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: Surface environments on Mars were almost certainly habitable in the past [1−3], but severe environmental changes [1−3] left conditions that today are likely inhospitable, at least for Terran life [4−6]. Thus, if life ever existed on Mars, and it was present near the surface, its evolution would have been shaped by a very different environmental history than that of Terran life [1−3], perhaps potentiating a capacity for Martian life to adapt to the hostile surface conditions. In this case, life might persist near Mars's surface, i.e., within a couple of meters of the surface.

The greatest barriers to persistence in near-surface environments on Mars would likely be desiccation and ultraviolet (UV) radiation [5−7], particularly the highly mutagenic UV-C wavelengths. The physiology and genetics of tolerance to these two stressors are fairly well understood, but not their evolutionary dynamics. We therefore carried out an evolution experiment in which replicate populations of *Escherichia coli* were propagated under stress from desiccation and UV-C.

Materials and methods: Four groups of six clonal populations of *E. coli* were evolved for 500 generations under one of four conditions: desiccation only, UV-C only, both stressors combined, or no stress (control). The treatment groups experienced daily pulses of the respective stressor. Fitness of the groups under each stressor was assayed by competing their stress-evolved populations against the ancestor.

Results: We concluded that tolerance had evolved if the mean fitness of the treatment group significantly increased relative to the control group when exposed to the respective stressor. All groups evolved tolerance to the treatment stressor, more than doubling their fitness. Cross-tolerance, i.e., tolerance to the non-treatment stressor, did not evolve; e.g., the desiccation-treated group was not tolerant of UV-C. However, the group treated with desiccation and UV-C combined evolved tolerance to both stressors. Increases in survival, rather than recovery, were key to evolving tolerance in all three treatment groups.

Discussion: Tolerance to desiccation and UV-C evolved within a mere 500 generations, in an organism that is very sensitive to these stressors (ancestor: 1.2% and 2.5% survival, respectively). The tolerance levels of the evolved populations to these stressors would be insufficient for persistence near the surface of Mars, although further evolution could potentially do so. Nevertheless, our results demonstrate that tolerance to desiccation and UV-C can evolve rapidly, even absent the history of severe environmental changes that would have shaped possible Martian life. Moreover, adapting to both stressors simultaneously does not appear to be overly difficult. However, our results also show that cross-tolerance to desiccation and UV-C, as occurs in some organisms, e.g., *Deinococcus radiodurans* [8], does not necessarily evolve.

Concerning other stressors present at the surface of Mars, experimentally evolved tolerance to freeze–thaw and hypobaricity can occur quickly [9, 10], and some microbes can grow at Mars-level hypobaricity [11−13]. Experimentally evolved tolerance to ionizing radiation can also occur quickly [14], and microbes such as the aforementioned *D. radiodurans* could remain viable in a dormant state for millions of years within a couple of meters of Mars's surface [15−17]. Furthermore, a few millimeters of regolith can attenuate UV-C [18−20]. However, growth and reproduction under all of Mars's surface stressors combined has yet to be demonstrated.

Conclusion: Our results support the possibility that extant life could be found in near-surface environments on Mars, provided that the environmental dynamics allowed adaptation to one or two stressors at a time as conditions deteriorated at the surface. One environment where such dynamics might have played out is water ice-bearing permafrost in Mars's polar regions. There, meltwater may have formed, and may still form, during periods of high obliquity, perhaps sustaining growth and reproduction [21−23]. Thus, psychrophiles tolerant of desiccation, freeze–thaw, hypobaricity, and radiation might persist near the surface of Martian permafrost, lying dormant until the next period of high obliquity.

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