Ecology Letters, (2009) **12**: xxx–xxx doi: 10.1111/j.1461-0248.2009.01350.x

LETTER

Evolutionary rescue can prevent extinction following environmental change

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Abstract

The ubiquity of global change and its impacts on biodiversity poses a clear and urgent challenge for evolutionary biologists. In many cases, environmental change is so widespread and rapid that individuals can neither accommodate to them physiologically nor migrate to a more favourable site. Extinction will ensue unless the population adapts fast enough to counter the rate of decline. According to theory, whether populations can be rescued by evolution depends upon several crucial variables: population size, the supply of genetic variation, and the degree of maladaptation to the new environment. Using techniques in experimental evolution we tested the conditions for evolutionary rescue (ER). Hundreds of yeast populations were exposed to normally lethal concentrations of salt in conditions, where the frequency of rescue mutations was estimated and population size was manipulated. In a striking match with theory, we show that ER is possible, and that the recovery of the population may occur within 25 generations. We observed a clear threshold in population size for ER whereby the ancestral population size must be sufficiently large to counter stochastic extinction and contain resistant individuals. These results demonstrate that rapid evolution is an important component of the response of small populations to environmental change.

Keywords

Adaptation, environmental change, experimental evolution, microcosm, population, *Saccharomyces cerevisiae*.

Ecology Letters (2009) 12: 1–7

INTRODUCTION

Rates of biodiversity loss are greater than at any other time in human history. Species extinctions are estimated to be a 100 to a 1000 times the background rate (May & Lawton 1995) and population extinction 10 to a 100 times more (Hughes *et al.* 1997). Mitigation of this loss requires a theory of extinction that integrates both ecological and evolutionary responses of populations to rapid environmental change (Lynch & Lande 1993; Gomulkiewicz & Holt 1995; Holt & Gomulkiewicz 2004; Orr & Unckless 2008). Current theory suggests that evolution may have no effect, enhance, or even hamper long-term persistence in the face of environmental change (Ferrière *et al.* 2004). We used experimental evolution to test the existing predictive framework and reveal the conditions under which evolution can rescue declining populations due to rapid environmental change.

A population exposed to rapid and sustained environmental change will decline geometrically if it is sufficiently maladapted (fitness < 1) and will rapidly become extinct. Before this happens, however, resistant types that are present in the population, or that appear by mutation, may proliferate and restore population growth. The key issue is whether the adjustment of population growth due to evolution is sufficient to counter the rate of decline due to environmental stress. In many cases, adaptation may be partial and evolution will only slow the decline to extinction. However, if adapted types can increase fast enough then the population will spend little time at or close to a critical abundance where it is prone to extinction due to demographic and environmental stochasticity. The populations that undergo evolutionary rescue (ER) have a distinct signature, where population dynamics are typified by a *U*-shaped curve (Gomulkiewicz & Holt 1995; Holt & Gomulkiewicz 2004): a geometric decline, as susceptible individuals are killed, followed by a nearly geometric recovery as viable individuals reproduce. Extinction under environmental change is therefore a race

between demography and adaptive evolution (Maynard Smith 1989).

If some quantitative character is responsible for resistance, the probability of rescue depends on the severity of the stress and on the quantity of genetic variance present in the ancestral population (Lynch & Lande 1993; Lande & Shannon 1996; Barrett & Schluter 2008). If the ancestor is isogenic the probability that it will adapt soon enough to avoid extinction depends on the mutation supply rate (Bell & Collins 2008): for a population of size N_0 with current rate of increase $r_0 < 0$ that experiences U mutations per genome per generation, of which a fraction φ are beneficial with $r_1 > 0$, the probability that a beneficial mutation will spread before the population becomes extinct is $2N_0U\varphi\ (r_0–r_1)/r_0$ (Bell 2008; Orr & Unckless 2008). In either case, it is the size and variability of the ancestral population that determine whether or not it will be able to adapt.

Current theory emphasizes the importance of population size for ER (e.g. Holt & Gomulkiewicz 1997; Willi *et al.* 2006). The simplest model would be a population of size N in which mutations capable of rescuing it from a stress of given severity were present at frequency f; the population would then be likely (but not certain) to adapt if $Nf > 1$. In practice, a somewhat larger value will be required, because the very few resistant individuals originally present may be lost by demographic stochasticity despite their superiority. To test this theory requires that we can estimate f and manipulate N in the face of an environmental stress of known effect. We report here a test using yeast as a model system (Replansky *et al.* 2008), methods in experimental evolution and a robotic liquid handling system to attain the high levels of population replication required to estimate extinction probability as a function of population size in stressful and benign environments.

