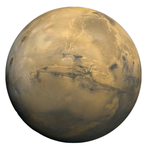
Experimentally Evolved Tolerance to Desiccation and UV-C Radiation in *E. coli* and Its Implications for Extant Life in Martian Near-Surface Environments



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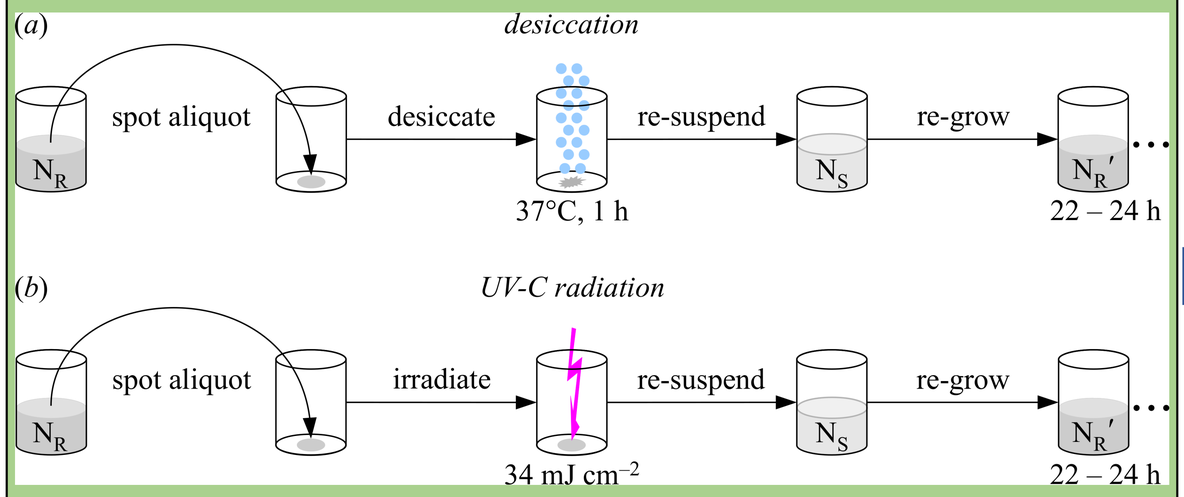
Introduction

