Disentangling the multiple drivers of ecological adaptation in a microorganism

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Abstract

The survival and reproduction of living organisms depend on their ability to achieve an adequate balance between energy intake and energy expenditure. Multiple quantities contribute to this energetic balance, such as the feeding rate, and the allocation of available energy to growth, maintenance, movement and reproduction. Given that many of these quantities scale in a predictable way with the size of the organism and with environmental parameters, by quantifying these scaling relations it should be possible to predict how organisms may adapt to novel environmental conditions in terms of adjusting their morphology, physiology, and behaviour. In a series of experiments, we adapted axenic experimental populations of the ciliate *Tetrahymena pyriformis* to different environmental conditions of temperature (15°C, 20°C and 25°C) and resource levels (50%, 100%, and 200% of a standard protein solution). We measured population growth, metabolic rate (from respiration), cell size, and movement speed (from video-tracking). On a very short time scale, movement speed and metabolic rate increased with environmental temperature in a way that can be predicted from simple physical scaling relations such as the Boltzmann-Arrhenius equation and the 'viscous drag' impacting movement. However, soon after the introduction of tetrahymena into a novel environment, all the

measured quantities were further modulated in a direction that likely provided better biological fitness in the new environment. We discuss our results in terms of both theoretical and experimentally observed scaling relations of individual quantities, combined with reasonable optimisation principles.

1 Introduction

Living organisms can vary hugely in size, morphology, and functional or behavioural traits, indicating that variation along such phenotypic axes is weakly constrained by evolution: organisms large and small, slow-moving and fast, can coexist on the same planet for millions of years, all having similar fitness.

However, variation along other phenotypic axes is heavily constrained, and when variables such as metabolic rate, body size, generation time, and movement speed are plotted one against the other, different organisms all cluster together along relatively narrow curves [1, 2]. These narrow 'inhabitable regions' of the phenotypic space likely reflect boundaries between regions that are physically unattainable (for example because either diffusion or transport of molecules imposes an upper limit on biological rates) and regions that are physically possible but biologically inefficient.

When the physical parameters of the environment change, for example if temperature increases or nutrient resources are depleted, these boundaries shift, and empirical evidence across a range of species suggests that organisms respond with consistent changes in their development [3, 4, 5], in their metabolism [6, 7], and in their movement or behaviour [8].

Unicellular organisms are particularly well-suited as a model for understanding environmental adaptation over time scales ranging from one to several generations because of their small size and relatively short generation time, which make them suitable candidates for controlled laboratory microcosm studies [9]. Microcosm studies have allowed identification of some of the elements that mediate the adaptation of microorganisms to their environment. There is evidence – for example – that unicellular organisms change their phenotypic traits rapidly – traits like cell size and shape [10, 11], or the metabolic rate of individual cells [12] – in response to environmental conditions, and over ecological, non-evolutionary, time scales. In turn, these changes affect ecological quantities, such as population growth rate [12], car-

rying capacity, or predation risk [11]. Movement speed is also a temperature-dependent phenotypic trait with the potential to also affect – at least in theory – ecological variables such as the stability of predator-prey systems [8].

By testing cell populations across a wide range of different environments, microcosm studies have also pointed to possible explanations of why phenotypic changes occur at the individual level. For example, Padfield et al.[13] studied the adaptation of unicellular algae to different temperature conditions. In the absence of adaptation, with increasing temperature respiration increased faster than photosynthesis (the activation energy for respiration was larger than for photosynthesis). This difference caused a mismatch between energy acquisition and energy expenditure in warm environments. However, by selectively down-regulating respiration relative to photosynthesis, these organisms were able to increase their growth rate in high-temperature conditions that would have otherwise being limiting. All this happened over relatively short time scales of about 100 generations.

These and other similar studies have contributed to an increase in our phenomenological understanding of the phenotypic changes triggered by different environmental conditions. However, we have also started to realise that the adaptation of organisms to environmental conditions is a multifaceted process, not only because phenotypic changes produced at the individual level in turn also affect population- and ecosystem-level variables, but also because multiple environmental parameters interact with each other in driving adaptation. For example, the temperature that maximizes the growth rate of organisms is not independent of the concentration of available nutrients in the environment [14, 15].

