nature portfolio

Corresponding author(s): DBPR NCOMMS-21-49237

Last updated by author(s): Dec 23, 2021

# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☐ | ☒ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | No software was used |
| Data analysis | All statistical analyses were performed in R version 3.5.3. Packages used- stats, ARTool. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The datasets analysed during the current study are available in the Dryad repository, https://doi.org/10.5061/dryad.zs7h44j9b.
Temporary access link for reviewers: https://datadryad.org/stash/share/jYDduZ8xc6W8KSLCkffG6vvXjqlH9EVhnFMVLIn4I2k.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Study description | This study tests the hypothesis that bet-hedging can persist in the short term through the evolution of adaptive constraints. This is tested through experimental evolution of Saccharomyces cerevisiae across two sequential heat shock regimes that progressively decrease in the frequency of heat shocks. We predict the evolution of adaptive constraint in lines evolved in a regime consisting of low-frequency heat shocks. The 8 evolved replicate lines from both regimes and the ancestral strain are first assayed for heat shock tolerance and fitness under benign conditions to verify successful evolution across regimes. Then, to assay for evolutionary constraint, the 8 replicate lines from both regimes are further evolved under constant benign conditions and assayed for the loss of heat shock tolerance (and concomitant gain of fitness under benign conditions). These results are observed as the reduction of heat shock tolerance (and gain of relative fitness) as a function of the regime evolved in (high or low frequency heat shocks), time evolved under constant benign conditions, and the interaction of these two factors. |
| Research sample | Research sample is a thermotolerant Saccharomyces cerevisiae strain T1 (S288C background- (MATα SUC2 gal2 mal2 mel flo1 flo8-1 hap1 ho bio1 bio6)) previously adapted under constant high temperature (40 °C). Lines evolved using this thermotolerant strain across two sequential heat shock regimes are also measured. |
| Sampling strategy | All populations were homogenized with vortexing prior to sample collection (for both for heat shock tolerance assays and for competitive fitness assays). Viability counts (post heat shocks) and colony counts (for estimates of proportions in competition experiments) were performed while ensuring adequate total count numbers per plate. |
| Data collection | For a given assay, all samples were plated and measured simultaneously. Shravan Raghu (corresponding author) recorded all data. Following heat shock experiments, viable counts of the samples from the 8 replicate lines from both regimes were estimated by visual colony counting on solid YPD agar. For competition experiments, initial and final proportions were estimated by plating on solid YPD agar with and without G418 (a drug marker for the reference strain). Subsequently, plates were incubated and colonies were visually counted. |
| Timing and spatial scale | All data was collected between July and September 2019. All populations, at the end of experimental evolution, were frozen as glycerol stocks. Assays were performed using the frozen glycerol stocks as samples. |
| Data exclusions | No data was excluded from the study |
| Reproducibility | Methods to do so are described in detail. Frozen glycerol stock of all the samples are available for future studies, including reproducibility tests. |
| Randomization | The thermotolerant ancestor T1 was plated on YPD agar and a single colony was inoculated and used for experimental evolution. Eight replicate lines were inoculated using this culture. No covariates were controlled for as this study compared evolved lines subjected to the same background laboratory conditions. |
| Blinding | Blinding is not applicable to this study because the number of cells being transferred in a quantitative aliquot cannot be ascertained by the experimenter, and final survival and growth assays are strictly quantitative and not subject to interpretation. |

Did the study involve field work? ☐ Yes ☒ No

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |