The robustness of thermal performance curves limits adaptation in growth rate of wild bacterial strains

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Abstract

Thermal adaptation of organisms is a property emerging from the complex interplay of biophysical constraints and selective forces. The shape of thermal performance curves has been well investigated but we lack knowledge of how they may evolve. Two extreme cases can be expected: i) under the hypothesis of local adaptation, species should shift their thermal performance curves and have an optimum at the temperature at which they evolve, or ii) under the hypothesis of thermodynamical constraints, universal biophysical rules dictate a fixed performance curve with an optimum at warm temperatures. We perform an evolutionary experiment to test these two hypotheses on the thermal response of bacteria growth rate, expecting a strong evolutionary response of the thermal performance curve. We use four wild bacterial strains and allow them to evolve at ten different temperatures (ranging from 8.5 to 40°C) to subsequently measure their growth rate at these ten temperatures. We investigate the difference in growth rate between evolved lines and their ancestors. We observe signs of adaptation, as growth rates of evolved and ancestral strains exhibit small but significant differences. Our analysis shows however that the shape of the thermal performance curves does not systematically vary between evolved and ancestral strains, and none of the evolved lines have an optimal growth rate at the evolution temperature. One strain grows significantly faster than its ancestor at the temperature of evolution, but we find that for other strains, evolution leads to faster as well as slower growth rates. These differences are repeated between evolutionary replicates, suggesting they are selected. Our study demonstrates that adaptation does not always overcome thermodynamical constraints on growth rates, and helps to better understand how microbes will respond to temperature changes.

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1 Abstract

Thermal adaptation of organisms is a property emerging from the complex interplay of biophysical constraints and selective forces. The shape of thermal performance curves has been well investigated but we lack knowledge of how they may evolve. Two extreme cases can be expected: i) under the hypothesis of local adaptation, species should shift their thermal performance curves and have an optimum at the temperature at which they evolve, or ii) under the hypothesis of thermodynamical constraints, universal biophysical rules dictate a fixed performance curve with an optimum at warm temperatures. We perform an evolutionary experiment to test these two hypotheses on the thermal response of bacteria growth rate, expecting a strong evolutionary response of the thermal performance curve. We use four wild bacterial strains and allow them to evolve at ten different temperatures (ranging from 8.5 to 40°C) to subsequently measure their growth rate at these ten temperatures. We investigate the difference in growth rate between evolved lines and their ancestors. We observe signs of adaptation, as growth rates of evolved and ancestral strains exhibit small but significant differences. Our analysis shows however that the shape of the thermal performance curves does not systematically vary between evolved and ancestral strains, and none of the evolved lines have an optimal growth rate at the evolution temperature. One strain grows significantly faster than its ancestor at the temperature of evolution, but we find that for other strains, evolution leads to faster as well as slower growth rates. These differences are repeated between evolutionary replicates, suggesting they are selected. Our study demonstrates that adaptation does not always overcome thermodynamical constraints on growth rates, and helps to better understand how microbes will respond to temperature changes.

Keywords : temperature, evolution, growth rate, thermal performance curve, adaptation

2 Introduction

Thermal adaptation plays a key role in determining species distributions and the eco-evolutionary dynamics of their interactions (Hoffmann and Sgró, 2011; Araújo et al., 2013). It has been a focal concern of biologists for decades and remains today a question of particular interest in view of current climate change (Walther et al., 2002). Temperature determines the pace of life, from the rate of biochemical reactions within individual cells to the distribution and functioning of biodiversity across the globe. The relationship between biological rates and temperature is usually characterized by a unimodal function, with a peak at an optimal temperature, and graphically depicted by a thermal performance curve (TPC hereafter) (Huey and Berrigan, 2001). TPCs have indeed been widely used to characterize the temperature dependence of biological rates of many organisms from bacteria to ectotherms (Gillooly et al., 2001; Savage et al., 2004). However, how adaptation to changing temperatures can modify TPCs has raised less attention. Two main scenarios can be expected : i) under the hypothesis of local adaptation, species should shift their thermal performance curves and have an optimum at their evolution temperature, or ii) under the hypothesis of thermodynamical constraints, biophysical rules dictate a boundary TPC with an optimum at warm temperatures.

A key biological rate is r, the instantaneous per capita growth rate (Wiser and Lenski, 2015). Growth rates of most organisms are closely related to their metabolic rate and typically depend upon temperature (Gillooly et al., 2001; Brown et al., 2004; Savage et al., 2004). By definition, local adaptation implies that species should grow optimally under conditions where they are most commonly found. Previous studies were able to show that adaptation to changing temperatures can occur rapidly (e.g. Bennett et al. (1992); Leroi et al. (1994); Vasi et al. (1994); Travisano and Lenski (1996); Mongold et al. (1996); Cooper et al. (2001); Saarinen et al. (2018)). However, submaximal growth rates are also frequently observed (Dmitriew, 2011) and thermophilic species are often found in non-extreme environments (Low-Décarie et al., 2016). Being able to grow fast indeed implies to allocate more resources to biosynthesis, potentially facing trade-offs with other biological functions (Gounand et al., 2016). Some studies also suggest that bacteria optimal growth temperatures are not correlated to the temperatures of their original sites (Préfontaine et al, in prep.) and that adaptation does not overcome thermodynamical constraints (Frazier et al., 2006).

Bacteria have been widely used in laboratory experiments to investigate adaptive responses (Bennett et al., 1992; Lenski et al., 1991; Bronikowski et al., 2001; Buckling et al., 2009; Kawecki et al., 2012). They offer significant advantages for experimental studies due to their short generation time and small size. The effect of controled environments on populations replicated from a common ancestor can be studied, allowing direct comparisons between ancestors and derived populations (Wiser and Lenski, 2015). Most studies use laboratory strains, whose advantage is being well-known and easily culturable (Kawecki et al., 2012).

However, results obtained with laboratory strains may not necessarily hold for wild strains (Buckling et al., 2009). It is hence also necessary to conduct experiments on wild populations.