The complex relations between environmental parameters (such as temperature and available resources), the phenotypic changes induced by those parameters, and fitness, [16] imply that a detailed mechanistic understanding of these relations is necessary if we want to make generalisable predictions on the effects of environmental changes.

To contribute to fill this gap, we performed an extensive study in which experimental axenic populations of a microorganism –the ciliate *Tetrahymena pyriformis*– were experimentally acclimated over ecological (non-evolutionary) time scales to a range of different environmental conditions characterised by different temperature and concentration of nutrients. We measure how the acclimation process affects reproduction rate, metabolic rate (respiration), movement speed, and organism size (cell volume). We interpret our results

on the basis of the idea that the physical parameters of the environment affect the organisms directly (for example, through the effect of temperature on metabolic rate) and alter the costs and benefits of behaviour (for example, water viscosity affects the cost of movement, and the availability of nutrients determines the energetic return of foraging). We can then try to understand the patterns of adaptation over longer time scales by assuming that tetrahymena aim at maximizing *per capita* growth rate in the environment in which they are adapted, and considering how different phenotypic changes make this possible.

2 Methods

2.1 Cell cultures

Axenic cultures of *Tetrahymena pyriformis* strain 1630/1W were obtained from CCAP (`http://www.ccap.ac.uk`) and cultured at 20 °C in a Proteose peptone - yeast extract medium (20 g Proteose peptone + 2.5 g yeast extract in 1 L deionised water). In the following of this paper, we will refer to this "standard" medium composition as the 100% concentration, to distinguish it from a 50% medium (with half the concentration of Proteose peptone and yeast extract) and a 200% medium with double concentration.

2.2 Experiment design

Cells were maintained in exponential growth phase at 20°C and 100% medium concentration for about 50 days. Subsequently, cultures were split across 9 different treatments (3 temperature conditions x 3 medium concentrations) and adapted to the new conditions for at least three weeks, in four replicates for each condition (see fig. 1 for a schematic diagram of the experiment). At the end of the adaptation period, cultures were further split across a wide range of temperature conditions (keeping the same medium concentration at which they were adapted) to characterise thermal response curves for different biological quantities (respiration, movement speed, and population growth rate). Specifically, we measured acute thermal responses for respiration and movement speed (from 1.5 to 3 minutes after cells were moved to the new temperature for speed recordings, and from approximately 30 minutes to three hours for respiration), and we measured long-term responses over a few

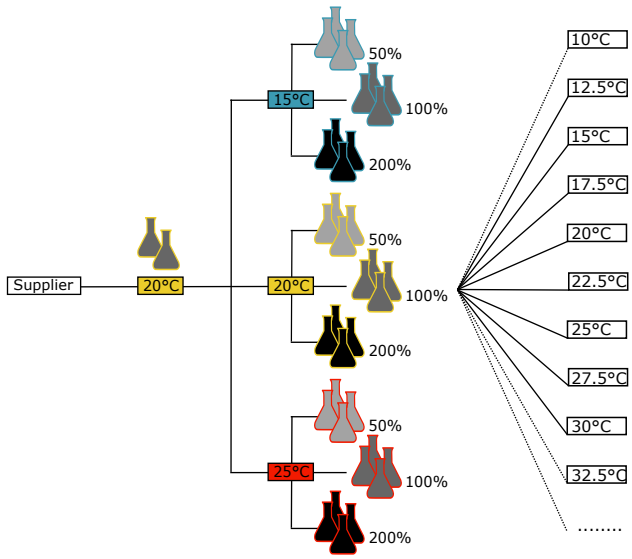

Figure 1: Schematic diagram of the main adaptation experiment. After an initial adaptation at 20°C and 100% culture medium, cells were split across combinations of medium concentration and temperature. At the end of the adaptation period, cultures were further split across different temperatures to assess thermal response curves for various measures of performance.

days (2 to 6 generations, or 3.5 days on average, with differences depending on incubation temperature) for population growth, long-term movement speed, and cell volume changes.

2.3 Population growth

Assuming exponential growth throughout the experiment, the growth rate, expressed in number of generations per day was calculated as $\log_2(N(t)/N(0))/d$ where $N(0)$ is the density at the beginning of a subculture, $N(t)$ is the final density of the subculture, and d is the length of the subculture period in number of days.