METHODS

Yeast strains, salt tolerance and population recovery

We cultured the haploid wild-type strain of baker's yeast (*Saccharomyces cerevisiae,* Meyen) BY4741 MATa his3d1 leu2d0 met15d0 ura3d0 as our base population in the experiments. Reproduction was asexual during these experiments because there was only a single mating type in the populations. Salt causes osmotic and ionic stress that reduces growth (Ferrando *et al.* 1995; Hohmann 2002); concentrations in excess of 10 g L^{-1} inhibit growth rate and yield and a concentration of 150 g L^{-1} or more was lethal to our standard wild-type strain (Fig. S1 of Appendix S1). We chose 125 g L^{-1} NaCl as a highly stressful environment in which the population survives for a time but cannot be propagated indefinitely by serial transfer with modest rates of dilution.

Experiment 1: Population rescue following environmental change

We tested whether a large population could recover from an abrupt salt stress by culturing four replicate populations with an initial population size of $\approx 6 \times 10^5$ in 125 g L^{-1} NaCl. Cell density was measured eight times over 5 days by optical density using a spectrophotometer (OD 600 nm) to track population size.

Experiment 2: Population size and the probability of adaptation

To establish the adaptive potential of populations of different initial size, we constructed a dilution series in high-salt medium to span a wide range of densities on microtitre plates and hence a corresponding range of population sizes in the wells. The first level comprised very large populations each of which would include a substantial fraction of all possible single-nucleotide mutations ($n \approx 10^7$ cells). The 10th and final level comprised very small populations of only a few cells ($n < 10$ cells). This dilution series was constructed in two environments: normal growth medium and high-salt medium.

The central 60 wells of a 96-well plate were each inoculated with 10 μL of a 24 h culture of yeast in yeast proteose dextrose (YPD) supplemented with amino acids. This was then used to construct a dilution series in YPD, reducing the number of cells inoculated at each step by a factor of one-quarter. The number of cells transferred at each dilution level was calibrated by estimating the cell density of the 24 h culture by plating. Three independent trials yielded estimates of 5.9×10^7, 5.99×10^7 and 5.53×10^7 cells mL^{-1}, so the initial density was taken to be 6×10^7 cells mL^{-1}. The plates were then used to inoculate a parallel set of plates supplied with the same medium supplemented with NaCl at a concentration of 125 g L^{-1}. Each plate was incubated at 28 °C for 6 days then used to inoculate a second plate with the same medium that was grown for a further 6 days. This second plate transfer and growth phase allowed us to check whether adapted populations (those that had grown), were capable of increasing again from rare. Cell density was measured by optical density using a spectrophotometer (OD 600 nm). Plates were manipulated and scored with a Biomek FX liquid handling system served by a SAMI robot (Beckman Coulter Inc., Fullerton, California, USA). The probability of ER at a given dilution level was then estimated as the frequency of growth among the 60 re-inoculated wells at the end of the experiment. The whole experiment was then repeated to confirm our result.

Experiment 3: The genetic basis of adaptation

We performed this experiment to investigate the genetic basis of the adaptation we observed in Experiments 1 and 2. Using the annotated yeast genome (http://www.yeastgenome.org/), we identified 14 open reading frames (ORFs) whose phenotypes included cation regulation and halosensitivity. Deletion strains (Table S1) were extracted from the Mata haploid single deletion set. We then repeated our initial experiment with strains deleted for each of these ORFs, using an inoculum known from the previous experiments to be sufficient to guarantee ER of the intact wild-type strain. We reasoned that if a deletion strain succeeded in adapting to high salt, the gene deleted was unlikely to have contributed to rescue in the main experiment.

Statistical analysis

The response variable was the proportion of the 60 replicate populations at each level of dilution that had grown by the end of Experiment 1. These were analysed as proportions in a generalized linear model (logistic regression) with binomial errors with initial inoculum size as a continuous predictor variables and treatment (salt addition vs. unsupplemented YPD control) as discrete explanatory variables. Analyses were performed in R (R Development Core Team 2008).

