dextrose (YPD) and amino acids supplemented with NaCl. The 60 populations of both species were then transferred to nine different concentrations of salt (0, 2, 5, 10, 20, 40, 80, 120 and 150 g l⁻¹). Five μl of all populations was transferred every 72 h to another plate with the same concentration of salt for a total of eight transfers. On the ninth transfer all populations were exposed to NaCl of 150 g l⁻¹. All populations were then transferred a further four times every 72 h to allow population growth at this high salt concentration. After the final transfer the plates were left to grow for a total of 12 days to confirm the fate of the population (persistence or extinction). Plates were manipulated and scored with a Biomek FX liquid-handling system served by a SAMI robot (Beckman Coulter Inc., Fullerton, CA, USA). The whole experiment was then repeated to confirm our result; thus the entire study manipulated 2160 distinct populations.

(b) Statistical analysis

We estimated the effect of salt concentration on population fitness and probability of ER before (phase 1) and after (phase 2) transfer to 150 g l⁻¹. Population fitness during phase 1 was estimated as the change in population yield over eight transfers, as measured by optical density with a spectrophotometer. We tested the statistical significance of a quadratic response in the change in population yield with a general linear model with salt concentration as the predictor variable and strain as a covariate. For phase 2, we analysed the proportion of the 60 replicate populations at each level of salt concentration that had exhibited growth after four cycles at 150 g l⁻¹. We scored a rescue event when the final yield of a population after the last transfer at 150 g l⁻¹ was greater than OD > 0.5. An OD of 0.5 is in the 99th percentile of the readings from the outer blank wells, and is thus a reliable indicator of yeast growth. The probability of ER was estimated as the frequency of rescue events among the 60 re-inoculated wells after the fourth and final transfer to 150 g l⁻¹. The difference in the proportion of rescue events observed when all populations were transferred to 150 g l⁻¹ was analysed with a generalized linear model with binomial errors, with salt concentration as a continuous predictor variable and species as a discrete covariate (data from both experiments were pooled). Finally, we used the rate of increase in population yield to evaluate the fitness of populations over the course of the four transfers at 150 g l⁻¹ of salt. We tested statistical significance of a quadratic response with a general linear model with either prior salt concentration or population yield just prior to transfer to 150 g l⁻¹ NaCl as predictor variables. Data are available in the electronic supplementary material. Analyses were performed in R v. 2.13.1 [19].

3. Results

We report the response of each species over the two distinct selective phases of the experiment.
(a) Phase 1: response to chronic salt stress
Over time many of the populations adapted to high salt concentrations (figure 1). This is indicated by the persistence and even growth (rate of change of yield more than unity) of the populations in presence of the salt despite the regular transfers. There was a marked difference between the two species in their response to selection during phase 1, with \textit{S. cerevisiae} showing a weak negative slope, while \textit{S. paradoxus} showed a significant quadratic relationship (figure 1; species \( \times \) salt\(^2\) interaction for rate of change \( t = 81.48, p < 0.001 \)). An interesting case is 120 g l\(^{-1}\) NaCl: fitness depended on species identity, yield and the concentration of salt experienced in phase 1 of the experiment for \textit{Saccharomyces cerevisiae} (filled circles) and \textit{Saccharomyces paradoxus} (open circles) in (a) experiment 1 and (b) experiment 2. The horizontal lines indicate the optical density for no detectable yield.

(b) Phase 2: population fitness and rescue in response to lethal salt
The demographic response to transfer into 150 g l\(^{-1}\) NaCl was markedly affected by the degree of salt stress experienced in phase 1 of the experiments, and this response was consistently different between the two strains of these species (figure 2).