2.4 Respiration

Oxygen consumption was measured using a 24-channel PreSens Sensor Dish Reader and 2 ml oxygen sensor vials from PreSens (`https://www.presens.`

de/) inside thermal cabinets at the desired temperature (see SI S.I.A for details of the experimental procedure). Population-level respiration rate was then converted to rate of energy consumption per individual cell by accounting for population density in the vial, and assuming an equivalence 1 mol O_2 = 478576 J.

2.5 Imaging

Tetrahymena cultures were imaged under the microscope in cell-counting slides (BVS100 FastRead), which provide a constant depth of 100 μm (details of the procedure in SI S.I.A). A microscope camera (Lumenera Infinity 3-3UR `https://www.lumenera.com/infinity3-3ur.html`) was used to record videos at 30 fps. Temperature while imaging under the microscope stage was controlled using a temperature-controlled stage (Linkam PE120 Peltier System).

2.6 Video tracking

We used a custom-made software (available at `https://github.com/pernafrost/Tetrahymena`) written in python and based on the opencv, traktor, and scikit-image libraries to extract trajectories and measurements of cell size and morphology directly from the videos. The software returns measurements for each 'tracked particle', where a particle generally corresponds to one individual cell. However, there isn't a perfect one-to-one correspondence between tetrahymena cells and particles, in fact, a cell moving in and out of the field of view of the microscope camera would be labelled as a different particle, and occasionally the identity of two cells swimming close to each other and overlapping in the images were swapped. Given the very large number of individual measurements both across and within experimental conditions, we ignored this small intrinsic potential for pseudo-replication in our analyses.

2.7 Movement analysis

The instantaneous movement speed of each tracked particle was measured from the trajectories as the displacement per unit time of the centre of mass of the particle, measured over a time scale of 1/10 of a second (3 frames).

We took the median of all the instantaneous speed values along the trajectory as the individual speed of the particle. We excluded from the analysis particles that did not move and cells with an unnaturally small size that might have been incorrectly detected in the image. Unless otherwise specified, in the analyses and in the figures presented here we focus on the top 20% faster moving cells within each tested experimental condition. This is because fastest moving cells are more likely to be representative of the intrinsic limits of tetrahymena swimming, while slowly moving cells might be stuck temporarily against the microscope slide or they might be moving at slow speed for reasons independent of their ability to move faster. (Details of movement analysis in SI S.I.A)

2.8 Estimation of cell volume

The video-tracking software fits an ellipse around each segmented tetrahymena cell. Cell volume V was estimated based on the major and minor axes of the best fitting ellipse (respectively l and w) using the formula $V = 4/3\pi l w^2/8$.

2.9 Fitting thermal response curves

Respiration, movement speed, and population growth were analysed by fitting a slightly modified version of the Sharpe-Schoolfield model for thermal response [17, 18] implemented in the rTCP R package ([19]). The model assumes that the rate $R(T)$ of the process at a given temperature increases following an Arrhenius-type relation with temperature, but also accounting for the plateauing and typically fast decrease of biological rates at very high temperature (See SI for the full details of the Sharpe-Schoolfield model in its rTPC implementation). In the analyses that we report here, we focus on this Arrhenius part of the curve, which can be summarized as

$$R(T) = R_0 e^{-\frac{E}{k_B}\left(\frac{1}{T}-\frac{1}{T_0}\right)} \tag{1}$$

and is described by two parameters: a value of activation energy E, which describes how fast the rate increases with increasing temperature, and a reference rate R_0, which is the rate of the process at the reference temperature T_0. Throughout all our analyses we set $T_0 = 20\,^\circ\mathrm{C}$ (293.15 K). k_B is the Boltzmann constant.