RESULTS

Evolutionary rescue

When a large population is exposed to high concentrations of salt, the number of viable cells declines geometrically over 24 h to ~10% of its initial value, but then stabilizes and subsequently increases geometrically to high values (Fig. 1). Variation in the duration of the valley and the rate of increase thereafter were surprisingly consistent across our replicate populations. This is a clear example of the *U*-shaped curve hypothesized by Gomulkiewicz & Holt (1995) to characterize ER: the dual process of collapse under stress followed by selection leading to renewed adaptation and subsequent growth.

The recovery of the population indicates that it contained a small number of resistant cells at the outset which were able to proliferate as the majority of susceptible cells were dying. The initial frequency of resistant cells can be estimated in two ways. The first is to extrapolate the exponential part of the *U*-shaped recovery curve backwards in time to obtain an estimate of the initial size of the proliferating subpopulation. This gave an estimate of 0.02 for the initial frequency of resistant cells. The second way is to spread a sample from the base population on high-salt plates, which reveals the clear difference between susceptible cells, which remain singletons and resistant cells which give rise to small colonies (Fig. S2, see Appendix S2). Two experiments gave estimates of 0.0155 (SE 0.0016) and 0.0350 (SE 0.0027), with an average of 0.025. Hence, our base population contained ~2–2.5% of resistant cells able to grow at high levels of salt.

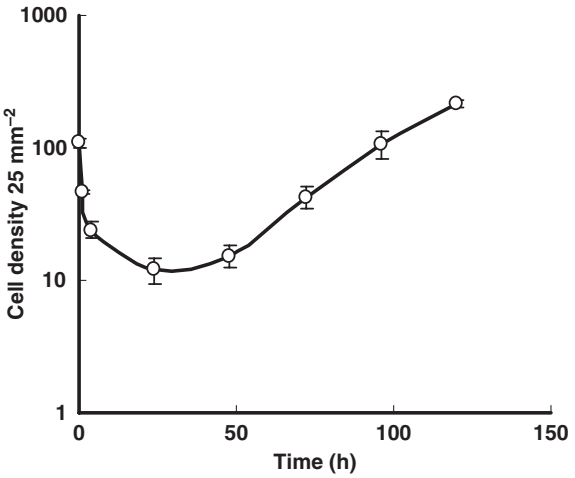

Figure 1 Collapse and recovery of yeast populations (mean ± 1 SE) exposed to high-salt concentration. The concentration used was 125 g L^{-1} of NaCl in yeast–peptone–dextrose medium. The initial inoculum was 10 μL of a 24-hour YPD culture into 140 μL medium, comprising $\sim 5 \times 10^5$ cells, with 4 replicate populations sampled on each occasion. Cell counts made on a 5×5 mm^2 randomly selected on the surface of the YPD plate.

At the smallest population size (highest dilution) some wells may grow, but will suffer rapid extinction even in the normal medium, because of demographic stochasticity (others may fail because they do not receive any cells at all). At the lowest dilution level, so many cells are delivered that adaptation to high salt is almost certain and both control and high-salt populations will grow. Between these two extremes exists a threshold where the high-salt populations fail to grow although the control populations grow normally, which marks the limit of ER. Because our simple theory predicts that adaptation requires $Nf > 1$, while observations show that $f \approx 0.02$, we expect that this population threshold will lie near $n \approx 50$ cells, corresponding to level 8 of the experimental dilution series.

The effect of population size

During Experiment 2 and in the absence of salt, extinction due to demographic extinction occurred under the highest dilution levels: at levels 9 (~12 cells) and 10 (~3 cells), 5% and 30% of populations went extinct respectively (Figs 2 and 3). In high-salt medium, the final densities were also bimodal, with growth in some but not in others (Fig. 2).

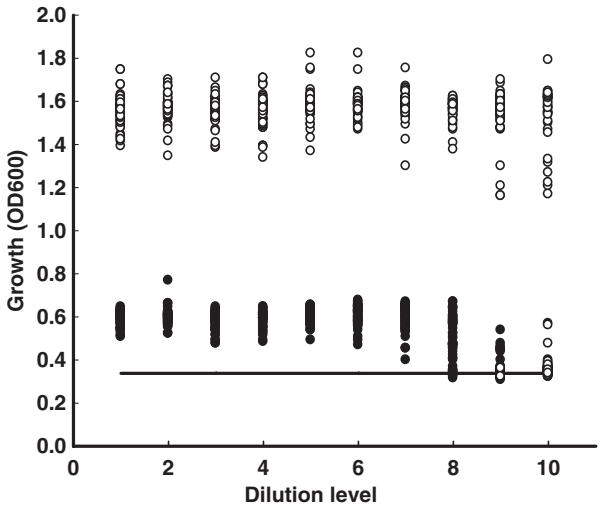

Figure 2 Final population densities achieved by control (open circles) and salt-exposed populations (filled circles) in relation to initial population size (dilution level, where level 1 had the largest populations, see Methods). All replicate populations are shown in this figure. Growth was measured as optical density (OD 600 nm). The solid line indicates the mean value of the blank wells that contained no cells.