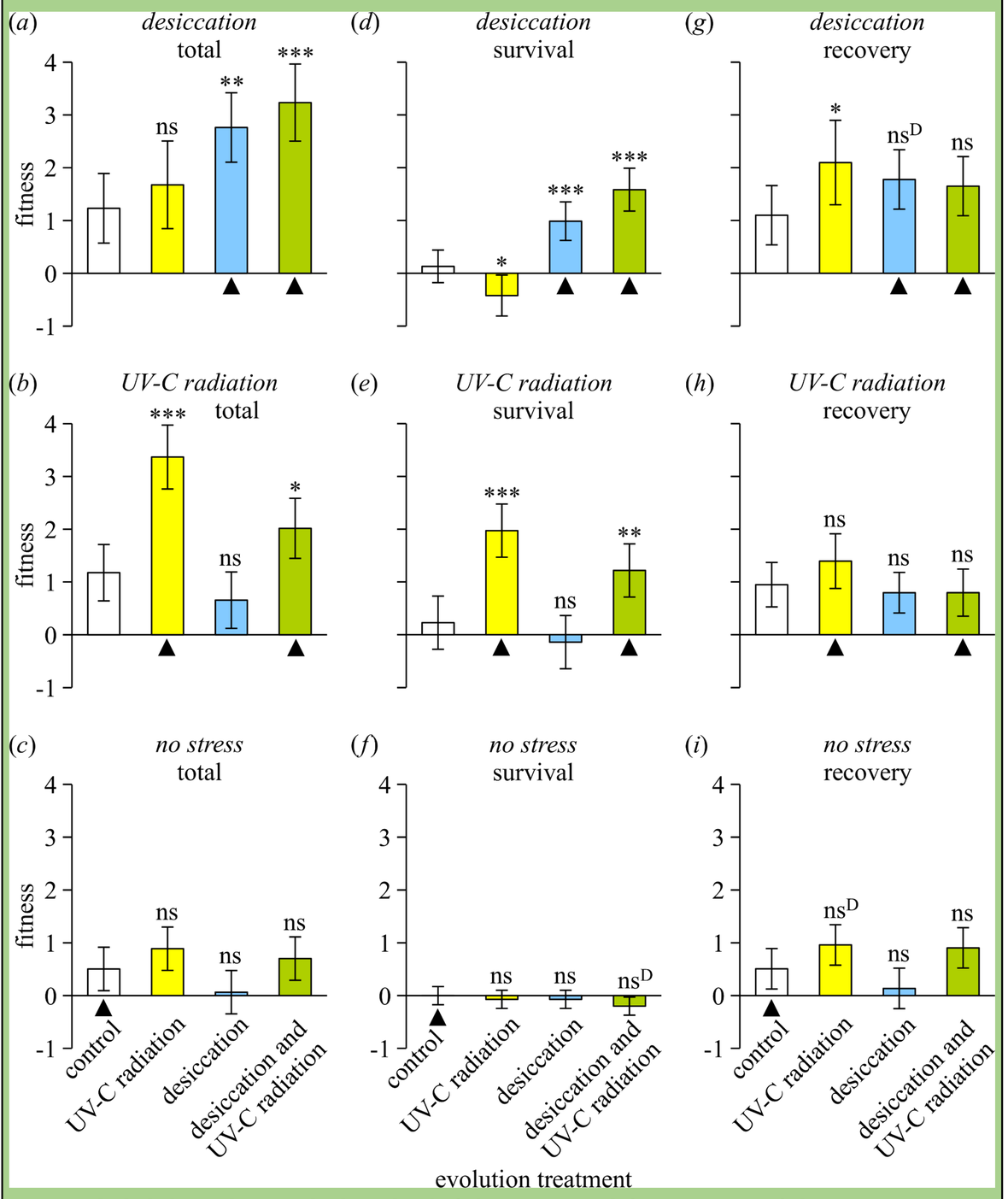
If life ever existed on Mars, and it was present near the surface, its evolution would have been shaped by a very different, and much more extreme, environmental history than that which has shaped Terran life [1–3]. The evolutionary dynamics that may have resulted from Mars's unique environmental history perhaps potentiated a capacity for Martian life to adapt to today's hostile surface conditions. In this case, life might persist near Mars's surface, i.e., within a couple of meters. Desiccation and UV-C radiation would likely be the greatest challenges to persistence near Mars's surface [4–6]. We therefore carried out an evolution experiment with *Escherichia coli* to investigate the evolutionary dynamics of *de novo* tolerance to those stressors.

Experimental evolution under stress from desiccation and UV-C



Four groups of 6 clonal populations of *E. coli* were evolved for 500 generations under one of four conditions: (*a*) desiccation only, (*b*) UV-C only, both stressors combined, or no stress (control). Fitness relative to the ancestor was assayed in two phases, survival (N_S) and recovery (N_R'), which were summed to give total fitness; N_R, initial cell density.

Desiccation and UV-C tolerance evolved within 500 generations



We concluded that tolerance had evolved if the mean total fitness (*a*–*c*) of the treatment group significantly increased relative to the control group (white bars) when exposed to the respective stressor. The fitness assay stress and phase (survival, *d*–*f*, and recovery, *g*–*i*) are given above each panel. Error bars represent 95% CIs: *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$; ***, $P \leq 0.001$; "ns", no significant difference; "ns^D", the unadjusted *P*-value was marginally significant, but the Dunnett-adjusted *P*-value was not. A triangle indicates that the fitness assay stress was also the treatment for that group during the evolution experiment.