In this study, we investigate the evolutionary response of growth rates of wild bacterial strains, sampled from pitcher plants. We use four wild bacterial strains and allow them to evolve over multiple generations at ten different temperatures (referred to as 'temperature of evolution', T_{evo}, throughout the study, ranging from 8.5 to 40°C, or 281.5 to 313 K). We subsequently measure the TPCs of ancestors and evolved populations with assessment of their growth rate at the same ten temperatures (referred to as 'temperature of incubation', T_{incu}). We first explore how the parameters of the TPC, characterized by the initial exponential increase (i.e. activation energies) and optimal temperatures, vary with evolution temperature. We expect a strong evolutionary response leading to malleable shapes of the TPCs. We further evaluate the consistency of the evolutionary response, expecting that selection minimizes variability among replicates of the same evolutionary treatment relative to the variability among evolutionary treatments. Our experiment allows us to evaluate the relative importance of thermodynamical constraints and adaptation in the thermal response of biological rates.

3 Material and methods

3.1 Study system

Bacterial strains were sampled from the carnivorous purple pitcher plant (*Sarracenia purpurea L.*), which is widely distributed across North America and used as a model system (e.g. Miller et al. (2002); Kneitel and Miller (2002)). This carnivorous plant lives around 50 years and produces a rosette of leaves that are modified into pitfall traps. New leaves are produced each year and successions of microbes and invertebrates rapidly take place following opening. The entire community consists of detritus-based food webs composed of bacteria, protozoa and arthropod larvae (Miller et al., 2002; Paisie et al., 2014). Homogenized water collected from the leaves was filtered and transported to the lab. Samples were spread and grown on Nutrient Agar (NA) plates and strains were differentiated according to their morphotype. Bacterial colonies were isolated on NA plates with a striation technique and stored at -80 °C in Eppendorf tubes containing 2ml of Nutrient Broth (NB) and glycerol 60 % v/v. See Préfontaine et al (in prep.) for details on the sampling and isolation protocol.

3.2 Experiment

3.2.1 Evolutionary phase

Four randomly chosen strains of bacteria (the ancestors, see table S1) were cultivated at 10 temperatures (8.5, 12, 15.5, 19, 22.5, 26, 29.5, 33, 36.5, 40°C, or from 281.5 to 313 K) during 60 days (from November 27th 2019 to January 26th 2020) in 96-well plates. We refer to these temperatures as 'temperature of evolution' T_{evo} throughout the text. Three replicates were cultivated for each of the four ancestors at each temperature of evolution, giving a set of 120 populations in total. One 96-well plate was incubated at each of the 10 evolutionary temperature containing the three replicates for each of the four ancestors. 15 µL of cultures (50 µL if the bacteria density was low) was transferred every two days into new plates containing 125 µL of fresh NB broth. Blanks were set on plates between each well containing bacteria to avoid contaminations. Contaminations were checked at every transfer by measuring optical density with a spectrophotometer (TECAN) at 600 nm wavelength. Bacteria were stored at the end of the evolutionary period at -80°C in 96 well plates containing 80 µL of bacterial broth and 120 µL of glycerol to a final concentration of 60% v/v. Note that some populations went extinct during the evolutionary experiment, mostly at 8.5 and 40 °C.

3.2.2 Thermal performance curves

To compute TPCs, we measured growth rates at the 10 incubation temperatures T_{incu}. Growth rates of a batch of 6 randomly chosen populations were measured at a time, using the procedure described below. The same procedure was used for every batch until all populations were measured at every T_{incu}. Frozen bacteria were first activated with overnight incubation at 19°C in plates containing 200 µL of NB broth. 20 µL of bacterial broth was then transferred into a deep well plate containing 250 µL of NB broth to start the growth phase. This plate was then incubated at a randomly selected temperature (T_{incu}) for 50 minutes. 100 µL of bacterial broth was then transferred into a deep well plate to which 50 µL of SYBR Green I was added (to reach a final concentration of $\sim$ 3X). The plate was kept 20 minutes in the dark at room temperature before being placed into a BD Accuri C6 cytometer for count measurements. The second measurement was performed using the same methodology after a total of 2h05 of incubation at T_{incu}. TPCs were measured once for each evolved population and three measurement replicates were done for ancestral strains.

Bacteria counts were distinguished from noise using FL1-H fluorescence parameter against FSC-H parameter with a manual rectangular gate used for all bacterial strains. Abundance N_t was measured as the number of events inside the gate. Growth rates were estimated as

$$r = \frac{ln(N_1) - ln(N_0)}{t_1 - t_0} \quad (1)$$

where r is the growth rate in h^{-1} (figure 2A), N the number of events included in the gate (N_1 at t_1, N_0 at t_0), t_1 and t_0 respectively the times of measurement in hours.

We computed the thermal performance curves (hereafter TPCs) for each bacterial strain evolved at a given temperature T_{evo} (see figure 1 for an illustration of a TPC). The densities of some populations were not different from noise due to extinction, lack of growth or fluorescence. We removed these populations using the 95% quantile of the noise distribution (compiled from all blanks measured with the cytometer) as a threshold. Outliers also occurred due to measurement errors with the cytometer and were removed from the analysis. These were identified using a threshold of 2.5 times the standard deviation of growth rate values normalized for each bacterial strain, replicate and incubation temperature.

From these growth rate measurements we recorded two quantities characterizing the TPCs : 1) optimal temperatures T_{opt}, temperatures at which growth rates are maximal, and 2) activation energies Ea, which typically describes the thermal sensitivity of the rate of interest during the exponential phase.

We fitted the TPC as a unimodal function of growth rate (Low-Décarie et al., 2017) :

$$r(T) = a(T/293.15)(e^{b(1/293.15-1/T)})/(1 + e^{c(1/d-1/T)}) \quad (2)$$

where a, b, c and d are constants and T is temperature in Kelvin. The fit was performed by likelihood maximization using simulated annealing with a normal distribution of errors.