(i) Population growth
In both experiments, there were significant differences in the fitness of the populations that survived four transfers in 150 g l\(^{-1}\) NaCl. Fitness depended on species identity, yield and the concentration of salt experienced in phase 1 of the experiment (figure 3). We found that population yield was a good predictor of fitness following transfer into 150 g l\(^{-1}\) NaCl: \textit{S. cerevisiae} populations showed a significant quadratic response to yield (yield\(^2\) = 0.79, \( t = 9.44, p < 0.001 \)), with positive fitness at low and high yield. Fitness of \textit{S. paradoxus} populations also showed a significant quadratic response with yield (yield\(^2\) = 0.95, \( t = 7.53, p < 0.001 \)), but growth only occurred at yield OD > 1.2.

(ii) Evolutionary rescue
We observed numerous ER events following transfer of all populations to 150 g l\(^{-1}\) NaCl (figure 4). Analysis of covariance revealed that the proportion of ER events was markedly affected by the concentration of salt experienced by the populations in phase 1, although the species showed opposing responses (figure 5): \textit{S. cerevisiae} showed an increasing proportion of rescue events with increasing salt concentration, while \textit{S. paradoxus} showed a decreasing
proportion of rescue events with increasing salt concentration (species × salt interaction: \( t = -2.43, p = 0.02 \)).

4. Discussion

These results build on our previous findings [8–10] that the likelihood of ER depends upon population size and degree of stress experienced prior to abrupt environmental change. Here, we have shown that rapid adaptation and ER is possible following an abrupt shift to a historically lethal concentration of salt, especially when the populations have experienced prior selection on salt (figures 4 and 5).

Figure 3. Relationship between the rate of change in yield for each population during phase 2 of the experiment (four transfers at 150 g l\(^{-1}\) (NaCl)) and the population yield measured on the final date of phase 1. (a) and (b) show the data from experiment 1, while (c) and (d) show the data for experiment 2. Data points show a grey scale corresponding to the salt concentration experienced by each population during the phase 1 of the experiments (lightest grey = 120 g l\(^{-1}\), black = 0 g l\(^{-1}\)). The grey lines show the locally weighted polynomial regression (lowess) fit.

Figure 4. Example time-series showing evolutionary rescue (ER) of some of the *S. cerevisiae* populations selected at 120 g l\(^{-1}\) NaCl following transfer to lethal salt concentration (150 g l\(^{-1}\) NaCl) at transfer no. 8. Note variation in the transfer at which the minimum yield was observed, and the rate of population recovery.

Figure 5. The probability of ER (mean ± 95% CI) during phase 2 of the experiment as a function of the salt concentration at which the populations were selected during phase 1. *Saccharomyces cerevisiae* (filled circles) and *S. paradoxus* (open circles) in (a) experiment 1 and (b) experiment 2. An ER event was scored if the final OD > 0.5 (0.5 is in the upper 99th percentile of the readings taken from outer blank wells of the plates).
by imposing selection at sublethal concentrations of salt. We have also shown species-specific differences in ER: *S. paradoxus* showed only a marked increase in the probability of rescue when selected at low salt concentrations, while *S. cerevisiae* showed the opposite trend (figure 5). At this time, we have used only a single strain of each species, and so further experiments are required before we can interpret the effects as species level differences in the probability of ER.

We found good evidence for the first aspect of the theory we tested. The increased probability of ER in this context may have been an indirect response to historical selection in less stressful saline conditions. We hypothesize that the frequency of ER was increased by prior selection in a stressful environment because beneficial mutations conferring tolerance to conditions lethal to the ancestor spread because of their correlated advantage at sublethal levels of stress, as argued by Samani & Bell [10]. The indirect response to selection $R'$ of a trait $y$ (e.g. growth in hypersaline conditions) is equal to the direct response $R$ of a trait $z$ (e.g. growth at lower salinity) reduced by the genetic correction of $y$ with $z$ and scaled by the ratio of their genetic standard deviations: $R' = r_{yz}(\sigma_y / \sigma_z)R$ [20]. The overall indirect response integrates the direct response to all prior states of a harsh environment. The ER we observed in extreme salt stress was thus probable because of the cross-environmental genetic correlation of beneficial mutations selected at lower salt concentrations.