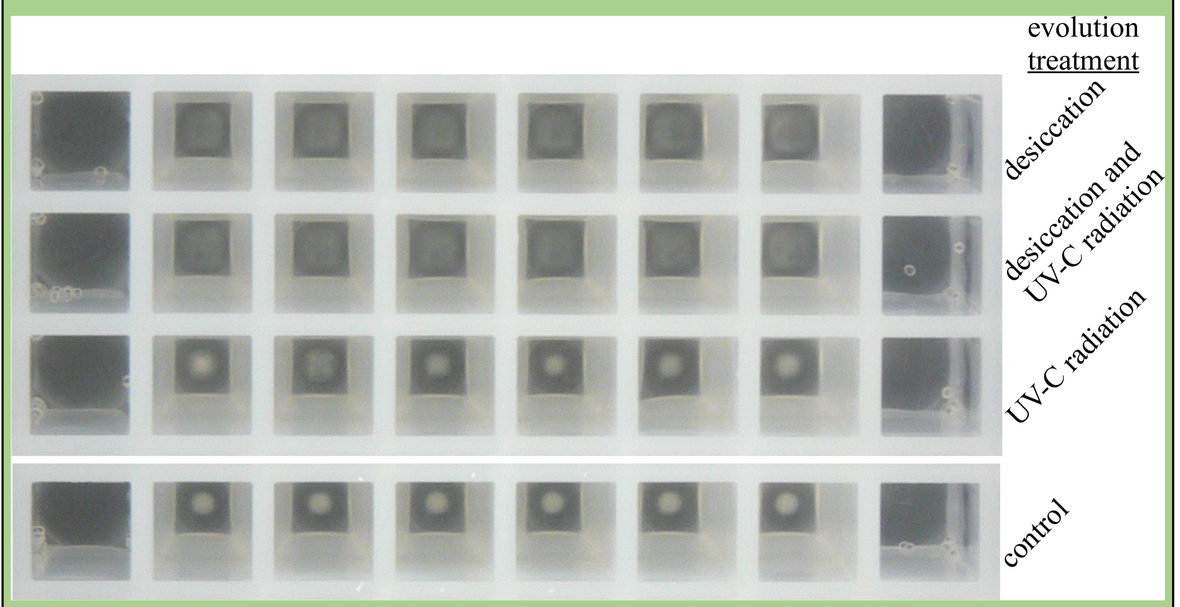
Experimentally evolved tolerance to desiccation and UV-C

- Tolerance to desiccation and UV-C evolved rapidly in an organism sensitive to those stressors
- Cross-tolerance did not evolve; tolerance to both stressors required dual treatment (fitness *a*,*b*)
  - Lack of cross-tolerance suggests different mechanisms for desiccation and UV-C tolerance
- Increases in survival were key to evolving tolerance in all treatment groups (fitness *d*,*e* vs. *g*,*h*)
- UV-C tolerance incurred a survival trade-off under desiccation, but gained recovery (fitness *d*,*g*)
- Desiccation-treated populations evolved a unique phenotype involving diffuse cell pellets

Could extant life exist in Martian near-surface environments?

- Tolerance levels that evolved in this study would be insufficient for persistence near the surface
  - Our experiment ran just 500 generations; further evolution might provide sufficient tolerance
- Experimentally evolved tolerance to other Mars-relevant stressors can also occur rapidly [7–9]
- Some Earth organisms can *survive* combinations of Mars's surface stressors, ***but not grow*** [4–6]
- ***Near-surface life might exist if conditions allowed adaptation to one or two stressors at a time***
- Conditions in polar permafrost may have allowed stepwise adaptation to multiple stressors
  - If so, life might persist there if high obliquity intervals are less than a couple of Myr [10–12]

Populations evolved under desiccation form diffuse cell pellets



Populations that evolved under stress from desiccation (upper two rows) form diffuse cell pellets after settling overnight in spent growth medium (view is from the top, looking down into wells). We have not yet investigated this diffuse pellet phenotype, but we hypothesize that it involves a change in the production or composition of exopolysaccharides. ***We welcome other hypotheses*!**

References *(with clickable links)*

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