Optimal temperature T_{opt} was simply calculated as the temperature at which the predicted growth rate from equation 2 is maximal under the range of measurement temperatures. As many populations had a strictly exponential growth under this temperature range, we could not always extrapolate their optimal temperatures from equation 2 and considered that they had an optimal growth at the maximal measurement temperature (i.e. 313 K).

Parameter b describes the exponential rise in equation 2 but it does not correspond exactly to the activation energy Ea as defined by the metabolic theory in ecology (Gillooly et al., 2001) and its estimation can be quite sensitive. Hence, to characterize the exponential rising phase of the TPCs and get robust estimates of activation energies comparable to typical values (e.g. Savage et al. (2004); Dell et al. (2011)), we next fitted the exponential rising phase of the TPCs only (when $T < 300K$). We fitted the exponential rising phase of growth rate r with temperature T with the Boltzmann-Arrhenius model (Gillooly et al., 2001;

Brown et al., 2004; Savage et al., 2004):

$$r(T) = r_0 e^{Ea(T-T_0)/(kTT_0)} \quad (3)$$

where r_0 is an organism- and state-dependent scaling coefficient, T is temperature in Kelvin, T_0 is the temperature of reference at which the rate equals r_0, k is the Boltzmann constant ($8.617.10^{-5}$ $eV.K^{-1}$) and Ea is the activation energy in eV (electronvolts). The activation energy Ea is the rate at which $r(T)$ exponentially increases with inverse temperature. We fitted the exponential part of the TPCs with equation 3 using a nonlinear least squares regression and extracted the activation energy Ea for each evolved population. We computed the difference in activation energies and optimal temperatures between evolved populations and ancestors as $\Delta Ea = Ea_{evo} - \overline{E}a_{ances}$ and $\Delta T_{opt} = T_{opt_{evo}} - \overline{T}_{opt_{ances}}$ where Ea is activation energy, T_{opt} optimal temperature and *evo* and *ances* stand for evolved and ancestral populations respectively. $\overline{E}a_{ances}$ and $\overline{T}_{opt_{ances}}$ are the averages across measurement replicates.

Difference in growth rate between ancestors and evolved populations

We computed the difference in growth rates between evolved and ancestral populations (figure 1 and 2B) as

$$\Delta r(T_{incu}, T_{evo}) = r_{evo}(T_{incu}, T_{evo}) - \overline{r_{ances}}(T_{incu}), \quad (4)$$

where $r_{evo}(T_{incu}, T_{evo})$ is the growth rate of evolved strains, at each temperature of incubation and evolution and $\overline{r_{ances}}(T_{incu})$ the ancestors growth rate at each temperature of incubation averaged across the different measurement replicates. An illustration of a TPC is given in figure 1, where we indicate how we compute Δ_r at different temperatures (e.g. at $T_{incu} = T_{evo}$).

3.3 Statistical analyses

We investigate if the shape of the TPCs (i.e. exponential rise and optimum) is evolutionary labile by testing if the activation energies Ea and the optimal temperatures T_{opt} are related to the temperature at which the populations evolved T_{evo}. In case of a labile TPC, we expect a high correlation between those parameters. We perform a linear mixed model (LMM) for growth rate against temperatures of incubation and evolution with bacteria identity as fixed effect (see figure S1 and table S2 in Supplementary Material).

Second, we investigate if there is an evolutionary response in growth rate by testing if ancestral and evolved lines, for each bacterial strain, show quantitative differences. We thus test if $\Delta r = 0$ (equation 4) at all

temperatures with a Student test for normally distributed data and a Wilcoxon test otherwise (the normality of the distributions were verified by a Shapiro-Wilk test, see table S3 for p-values). We also test a more specific prediction of evolutionary response by looking at whether evolved populations grow faster than their ancestors when incubated at their temperature of evolution, $T_{evo} = T_{incu}$ (i.e. a more moderate hypothesis of local adaptation), with the same approach (table S3). We finally test if evolved populations grow slower at temperatures different from their temperature of evolution ($\Delta r_{evo} = r_{evo}(T_{incu}, T_{evo}) - r_{evo}(T_{incu} = T_{evo}) < 0$).

We further evaluate with several tests the consistency of the evolutionary response (Δr) to make sure that it is not the result of measurement errors and stochasticity. We test two hypotheses for that case: first, that variations in growth rate will keep increasing with number of generations (true ongoing adaptive processes), and second, that variations among replicates of the same evolutionary treatments will be lower than variations among evolutionary treatments.

The first hypothesis is based on the idea that development and generation times decrease with higher temperatures (Gillooly et al., 2002; Charnov and Gillooly, 2003; Kingsolver and Huey, 2008). In that case, populations evolved at warmer temperatures should have more generations and hence should differ more than their ancestors (than populations evolved at colder temperatures). We thus expect $|\Delta r|$ to increase with the temperature of evolution. We tested this prediction using a GLMM for $|\Delta r|$ with evolution temperature as explanatory variable, with the date of measurement as random effects, and bacteria identity as fixed effects. We use a gamma distribution for residuals so that model criteria are satisfied (residuals uncorrelated with explanatory variables and homogeneously distributed as determined with Quantile–Quantile plots). We consider bacteria identity as fixed effects because considering them as random effects leads to singular fits, or to violation of the model criteria.

The second hypothesis implies that the variance among replicates of the same evolutionary treatments should differ from the variance among evolutionary treatments under a consistent evolutionary response. We therefore compute $var_{intra}(\Delta r|T_{evo}, T_{incu})$, the variance among the three replicates of Δr for a given temperature of evolution and incubation (i.e. in each cell of the heat map in figure 2B). We also compute the variance $var_{inter}(\Delta r|T_{incu})$ across all treatments at fixed T_{incu} (i.e. in each line in figure 2B). The mean of the ratios of these two variances, $\text{mean}_{T_{evo},T_{incu}}(var_{intra}/var_{inter})$, should be small if evolutionary replicates are more similar than populations evolved at different temperatures. A distribution of estimates of the mean of ratios is computed by bootstrapping with replacement for each bacterial strain. We compare this distribution to the one obtained when var_{intra} is computed with three randomly selected values of Δr for a given T_{incu}. All analyses are done using the R statistical software version 3.6.3 (R Core Team, 2020) and the lme4 package (Bates et al., 2015).

