

FLUCTUATING TEMPERATURE LEADS TO EVOLUTION OF THERMAL GENERALISM AND PRE-ADAPTATION TO NOVEL ENVIRONMENTS

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HYPOTHESES

Temperature fluctuations can select for thermal generalism^{1,2} that is hypothesized to lead to organisms' ability to invade novel environments³ and hosts⁴.

METHODS SUMMARY

We tested these hypotheses in 1000 generation long experimental evolution experiment by exposing 20 replicate populations of opportunistic bacterial pathogen *Serratia marcescens* either on temperature variations (daily variation between 24 and 38 °C, mean=31 °C, n=10) or constant temperature (31° C, n=10).

After ca. 1000 generations, maximal growth rates (OD) of evolved clones were measured in different temperatures, in different chemicals (DTT, H₂O₂) and against biological enemies (Predatory ciliate: *Tetrahymena thermophila*, Phage: PVV). Moreover, clones' virulence in *Drosophila melanogaster* insect host was determined.

RESULTS

In addition to better growth of strains evolved at fluctuating temperatures, across a range of temperatures, the growth was also better in novel environments; with parasitic viruses, and predatory protozoans. However, the strains from fluctuating environment were less virulent in *Drosophila melanogaster* insect host.

CONCLUSIONS

Whilst supporting the hypothesis that thermal generalism is paired with evolved tolerance across several novel environments, our results also indicate that a pathogen's performance outside the host can be traded off with virulence. These results show that predicted climate change driven thermal fluctuations⁵ can affect not only species' thermal tolerance but also invasiveness and virulence.

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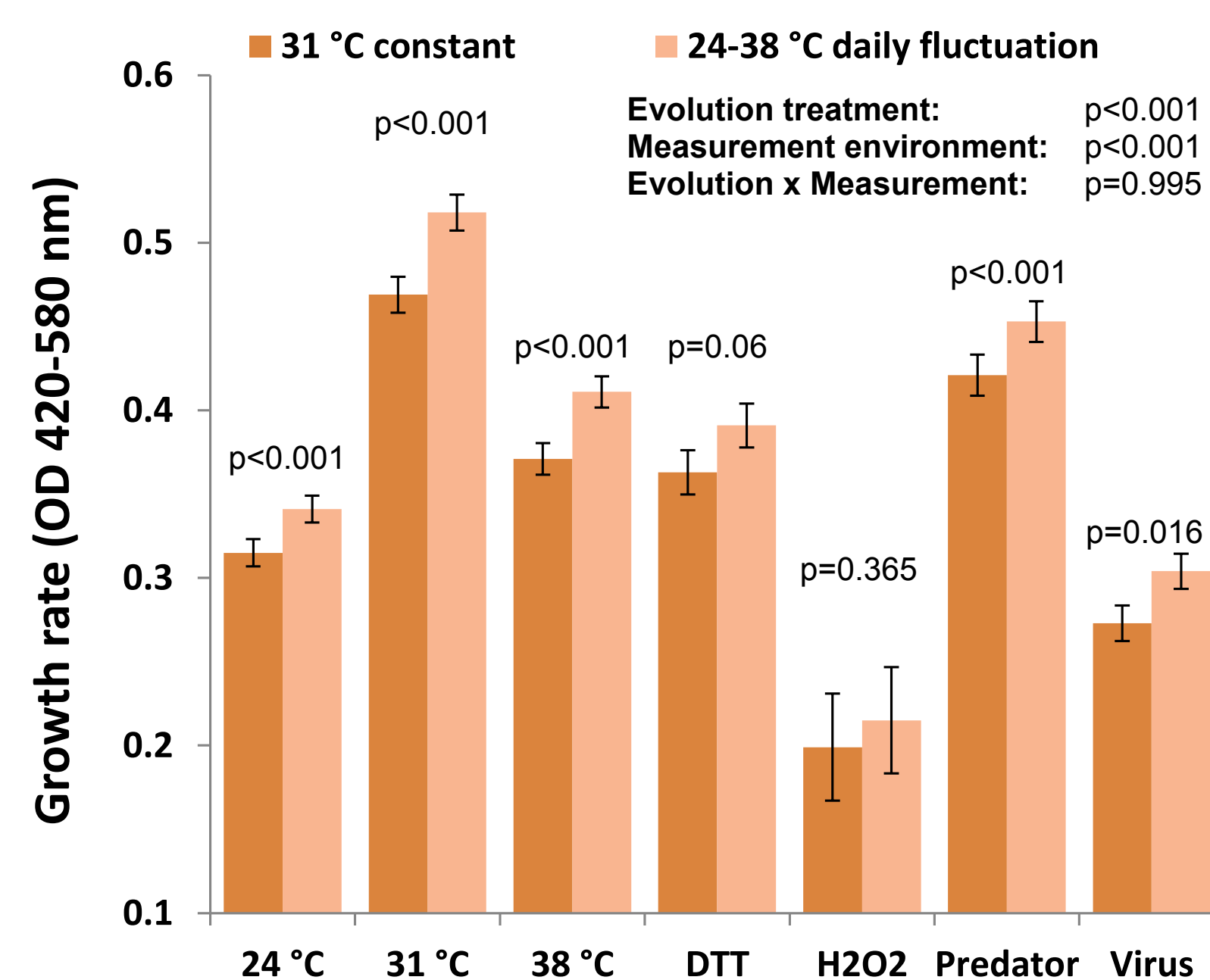


Figure 1. Maximal growth rate (± 2 s.e.) of *Serratia marcescens* -bacteria evolved either in constant 31 °C or in daily fluctuating temperature (between 24 and 38 °C). Predator: ciliate *Tetrahymena thermophila*; virus: PVV (*Podoviridae*).