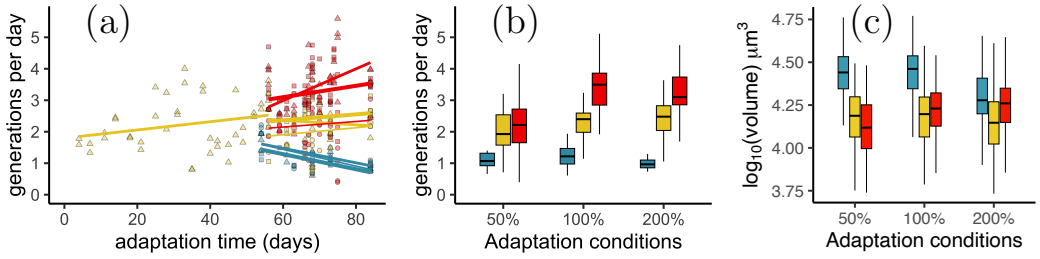

Figure 2: Adaptation to novel environmental conditions in tetrahymena populations. (a) Population growth rate of the mother culture (20 °C and 100% medium concentration, yellow line, before day 56) and of subcultures (starting from day 54). Growth rate is expressed in number of generations per day. Blue, yellow and red colours refer to temperature treatments (15, 20, and 25 °C, respectively); thicker lines represent cultures with higher medium concentration. Population growth rates increase through time (excluding at 15 °C) indicating adaptation and improved performance. (b) Growth rate during the subculture period (temporal data are combined together). Growth rate is higher at higher temperatures and largely unaffected by nutrient concentration, except at high temperature and low nutrients. (c) Volume of individual cells in each treatment at the end of the subculture period. Individual body size increases with decreasing temperature, particularly at 15 °C.In the boxplots, horizontal lines are medians, boxes extend from the 25th to the 75th percentiles, whiskers extend to 1.5 times the interquartile range. SI section S.II.A presents additional measurements of cell size and shape across experimental conditions.

3 Results

3.1 Population growth and individual cell size

Throughout the initial adaptation period at 20 °C, we observed a stable or slightly increasing *per capita* growth rate over time (fig. 2a). When cells were moved to the various experimental conditions with different acclimation temperatures and medium concentrations, the growth rate shifted to different baselines (some environments can support faster growth), and then it remained stable or increased slightly over time, compatible with the idea that tetrahymena improved their performance (measured as *per capita* growth) throughout the adaptation to the new environments. One exception were

cell populations growing at low temperature (15 °C), that seem to slightly reduce their growth rate over time. However, the rate of biomass production might still be increasing over time in this treatment if we consider that cells adapted at low temperature were simultaneously becoming larger in volume during this same adaptation period, as illustrated by their final volume (fig. 2c). These changes in cell volume across different adaptation conditions are particularly marked if we consider that cells must approximately double in volume during each generation before they reproduce by fission (this corresponds to a span of $\log_{10}(2) \simeq 0.3$ units in the logarithmic y-axis scale of the boxplot in fig. 2(c), and imposes a minimum boundary to the spread of data in each box), yet, in some nutrient conditions, recently divided cells at 15 °C are typically larger than cells cultured at 20 or 25 °C that are at the end of their cell cycle.

While temperature was a good predictor of *per capita* growth rate (fig.2b), the concentration of available nutrients did not have a strong effect on growth, suggesting that nutrients were not a limiting factor. Only at high temperature (25 °C) and low nutrients (50% medium) did cells show a slower growth, relative to conditions with the same 25 °C temperature but higher nutrient concentration.

The energy that individual cells can invest into fuelling population growth and biomass production can be thought as the result of a balance between energy intake from feeding F, and energy spent for basal metabolism (maintenance) B, and movement M:

$$\frac{1}{N}\frac{dN}{dt} = \frac{1}{C_t}\Big(F - B - P\Big) \tag{2}$$

where N is the population size (number of cells), F, B, and P have units of power (nanoWatts) and C_t can be thought as the energy amount required to form a new cell. In order to better understand the way how tetrahymena adapts to different environmental conditions, in the following sections we consider how these individual components of the tetrahymena's energy budget are each affected by temperature and resources.

3.2 Respiration and movement

It is well known that the metabolic rate of ectotherms increases with environmental temperature over a wide range of temperature conditions. In our data, the metabolic rate of tetrahymena –measured from oxygen consumption–