4 Results

Experimental evolution affected both the growth rate at temperature of evolution and at other incubation temperatures (figure 2A). The overall evolutionary response is positive, although a decrease in growth rate is obvious at several temperatures. The difference between evolved and ancestral populations Δr is on average close to zero, ranging from -0.95 to 1.14 and is quite heterogeneous across the different temperatures of evolution and incubation (see raw data on heat map of figure 2B). The effect of the temperature of incubation is however much stronger than the one of the temperature of evolution (see also figure S1 and table S2 in Supplementary Material).

4.1 No systematic change in the shape of the TPCs

Growth rate monotonically increases with incubation temperature T_{incu} (temperature at which growth is measured) for every population irrespective of their temperature of evolution T_{evo}, with sometimes a stabilisation or a decrease at the warmest temperatures (figure 3A). The shape of the TPC, as characterized by its initial slope (given by activation energies) and its mode, is conserved between evolved and ancestral populations and across evolutionary populations. ΔEa is centered around zero and there is no correlation between the activation energies Ea and the temperature of evolution T_{evo} ($cor(T_{evo}, Ea) = 0.03$), indicating that the initial slope (exponential regime) of the TPCs does not vary with T_{evo} (figure 3B). Further, $\Delta T_{opt} = 0$ for most populations and bacteria do not have an optimal growth rate at their temperature of evolution. There is no correlation between optimal temperature T_{opt} (temperature at which growth rate is maximal) and temperature of evolution ($cor(T_{evo}, T_{opt}) = 0.02$, figure 3C). Most populations grow the fastest at high temperatures ($T_{incu} > 300K$) no matter the temperature at which they evolved. The effect of the temperature of incubation is much stronger than that of the temperature of evolution (see results of the LMM in figure S1 and table S2 in Supplementary Material).

4.2 Evolutionary response of growth rate

The overall evolutionary response is positive (table S4), although a decrease in performance is obvious at several temperatures. The difference between evolved and ancestral populations Δr (equation 4) at all temperatures, is significantly different from 0 for three bacterial strains out of four (Wilcoxon test, strains 50, 52 and 210 are significant as indicated on figure 4A). The difference between evolved and ancestral populations evaluated at $T_{evo} = T_{incu}$, $\Delta r(T_{evo} = T_{incu})$ is however not statistically different from zero except for strain 52. For that strain, we also see evidence of a trade-off: populations tend to grow slower at temperatures above their temperature of evolution ($T_{incu} > T_{evo}$), when compared to populations evolved

at these temperatures ($T_{incu} = T_{evo}$) (see figure S4). For instance, when measured in warm treatments, populations evolved in those treatments tend to grow faster than populations evolved in cold treatments, although the converse is not true.

4.2.1 Consistency of evolutionary responses

We find that the relationship between the absolute amount of change $|\Delta r|$ (equation 4) and T_{evo} varies between the different bacterial strains. There is no significant effect for strain 406 and 50, an increase with temperature for strain 52 and a decrease for strain 210 (figure 5A, table S4). This result shows that populations evolved at warm temperatures are not necessarily more different from their ancestors than populations evolved at cold temperatures, except for strain 52. We assess the robustness of our observations with a comparison of the variances of Δr within and across evolutionary treatments T_{evo} to make sure that the evolutionary response we observe (Δr) is not the result of measurement errors and stochasticity (figure 5B). Ratios are significantly lower than the null expectation for bacterial strains 52 and 406, and the same trend is observed for bacterial strains 50 and 210. Evolved populations of strain 406 do not on average differ from their ancestor but mutations are less dispersed than the random null model, suggesting that growth rates experience adaptive constraints but that its adaptation is expressed in different directions (i.e. evolution might lead to faster or slower growth rates depending on treatments).

5 Discussion

We find that the shape of the thermal performance curves (TPCs) is conserved across temperature treatments, suggesting limited thermal adaptation on growth rate. Activation energies do not vary with temperature treatments and strains do not have an optimal growth rate near their evolution temperature. These results are consistent despite observations of a significant evolutionary response; that said, we observe important variation among the four wild bacterial strains under study.

5.1 Robustness of the shape of the TPC

Most populations grow faster at warmer temperatures irrespective of the temperature of evolution. The positive effect of temperature on growth rate could be explained by thermodynamical constraints (as reaction rates increase with absolute temperature) (Savage et al., 2004; Kingsolver and Huey, 2008). Thermodynamics have already been incorporated in the theory of metabolic scaling and used to develop models to describe biological rates as a function of body size and body temperature (Gillooly et al., 2001; Savage et al., 2004; Gillooly et al., 2002; Charnov and Gillooly, 2003). Despite the effect of the temperature of incubation

being stronger, we do observe a certain increase in growth rates with the temperature of evolution. Overall, growth rate is lower for populations evolved at cold temperatures than those evolved at warm temperatures. The hypothesis of 'hotter is better' (Bennett, 1987) has already been supported in previous studies on insects, where cold-adapted species grow slower than warm-adapted species when growth rate is measured in optimal conditions for each species (Frazier et al., 2006); on phages, where optimal temperatures were positively correlated with maximum growth rates (Knies et al., 2009), but also on trees, bacteria, reptiles, amphibians and fishes (Angilletta Jr et al., 2010). These results suggest that adaptation cannot completely compete against metabolic constraints and compensate the depressing effects of low temperatures on biological rates.