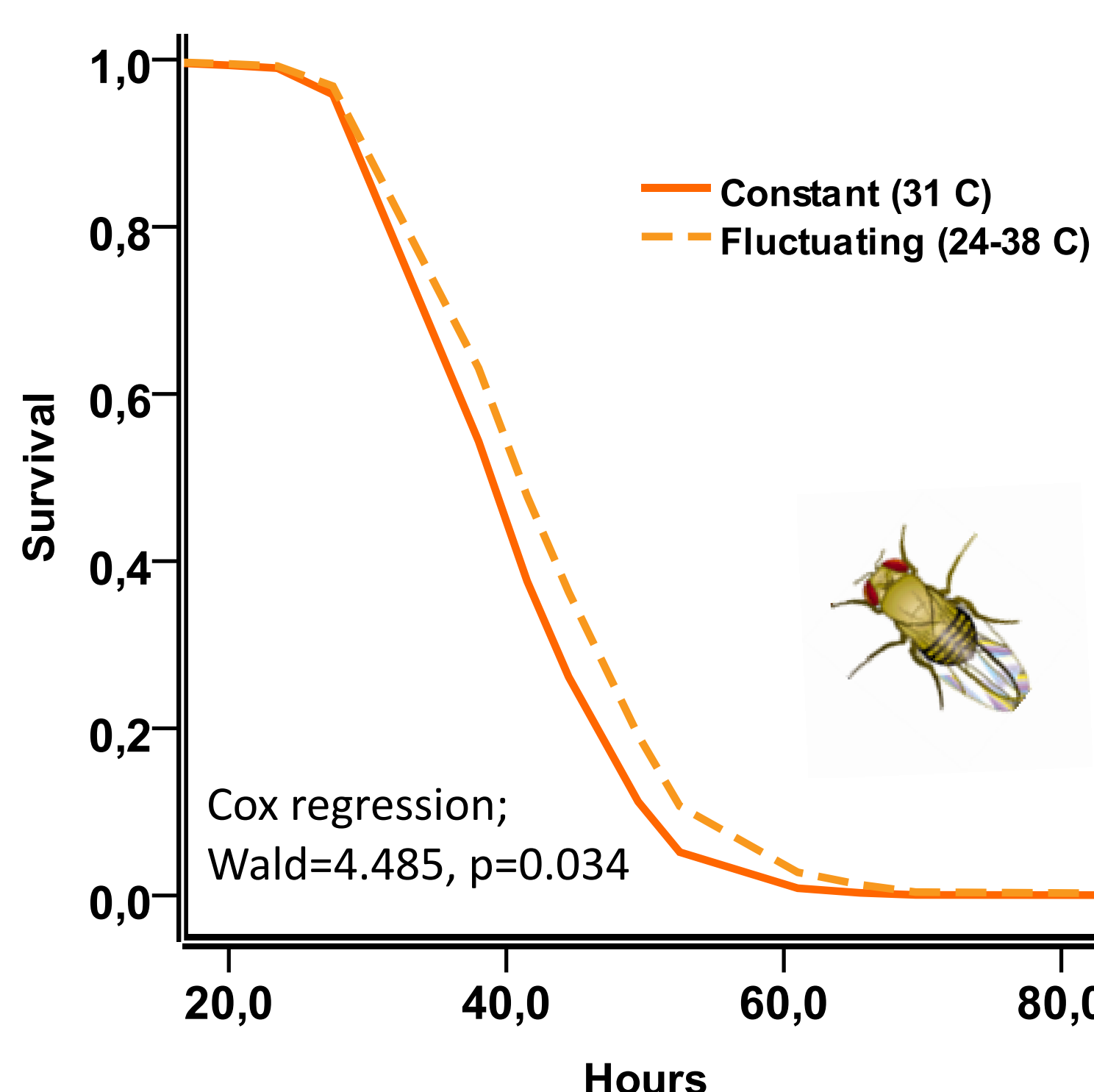


Figure 2. Cumulative survival of *Drosophila melanogaster* -flies infected with clones of *Serratia marcescens* -bacteria evolved either in constant 31 °C or in daily fluctuating temperature (24 and 38 °C).