The second aspect of our theory, that strains of species with lower tolerance to salt stress will have a lower probability of ER, was also upheld, although this prediction was only markedly apparent for historical selection at high salt concentrations (120 g l$^{-1}$ NaCl; figure 5), where *S. cerevisiae* enjoyed a substantially greater probability of ER than *S. paradoxus* when exposed to 150 g l$^{-1}$ NaCl. Our strain of *S. paradoxus* was less tolerant to salt than our strain of *S. cerevisiae* both before and after selection during phase 1 of the experiment, as indicated by the lower yields at all salt concentrations (figure 2).

The third aspect of our theory was also supported; population fitness over four transfers at historically lethal concentrations of salt was a quadratic function of population size (figure 3). We hypothesize that this fitness response to selection arose because of the interaction between population size at the time of exposure to lethal conditions, and the number and fitness of beneficial mutations that were present in the population transferred to the historical lethal conditions. Figure 2 indicates that population size just prior to transfer was negatively correlated with the salt concentration to which both species had adapted during phase 1 of the experiment. *Saccharomyces paradoxus* was only able to evolve viable populations under the conditions of weaker selection at more than 80 g l$^{-1}$ NaCl, while *S. cerevisiae* sustained viable populations throughout phase 1 of the experiment up to 120 g l$^{-1}$ NaCl. The largest populations of *S. cerevisiae* and *S. paradoxus* derived from low salt conditions, and a small fraction (10–20%) adapted to lethal salt conditions by virtue of their large size [7,8]. However, a surprisingly large fraction of the smallest populations of *S. cerevisiae* (40%) that had adapted to high salt (80–120 g l$^{-1}$ NaCl) underwent ER under lethal salt conditions (figure 5). Historical selection and adaptation to high salt seems to have established the cross-environmental genetic correlation required for persistence at 150 g l$^{-1}$ NaCl. There was no evidence for this in *S. paradoxus*, although this is in part because many fewer populations persisted at 120 g l$^{-1}$ NaCl.

The genetic basis of evolutionary adaptation of yeast to high saline stress is a complex polygenic trait that has been studied extensively at the physiological and molecular levels [18], but relatively little is known about the genetics of evolutionary adaptation to high salt stress. Yeast adaptation to high salt is a good model system for studying ER in eukaryotes, because fungi and plants share similar stress response pathways. Kvitek *et al.* [21] revealed substantial phenotypic and genomic variation within and between 52 *Saccharomyces* strains in their sensitivity to 14 environmental stressors (including NaCl). Bell & Gonzalez [8] identified candidate genes through functional gene profiling, but we have not sequenced populations to identify mutations or changes in gene expression. Dhar *et al.* [22] found evidence that adaptation to high salt stress in *S. cerevisiae* involved changes in gene expression and regulation, and an increase in genome size. A comparative analysis of the genetic and non-genetic basis of rapid adaptation to salt stress across yeast species will be an important direction for future experiments with this model system. Ultimately, this model system approach to validating the fast developing theory of ER should be extended to fast reproducing plants (e.g. *Arabidopsis, Lemna*) and animals (e.g. *Daphnia, Drosophila, Poeciliid* fishes). This development will allow experiments with organisms of sufficient complexity to allow tests of ER theory with organisms more akin to those of conservation concern. We believe that such experiments can provide the basis for the ongoing synthesis between ecology and evolution that is required to understand the impacts of anthropogenic stress on the Earth’s biodiversity.

The robot was programmed and operated by Ming Wang, G.B. and A.G. are supported by funding from the Natural Sciences and Engineering Research Council of Canada and by the Canadian Foundation for Innovation. A.G. is supported by the Canada Research Chair Program.

References

7. Martin G, Aquilée R, Ramsayer J, Kaltz O, Ronce O. 2012 The probability of evolutionary rescue: towards a quantitative comparison between theory and