The probability of growth was 1 for large populations, but fell abruptly in a sigmoidal fashion to nearly zero below the critical dilution level (Fig. 3). In the first experiment, adaptation to salt usually failed to occur above dilution level 8 (∼50 cells) and in the second above level 9 (∼12 cells). These values are close to the quantitative prediction of our elementary theory. Populations at higher dilution levels occasionally succeeded in adapting, which is expected to occur because of the stochastic variation in the number of resistant cells inoculated into each well. Logistic regression on the proportion of populations that grew at each dilution level fit these data well and revealed a highly significant effect of salt and initial density on the probability of persistence in both experiments (Table 1).

From the fitted models, we calculated that the initial population size required to produce a 50% probability of ER50 in the presence of salt was 526 individuals in Experiment 1 and 28 individuals in Experiment 2. However, comparison of the final densities in the two treatments revealed that populations adapted to salt did not grow as well as those in normal medium (Fig. 2). This fitness cost, measured as the difference in final density between normal and high-salt medium, was > 50%, even for those adapted populations that derived from the very largest initial populations. This result is also predicted by theory (Holt *et al.* 2005).

The results from Experiment 2 can also be used to provide a rough estimate of the beneficial mutation rate in

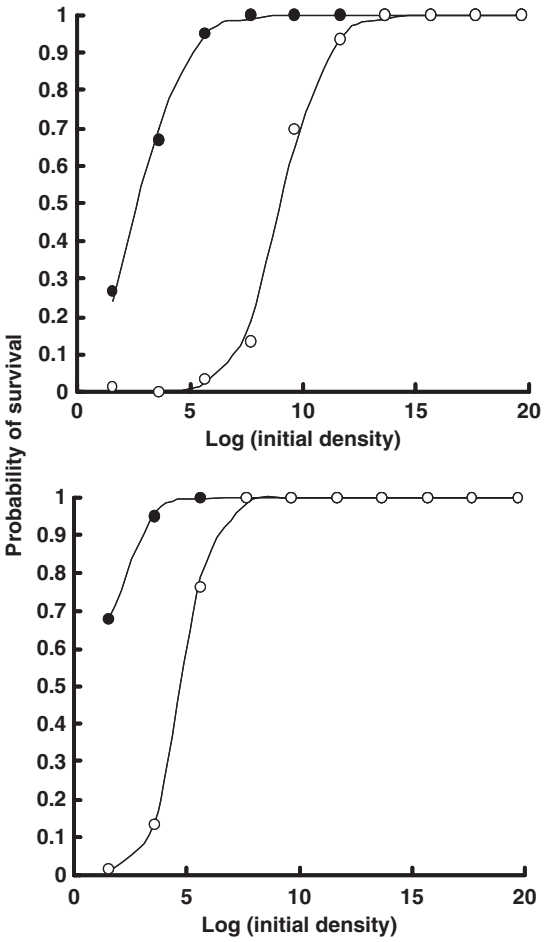

Figure 3 Probability of survival of salt-stressed populations in relation to the log of initial population size (dilution level). Two independent experiments in upper and lower panels. In each panel, the left-hand curve (filled circles) is for YPD, the right-hand curve (open circles) is YPD +125 g L^{-1} NaCl. Each point is based on 60 replicate populations. Lines are fitted logistic regressions see Table 1.