5.2 Differences in evolutionary responses between strains

Our results demonstrate that thermal adaptations can lead to a variety of responses in bacteria. One strain, strain 52, behaves in a more expected way. It shows multiple signs of local adaptation even though they are not strong enough to change the qualitative shape of the TPC: i) it grows faster than its ancestors at its evolution temperature and ii) performance decreases at other temperatures. This strain might still be undergoing adaptation: in that case the absolute amount of change $|\Delta r|$ should increase with the number of generations and therefore with temperature, assuming that generation times decrease at higher temperatures (Gillooly et al., 2002; Charnov and Gillooly, 2003; Kingsolver and Huey, 2008). We indeed see a positive correlation between Δr and T_{evo} for that strain. Thus, it is possible that local adaptation would have been more clearly observed in the longer term. We note that ancestor 52 grows slower than the other ancestral strains, which might explain why that strain shows clearer signs of adaptation.

Growth rate of evolved populations of strains 50 and 210 differ from their ancestors but we do not find clear evidence of adaptive mutations for these strains, suggesting that temperature induces weak or no selective constraints on growth rate in the conditions we evolved them. Finally, evolved populations of strain 406 do not strongly differ from their ancestor but mutations are less dispersed than the random null model, suggesting that it experiences adaptive constraints but that they select for faster or slower growth rates depending on temperature treatments.

5.3 Evolution might not necessarily lead to an increase in growth rate

Here, we measure the short-term growth of ancestral and evolved populations separately (i.e. not in competition assays). Selection however applies to the net growth rate of a mutant in the presence of the resident (i.e. ancestral) type over the fixation time. This is only identical to the instantaneous growth rate of the mutant

alone if there are no interactions, no density and time dependence. Thus we do not necessarily expect that adapted populations have faster growth rates than their ancestor when measured in isolation (i.e. intrinsic growth rates). Benton and Grant (2000) note that using growth rate as a measure of fitness assumes that life history is unaffected by density-dependent effects and that the environment is constant. Generally, adaptation is assessed through competition experiments (e.g. Lenski et al. (1991); Lenski and Travisano (1994)). For measurements performed only on one type, a variety of definitions of performance have been used, based on population growth, reproductive success, population size or other processes such as population extinction (Wiser and Lenski, 2015). They are context-dependent and one single measure cannot quantify the full extent of adaptation (Younginger et al., 2017; Benton and Grant, 2000; Amarasekare and Savage, 2012).

We cannot ascertain that our experimental setup (e.g. dilution cycles every 48h) prevents density- and time-dependent processes and favors types with fast growth in the short term (here measured over 2h). Conflicts among different traits may lead to long-term costs of growth, which implies that there is genetic variation in growth and submaximal growth rates can be often observed (Dmitriew, 2011). MacArthur and Wilson (1967) argued that selection should initially favor newly-arriving immigrants with fast growth rates (r-strategy) whilst, in the longer term (i.e. in environments not selecting for growth), selection should favor species able to survive and reproduce with limited resources (K-strategy).

As shown in our study, growth rates at the experimental temperature might evolve in both directions depending upon selective pressures. This is particularly obvious for strains 406 and 210, for which Δr is often negative, indicating that evolved lines grow slower than their ancestors. Moreover, despite the fact that our bacterial populations grow the fastest at warm temperatures, many populations had low densities at the end of the two months of the evolutionary phase at these warmest temperatures (especially at 40 °C). Hence, despite a fast short-term growth rate, populations had low densities in longer term, indicating potential costs of having a fast growth rate on the long term (an extreme case has been called ecological suicide when fast initial growth results in eventual extinction (Ratzke et al., 2018)). Other studies have demonstrated that high temperatures might favor slow-growing species (Lax et al., 2019).

It is also important to note that chance does still have important effects in evolutionary processes (Buckling et al., 2009). For instance, a study demonstrated that initially identical populations of E. coli grown in identical environments pursued very different evolutionary trajectories, in terms of fitness and cell size (Lenski and Travisano, 1994). Random mutations can be fixed in different populations altering the trajectory of evolution, which can diverge even more if subsequent mutations are contingent on prior ones (epistasis) (Buckling et al., 2009). Some of the variation observed in thermal responses between our bacterial strains might hence also be explained by initially random mutations that were fixed followed by epistasis processes.

5.4 Importance of the study system and of the experimental setup

Most populations did not grow optimally at the temperatures they were exposed to during the evolution experiment. Préfontaine et al (in prep.) did not find any correlation between climate of origin and TPCs characteristics using the same strains of bacteria isolated from pitcher plants. Yet, other studies revealed evidence of local adaptation in wild bacterial strains, with different optimum temperatures and tolerance ranges (Johnson et al., 2006; Yung et al., 2015). In the 90's, Richard Lenski and colleagues performed many experiments on the long-term adaptation of the bacterium Escherichia coli (e.g. Bennett et al. (1992); Bennett and Lenski (1993); Lenski et al. (1991)), showing that it can adapt rapidly to different temperature treatments. However, our results show that four wild strains of bacteria originating from the same environment respond differently to temperature treatments. We therefore argue that it remains important to study wild strains of bacteria, which could behave quite differently from lab-adapted organisms such as E.coli.

In particular, one aspect that might influence thermal adaptation in wild strains is temperature specialization versus generalism. For instance, thermal growth profiles of enteric bacteria from a free-living ectothermic host did not follow the variations of their host's body temperature (Bronikowski et al., 2001), suggesting that these bacteria are thermal specialists. In our case, previous studies on the purple pitcher plant showed that the inquiline communities were relatively homogeneous at large spatial scale (Buckley et al., 2010; Freedman et al., 2021), and therefore over a large range of thermal conditions. This homogeneity can be due to the particular relationship between the inquiline community and the host plant, which is not completely understood yet, and could suggest that bacteria are thermal generalists. Previous studies also demonstrated that bacteria from pitcher plants had higher densities in warmer treatments regardless of their temperature of origin, and were not specialized (Gray et al., 2016; Parain et al., 2016). Some of our strains might hence be thermal generalists which could explain that they did not widely diverge from their ancestors at any temperature.