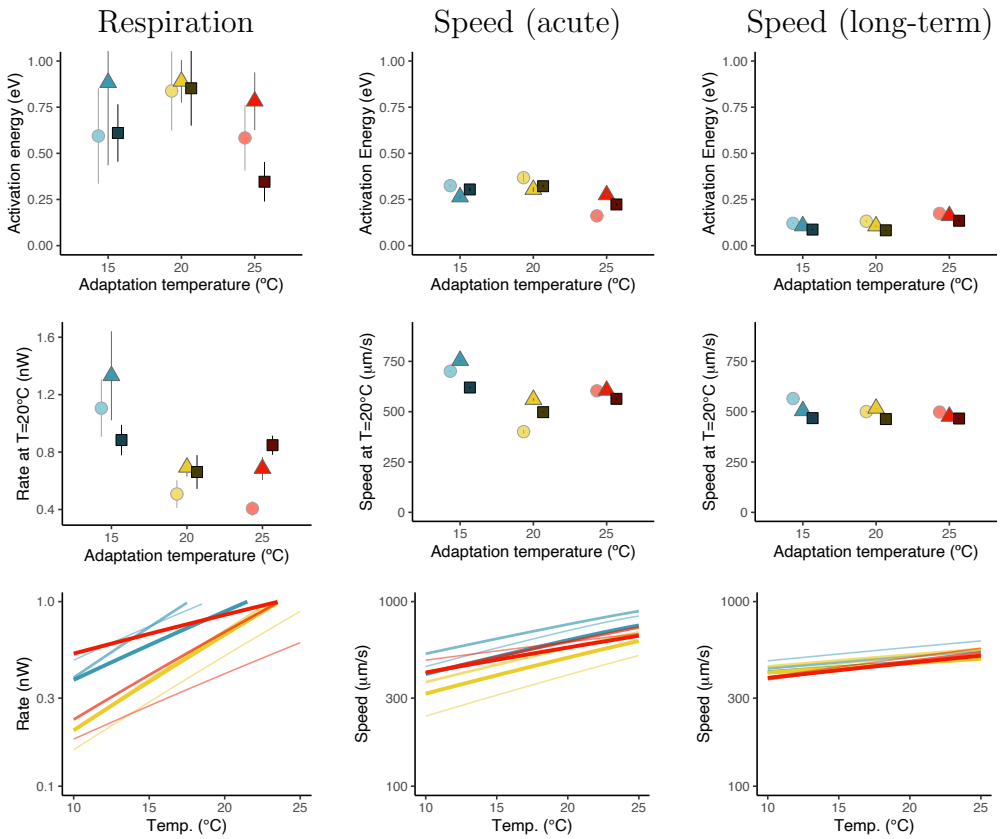

Figure 3: Thermal response curves for respiration and movement speed. Movement speed is further subdivided into an 'acute' response, where speed was recorded a few minutes after cells were moved to the new temperature, and a long-term response, a few days after cells were moved to the new temperature. Different color hues correspond to different temperature conditions (blue = 15 °C, yellow = 20 °C, red = 25 °C; bright colours correspond to low nutrient concentration: 50% medium, dark colours correspond to high nutrients: 200%). Error bars represent ±1 standard deviation, estimated from a bootstrap with 'residual' resampling in the rTPC package. Additional plots of these data and detailed thermal response curves for each condition are available as SI.

showed a clear increase with temperature, with values of activation energy E measured from respiration (top-left panel of fig. 3) broadly in the range of commonly reported values for a variety of organisms (0.6 - 0.7 eV [7]). We can't detect any clear differences in activation energy across adaptation treatments with different temperature or medium concentration, as indicated by the overlapping error bars in figure 3, but we do see a difference in the respiration rate at the reference temperature: cells adapted at 15 °C proportionally consumed more energy (fig. 3 left, second row), most likely reflecting the larger size of these cells (fig. 2c)

If a fixed proportion of metabolic energy is allocated to movement, the increase of metabolic rate with environmental temperature should be paralleled by a similar increase of movement speed. However, in general, we expect speed to increase with a lower activation energy than respiration because, in the low Reynolds number regime associated with tetrahymena swimming, the power P dissipated for moving in water at speed U increases proportionally to the square of speed ($P \propto U^2$, see SI sec. S.III), meaning that a 4-fold increase in the metabolic power invested into movement would lead only to a two-fold increase of swimming speed. This non-linear relation between power consumption and movement speed would lead to a predicted activation energy for speed about 1/2 the activation energy for respiration. In practice, however, the exact scaling between power consumption and speed is difficult to predict because most energy is dissipated within the ciliary layer with complex hydrodynamics [20, 21]. Nonetheless, our results (fig. 3 - central column) broadly confirm that movement speed increased with temperature during a short-term, acute, exposure, and that the activation energy measured for speed was consistently lower than the activation energy for respiration. The most notable effect of the adaptation process was that cold-adapted cells (at 15 °C) were comparatively faster than warm-adapted cells when tested at the same temperature, (avg. intercept of thermal performance curve for speed of cells adapted at 15 °C = 692 μm/s; speed of cells in the other conditions = 538 μm/s, fig. 3-middle column/middle panel), somewhat matching the higher metabolic rate (3-left column/middle panel) of these cold-adapted cells.