Table 1 Results from analysis of covariance with binomial response (logistic regression) for population survival for both runs of Experiment 2

	Estimate	SE	χ-value	Pr > |z|
Run 1				
Intercept	−13.33	1.92	−6.91	4.9×10^{-12}
Initial density	1.81	0.47	3.90	9.5×10^{-5}
Treatment	6.09	1.10	5.52	3.5×10^{-8}
Treatment × density	−0.31	0.34	−0.91	0.36
Run 2				
Intercept	−16.4	2.06	−7.94	2.0×10^{-15}
Initial density	1.12	0.25	4.35	9.2×10^{-6}
Treatment	6.85	1.10	6.23	4.6×10^{-10}
Treatment × density	−0.07	0.17	−0.41	0.68

our high-salt environment. The P_0 method (Luria & Delbrück 1943; Foster 2006) uses the proportion of populations that did not grow, and hence had no beneficial mutations; P_0 is the proportion of populations with no mutations. The method uses the zero-th term of the Poisson distribution: $P_0 = \exp(-m)$, where m is the number of mutations. So, $m = -\ln(P_0)$. From our experiments we have two values of P_0 from the 60 populations at high salt and the lowest dilution (largest population size): 0.8 and 0.9:

$$m = -\ln(0.8) = 0.223$$

$$m = -\ln(0.9) = 0.105$$

Dividing m by twice the initial population size (assuming mutations occurred at or during division) gives two estimates of the mutation rate:

$$0.223/1.2 \times 10^6 = 1.86 \times 10^{-7}$$

$$0.105/1.2 \times 10^6 = 8.78 \times 10^{-8}$$

These values are typical of mutation rates accurately estimated for *S. cerevisiae* in other selective environments (Lang & Murray 2008). Interestingly, these approximate mutation rates predict a frequency of 5% and 7% resistant cells for the initial population size we used in Experiment 1, and are close to the value of ~2% estimated by direct plating (Fig. S2). Further experiments are required to provide an accurate estimate of the per-base-pair mutation rates at the genes likely involved in salt tolerance.

We found that the growth of 12 of the deletion strains was similar to that of the wild-type control, whereas growth was severely reduced in one strain (*HAL5* deleted) and abolished in another (*PMP3* deleted) (Fig. 4). *HAL5* encodes a putative protein kinase, and its deletion is known to increase sensitivity to salt (Mulet *et al.* 1999). *PMP3* encodes a small plasma membrane protein concerned with the regulation of membrane potential (Navarre & Goffeau 2000). This experiment suggests that these two genes were primarily responsible for the adaptation that we observed.

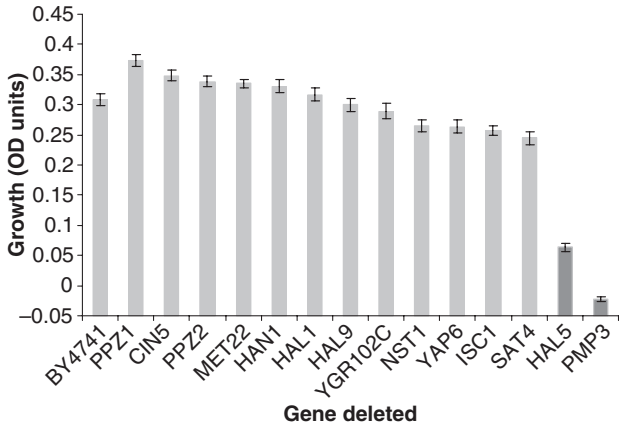

Figure 4 Adaptation of deletion strains to high-salt medium. Each value is the mean optical density (600 nm) for 60 cultures (± 2 SE) after 5 days' growth following re-inoculation. The wild-type is furthest to left. Dark grey bars indicate the deletion strains that showed little or no growth in the presence of high salt (see text for details).

DISCUSSION

Taken together these experiments provide the first experimental demonstration of ER and confirm that it is qualitatively and quantitatively consistent with the outcome predicted by theory (Gomulkiewicz & Holt 1995; Holt & Gomulkiewicz 1997; Bell 2008; Orr & Unckless 2008). In particular, we have shown that ER is typified by a *U*-shaped trajectory of rapid population decline followed by an exponential increase of adapted types within the population. The population therefore attains a minimum value which may pass close to a critical population size, where it is susceptible to extinction because of demographic and environmental stochasticity. The time taken to reach this critical population size will depend upon the initial population size and the rate of decline (the degree of maladaptation). We controlled the level of maladaptation and showed that initial population size is a crucial variable determining the probability of population extinction (Holt & Gomulkiewicz 1997).