The various outcomes we obtained and the relatively short period of evolution of the experiment might suggest that the experimental conditions limit the potential for adaptation. Previous studies on thermal adaptation propagated bacteria for 2000 to 50 000 generations (Leroi et al., 1994; Lenski and Travisano, 1994; Wiser and Lenski, 2015; Travisano and Lenski, 1996), which is significantly longer than in our experiment. However, these studies demonstrated that fitness evolved rapidly in the first thousand generations (Lenski et al., 1991; Lenski and Travisano, 1994; Bennett et al., 1992), which is more coherent with the duration of our experiment. We show in our study that most strains do significantly differ from their ancestors and that within strain replicates tend to perform the same. One strain has a clear signal of adaptation and we give evidence of selective constraints occurring on r suggesting that experimental conditions in principle

allowed for adaptation.

5.5 Different views of local adaptation

A strong hypothesis of local adaptation stipulates that a strain's optimal growth temperature is its evolution temperature (which implies a trade-off between performance at different temperatures). We rejected this hypothesis but there might be other aspects of local adaptation. A first possibility could be that adaptation to the growth medium and lab conditions would be the target of selection for these wild bacterial strains instead of the evolution temperature. In this case we would expect evolved populations to perform better than ancestors in all treatments. In our experiment, this might be the case for at least one strain (52). Another possibility is that there may be asymmetric trade-offs between adaptation to warm and cold temperatures. For two out of the four strains, cold evolved populations grow slower than warm evolved populations in warm treatments but we did not observe the reverse situation (i.e. warm evolved populations do not grow slower than cold evolved populations at cold temperatures). It has been shown that tolerance to heat is usually largely conserved across lineages, while tolerance to cold varies between species (Araújo et al., 2013). It remains uncertain whether changes conferring benefits in cold or warm environments have a negative effect on functions in the other environment. Another study showed that a fraction of their experimental lineages achieved low-temperature adaptation without detectable high-temperature trade-offs (Bennett and Lenski, 2007). Previous results also suggest that although general, trade-offs are not universal (Bennett and Lenski, 2007; Mongold et al., 1996; Yung et al., 2015; Bennett et al., 1992).

5.6 Conclusion

The results suggest that the shape of the TPCs is robust, and that evolution does not necessarily lead to faster growth. We argue that investigating other adaptive traits might be important to know if species adapt to changing temperatures and to estimate species survival chance under climate change (Bronikowski et al., 2001; Wiser and Lenski, 2015; Blanquart et al., 2013). Our study yields interesting results regarding the evolutionary thermal response of growth rate in wild bacterial strains. Evolutionary experiments with microorganisms are increasingly used to study various questions in evolutionary biology and have helped to better understand universal evolutionary principles (Buckling et al., 2009). Although the evolution of many plants and animals involve additional mechanisms such as sex, parental care or sexual selection which can limit the use of microorganisms as model systems (Kawecki et al., 2012), evolutionary experiments with microbes are powerful tools to better apprehend species evolutionary responses under climate change.

6 References

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7 Figures

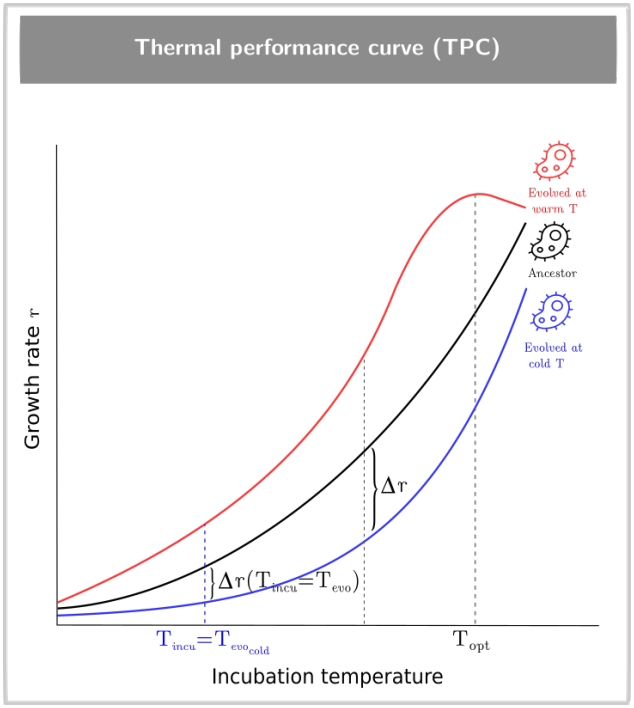

Figure 1: Example of thermal performance curves for an ancestral strain and its evolved strains.

Thermal performance curve (TPC) representing bacteria growth rate r (equation 1) according to the incubation temperature T_{incu} for an ancestral strain (in black) and its evolved strains (colored, blue for cold and red for warm temperatures of evolution T_{evo}). Δr is the difference between the growth rates of the ancestral strain and of the evolved strain at each temperature of incubation (equation 4). When $T_{incu} = T_{evo}$, growth rate is measured at the temperature at which the strain was evolved (diagonals on the heat-maps of figure 2). T_{opt} is the temperature at which growth rate is maximal.