The short-term thermal response curves for respiration and movement described above are likely to reflect simple chemio-physical changes in the rate of metabolic reactions, but they might not correspond to efficient energy allocation strategies for an individual. For example, an increase of movement speed at high temperature, and the associated increase of metabolic power

P allocated to movement (as in eq. 2) are only beneficial if they are also matched by a corresponding increase in energy intake from feeding F. However, this is unlikely to be the case for a number of reasons: first of all, the volume searched for food increases linearly with speed but, as we have seen above, the energy cost of movement increases with the square of speed (see e.g. [22, 23]). In addition, the actual capture of nutrient molecules partly depends on the diffusion of the nutrients themselves, meaning that an increase of speed potentially only leads to a less than proportional increase in the actual amount of food collected [24]. Finally, digestion time also limits the amount of nutrients that can be consumed, independently of the rate at which they are encountered. In relation to this, our respiration data indicate that, after adaptation, tetrahymena all have relatively similar metabolic rate each at its own adaptation temperature, meaning that the rate at which they digest food is likely also similar (see fig. 3 and SI fig. S3). Supplementary section S.III.B expands on these arguments. But, in short, the increase in movement speed with temperature observed under short-term exposure (fig. 3 - central column) is unlikely to be sustainable in the long term.

In order to test directly this prediction, we also measured movement speed of cells that were transferred to a different temperature condition for a longer period of a few days (average 3.5 days). Under these conditions, speed varied very little across different treatments, with only a slight increase of speed with increasing temperature and possibly a very small reduction at high nutrient concentrations (fig. 3 - right column).

3.3 Piecing together the puzzle

Cells adapted to different environments clearly had different generation times depending on temperature -and to a lower extent depending on nutrients concentration- as we have seen previously in figure 2. This can also be stated by saying that the *per capita* growth rate $\frac{1}{N}\frac{dN}{dt}$ (the left term in equation 2) is strongly temperature dependent. However, on the right side of eq. 2, temperature-induced changes to the three components of the energy budget of a cell (basal metabolism B, movement P and feeding F) were largely compensated by the adaptation process: speed was relatively constant on a long-term exposure (fig. 3 - right column), meaning that the energetic investment into movement P also did not vary much; differences in basal metabolic rate were partly compensated by changes in cell size, where larger cells at low temperature had similar respiration rate as smaller cells at higher tem-

perature (fig. 3 and SI fig. S3), with the effect of reducing the temperature dependence of B under long-term adaptation; finally, if both speed (which determines the rate of food encounter and attack), and metabolism (which might affect food handling and digestion time) only change relatively little after adaptation, we can imagine that variations in the energy intake from feeding F are also compensated by the adaptation process.

What can then explain a faster *per capita* growth rate at higher temperature, in spite of relatively limited variation in *per capita* energy intake and energy consumption $(F - B - P)$ = constant? Part of the answer to this question is in the remaining term of equation 2: $\frac{1}{C_t}$, the reciprocal of the energetic content of one cells.

One important way individual cells managed to contrast the effects of temperature on metabolic rate and movement was by changing their size. Larger cells naturally have a higher metabolic rate – simply because they are bigger. In general, larger cells can also swim faster, because while the viscous drag increases with a cell's linear dimension, the number of cilia and the metabolic power that support the movement scale with higher exponents of the linear dimension (e.g. as the surface or the volume). If the energetic content of biomass is similar for large and small cells, then it takes more energy to produce a larger cell, and -everything else being equal- it takes more time. In other words, we can expect a match between *per capita* growth rate, and cell volume.

Figure 4 reports thermal response curves for population growth and for the reciprocal of cell volume (which we assume proportional to $\frac{1}{C_t}$) in cells of each adaptation line that were transferred to new values of temperature for a period of the order a few days. All cell populations reproduced faster and developed smaller sizes when tested at higher temperature than at low temperature (positive activation energies for population growth and for the reciprocal of volume in fig. 4-top row). These graphs show that cells started changing their size rapidly in response to changing temperature (the curves for 1/volume show similar values of activation energy and similar values at reference temperature, irrespective of the original adaptation temperature of the cultures). Curves for population growth suggest that cold-adapted populations may be unable to start growing rapidly when they are exposed to higher temperatures (evidenced by their lower activation energy and lower growth at reference temperature).