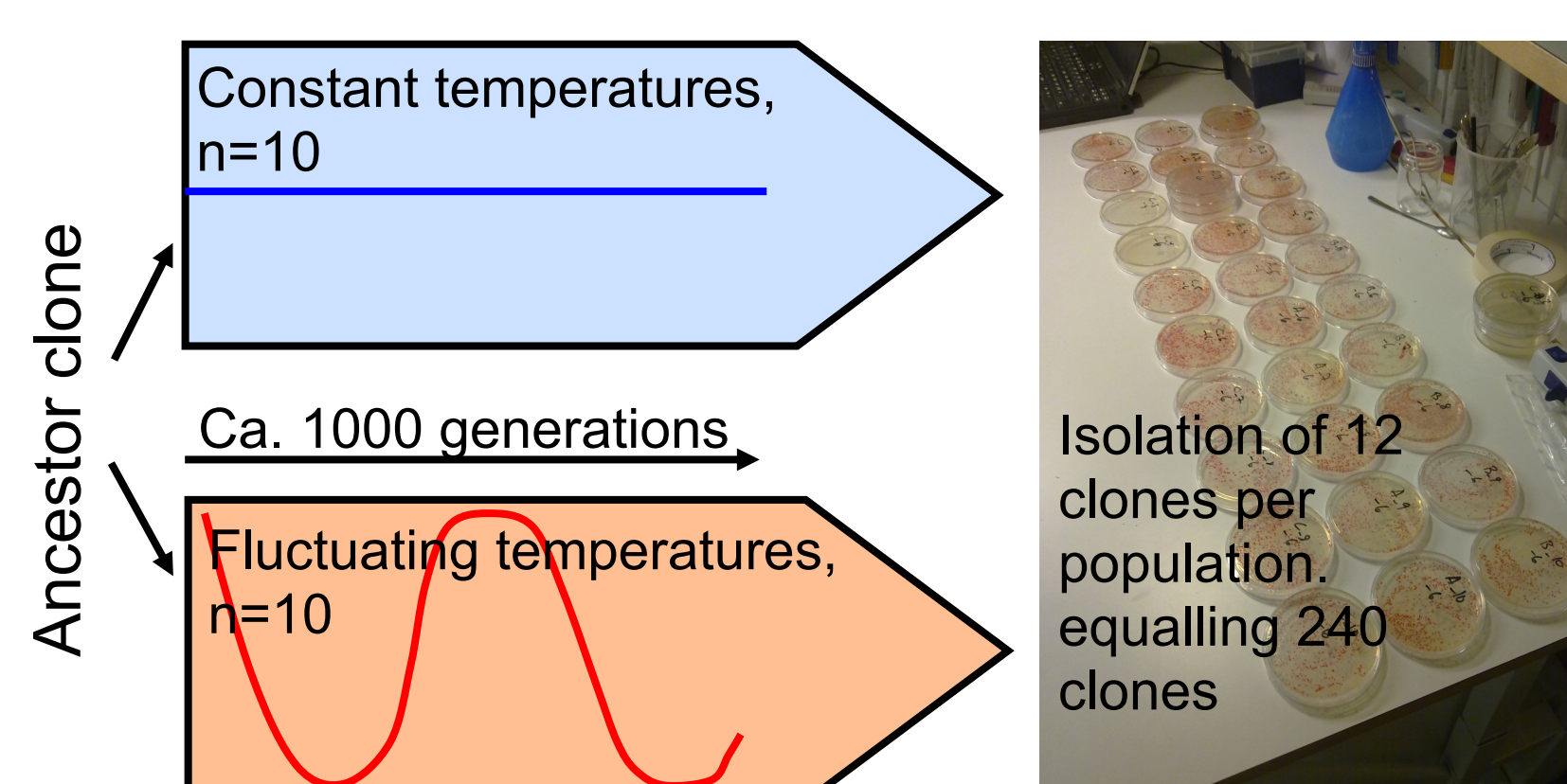


Figure 3. Natural variation of *Serratia marcescens* colonies expressing different levels of red pigment. Photomosaic of Darwin compiled from ca. 2000 colony photos was created using AndreaMosaic -software.

OVERVIEW OF EXPERIMENTAL EVOLUTION STUDY

We initiated the evolution experiment from a single *S. marcescens* colony that was grown overnight to high density. Twenty replicate populations were established on two 100-well spectrophotometer plates. We used two temperature-controlled spectrophotometers (Bioscreen C) to create the following temperature selection regimes: constant 31 °C, and daily fluctuating (from 24 to 38 °C, mean 31 °C). Replicated *S. marcescens* populations were propagated throughout the experiment in phosphate buffered pH 7.5 cereal leaf extract medium. Every 24 hours (± 1 h) 40 μ l of the populations were transferred into new wells containing 360 μ l of fresh medium, and returned to the spectrophotometer.

VIRULENCE ASSAY

After culturing bacteria for 24 hours in LB medium at 31 °C, we mixed 800 μ l of bacterial suspension with 800 μ l of 100 mM sucrose. This solution was absorbed into a cotton dental roll folded on the bottom of a standard fly vial. Next, we transferred ten 2-3 days old *Drosophila melanogaster* adults from a large laboratory colony to each vial and sealed the vials with cotton plugs. This was done for all the 240 bacterial clones and the death rate of flies were monitored over next 4 days, at ca. 6 hours intervals, at 31 °C.