As Maynard Smith (1989) emphasized, extinction under environmental change is a race between demography and adaptive evolution. We have shown that evolution can win this race, but that a 50% probability of adaptation may require many 100s of individuals even only in the presence of demographic stochasticity. Because we excluded environmental stochasticity our results are likely an underestimate of the population size required for ER in variable environments (e.g. Lande & Shannon 1996). Furthermore, our experiments revealed that salt tolerant cells existed at low frequency in our experimental populations. Recent theory (Orr & Unckless 2008) suggests that ER may be more difficult when there is no useful standing variation in the population and new mutations are required. Our rough estimates of the rate of beneficial mutation indicate that a sufficiently large population could contain enough useful variation to recover by natural selection. We assumed a single discrete change in environmental stress which models some aspects of environmental change better than others. However, environmental change may involve multiple stressors (e.g. temperature and CO_2) acting in synergy, and these experiments and current theory may not adequately predict the outcome of selection when adaptation involves multiple interacting dimensions. Further experimental work

is required to understand the role of these important complicating factors.

Methods in experimental evolution and an automated liquid handling system allowed us to manipulate the selective environment of many 100s of microbial populations. By manipulating the rate of environmental change, the severity of stress and the quantity of genetic variation (independent of population size), it will be possible to validate a general theory of adaptation and extinction. Such experiments are currently under way in our laboratory. Experiments like these are more difficult to perform with multicellular organisms but they are possible. Willi & Hoffmann (2009) parameterized a population model of persistence time using data from experimental populations of *Drosophila birchii* and environmental change in the form of heat-knockdown selection. Although they did not demonstrate ER their results suggest that larger populations tend to persist longer because they have higher reproductive output, lower stochasticity in growth rate and more additive genetic variance for heat-knockdown resistance. These results suggest that persistence in animal populations in the face of environmental change will be a function of population size.

Evidence from outside of an experimental context is ambiguous. Microbial populations often adapt rapidly to highly stressful environments, the classic example being antibiotic resistance in pathogenic bacteria (see Levin *et al.* 1997). Animals and plants may also adapt rapidly to anthropogenic stress, including heavy metal pollution (McNeilly & Bradshaw 1968), smoke damage (Kettlewell 1973), herbicides (Jasieniuk *et al.* 1996), pesticides (Georghiou 1972) and over-fishing (Handford *et al.* 1977). Thompson (1998) and Hendry *et al.* (2008) have reviewed and synthesized this literature. Nevertheless, they often fail to adapt. In naturally acidified lakes, for example, fish may adapt so as to be capable of foraging in water with pH as low as 3.5 (Hirata *et al.* 2003). This is a long-term response, however; in the short term, fish populations in lakes that have been rapidly acidified by smelter fall-out usually become extinct. In highly acidified lakes, with a concomitant burden of heavy metals, even large microbial populations are eliminated (Kwiatkowski & Roff 1976). The evolution of heavy metal tolerance among plant populations growing on old mine tailings is a classical example of rapid natural selection. Nevertheless, Bradshaw (1991) has emphasized that only a minority of species in the original community consistently evolve high levels of tolerance, almost certainly through the selection of pre-existing genetic variation. Most species lack this variation, and despite their large populations become extinct at heavily polluted sites. For this reason, Bradshaw & McNeilly (1991) were skeptical that most species would succeed in adapting to global change.

Hence, a general theory of ER will be indispensable for predicting the broad consequences of global change. The crucial parameters of a general theory will be the rate at which the stress is applied, the overall severity of the stress, the size of the affected population, and the amount of genetic variation available to natural selection. These conclusions hold across a broad range of models (Gomulkiewicz & Holt 1995; Holt & Gomulkiewicz 1997; Willi *et al.* 2006; Bell 2008; Bell & Collins 2008; Orr & Unckless 2008) but it remains to be seen to what extent they will be verified under experimental conditions in the laboratory or the field. Evolution experiments like those described here will no doubt play a key role as the theory of ER is developed, refined and applied to meet the many challenges of environmental change facing global biodiversity.

ACKNOWLEDGEMENTS

The robot was programmed and operated by Zhang Ming Wang, assisted by Anne-Marie L'Heureux. GB and AG are supported by funding from the Natural Sciences and Engineering Research Council of Canada and by the Canadian Foundation for Innovation. AG is supported by the Canada Research Chair Program.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 The population yield of our wild-type strain of *Saccharomyces cervisiae* across a broad range of salt concentration.

Figure S2 Photograph of susceptible cells and viable yeast colonies growing on salt supplemented agar.

Table S1 List of deletion strains used in Experiment 2.

Appendix S1 Further methodological details.

Appendix S2 Cell growth on high salt.

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First decision made 6 April 2009
Manuscript accepted 17 June 2009