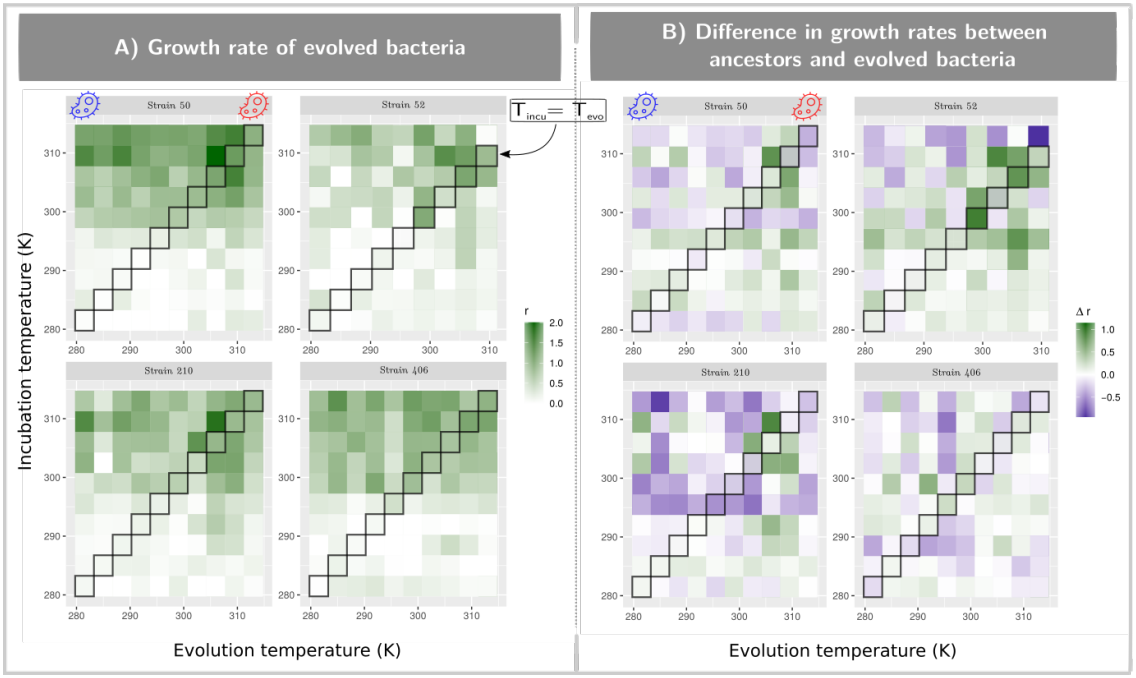

Figure 2: Heat-maps of raw data for r and Δr.

A) Heat-map of growth rates r averaged over the three replicates (raw data, $\overline{r} = 0.45 \pm 0.42$, mean $\pm$ sd) according to the temperatures of evolution and of incubation for each ancestral strain (color coded, see color key). Diagonal, emphasized by black squares, indicates situation where $T_{incu} = T_{evo}$. B) Heat-map of the evolutionary response. Color gradient indicates the difference between the growth rates of ancestors and evolved strains Δr averaged over the three replicates (raw data, $\overline{\Delta r} = 0.03 \pm 0.27$, mean $\pm$ sd) according to the temperatures of evolution and of incubation for each ancestral strain (color coded, see color key).

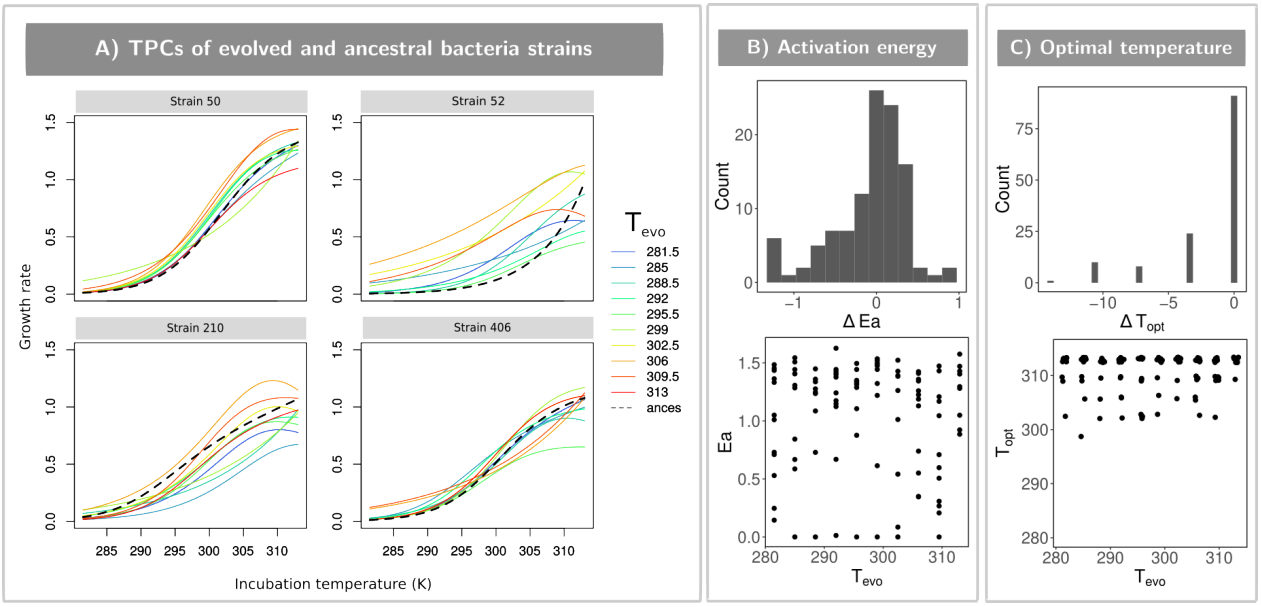

Figure 3: Robustness of the shape of the TPC.

A) Thermal Performance Curves (equation 2): Growth rate r according to the temperature of incubation (T_{incu}) for the different lines evolved at a given temperature of evolution (T_{evo}) and the ancestors (ances, black dotted lines) for each strain (50, 52, 210 and 406). Colors correspond to each temperature of evolution, see color key. B) Top panel: histogram of ΔEa, the difference in activation energies between evolved and ancestral populations, and bottom panel: activation energies Ea (eV, equation 3) according to the temperature of evolution T_{evo}. The correlation between the two variables equals 0.03. C) Top panel: histogram of ΔT_{opt}, the difference in optimal temperatures between evolved and ancestral populations, and bottom panel: jitter plot of optimal temperatures T_{opt}, temperature at which growth rate is maximal, according to the temperature of evolution. The correlation between the two variables equals 0.02.

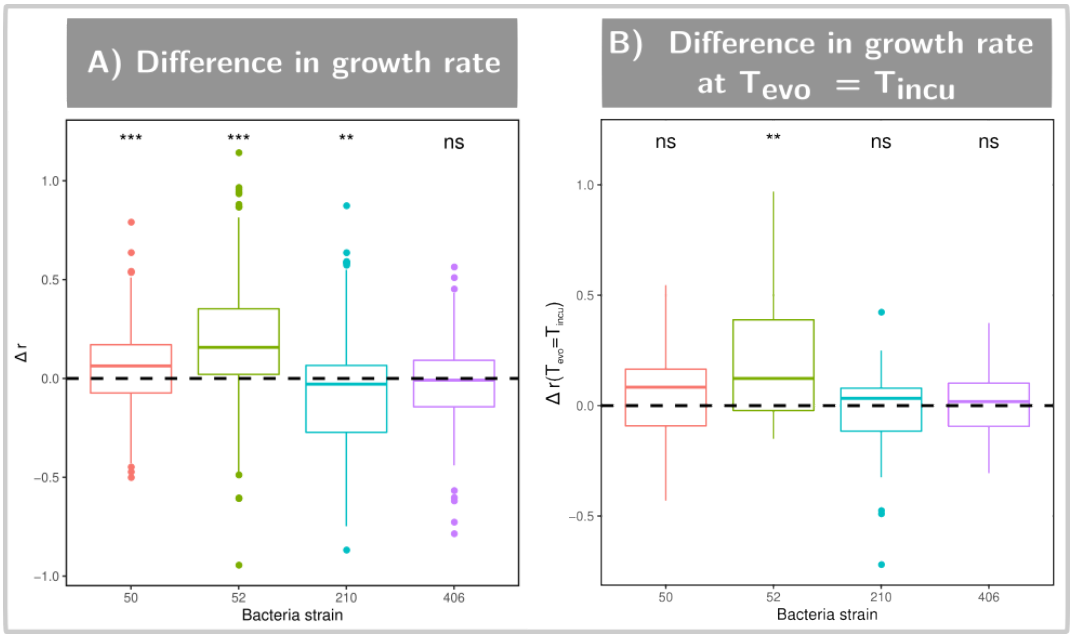

Figure 4: Difference in growth rates between evolved and ancestral strains.

A) Boxplot of Δr, difference in growth rate between ancestors and evolved populations (equation 4), for each bacterial strain (Wilcoxon two-sided test, significance indicated, $p_{value} < 0.05$). B) Boxplot of $\Delta r(T_{evo} = T_{incu})$, difference in growth rate between ancestors and evolved populations incubated at their temperature of evolution (i.e. diagonal on the heat map on figure 2B) for each strain (Wilcoxon or T test, significance indicated, $p_{value} < 0.05$).

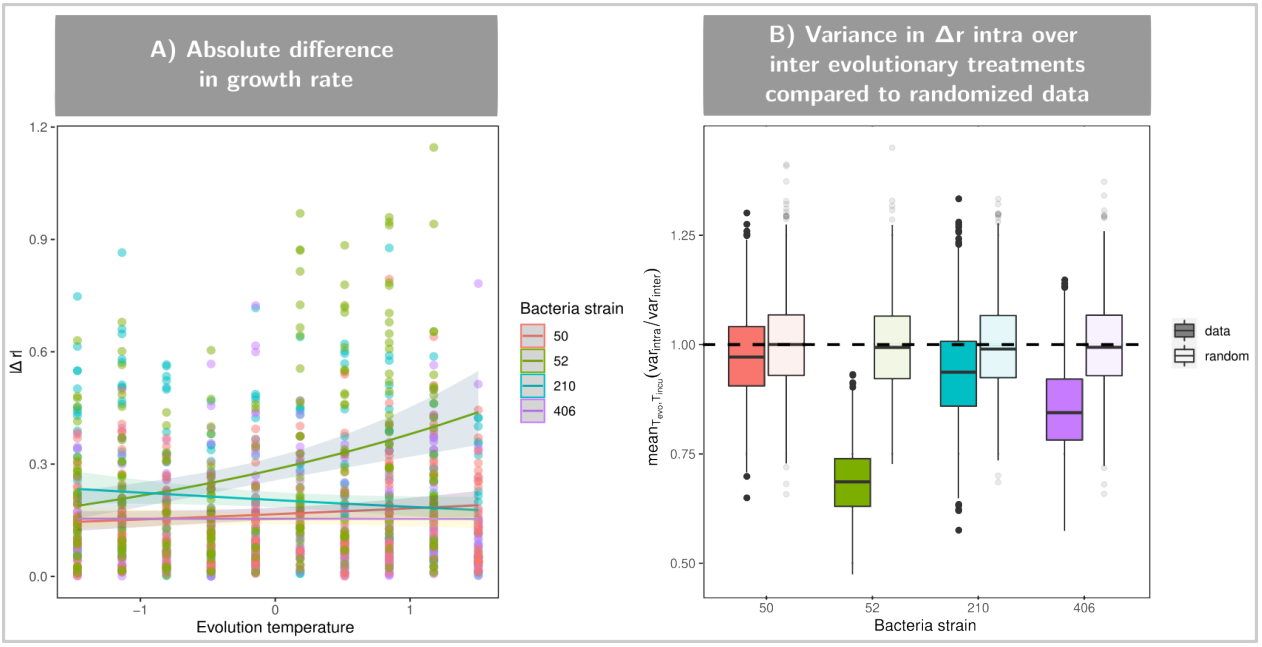

Figure 5: Selective constraints on growth rate.

A) $|\Delta r|$ according to the temperature of evolution. B) Ratio of variance var_{intra} of $\Delta r(T_{evo}, T_{incu})$ between replicates, over variance var_{inter} across all temperature treatments at fixed T_{incu} compared to randomized data for a given T_{incu}, for each strain.