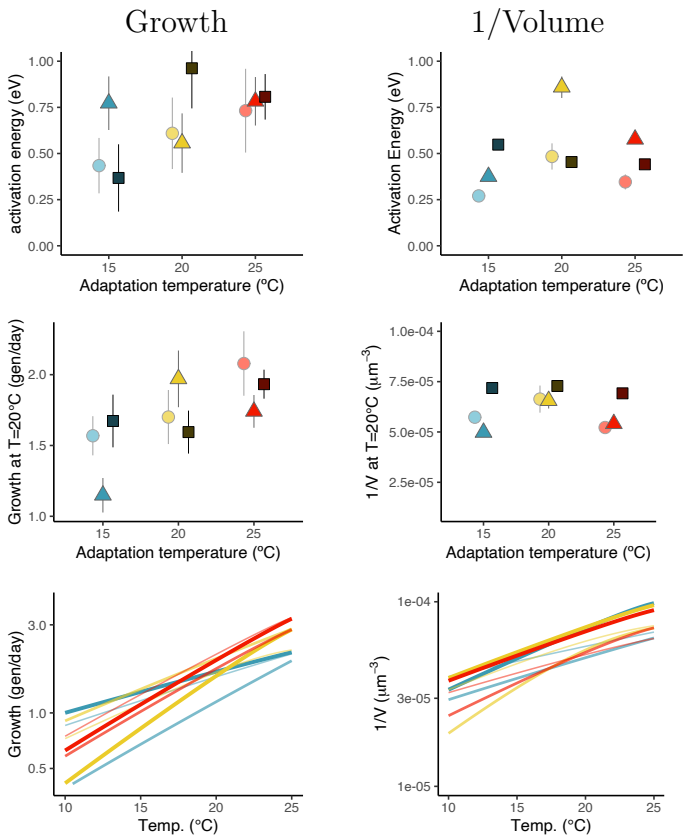

Figure 4: Rapid, and to some extent parallel, adaptation of population growth rate and the reciprocal of cell volume. Top row: activation energies for population growth and for the reciprocal of volume are both positive and of comparable magnitude (cells grow faster and develop smaller sizes as temperature increases). Middle row: growth rate (left) and reciprocal of volume (right) at the reference temperature of 20 °C. Colour scale as in figure 3. Volume here is measured over the entire short-term population growth experiment (approximately 2 to 6 generations), but most of these patterns are rapid and can be observed after one single day. Additional plots and detailed thermal response curves for each condition are available as SI.

4 Discussion

Changes in environmental conditions, and particularly in environmental temperature, had very different effects on our studied organisms depending on the time scale considered. On a short time scale, both respiration rate and movement speed increased sharply with increasing temperature. But these changes were short-lasting, and many of the short-term changes of metabolic rate and movement speed induced by the exposure to a new temperature condition were significantly attenuated over time scales of just a few days.

Acute thermal response curves measured from respiration (a proxy for energy consumption), and those measured from swimming speed showed similarities but also important differences. Cells that were cold-adapted, larger, had both higher respiration rates and higher movement speed, indicating that the phenotypic changes induced by environmental adaptation were detected by both measurements. Importantly, however, the values of activation energy measured from respiration and those measured from movement speed were different, and lower for speed than for respiration: when environmental temperature increased, respiration increased disproportionately more than swimming speed. Previous studies have also found relatively low values of activation energy for acute movement-speed responses in ciliates. For example, Glaser [25] reported speed values for paramecia that correspond to an activation energy of approximately 0.37 eV, slightly higher, but similar to our results.

Mechanistically, we can understand the mismatch of activation energies for respiration and for movement as a physical consequence of the quadratic scaling of movement speed with power consumption for swimmers in a viscous medium, but we remark that our values of activation energy for speed are not exactly half the activation energy for metabolic energy consumption (respiration). Many factors can contribute to this discrepancy, ranging from noise in the data, to changes in cell size and shape with temperature, to complex hydrodynamics of ciliary beating. We indeed call for further studies to establish a more precise relation between activation energy for speed and for respiration across more species of unicellular swimmers, and across different experimental conditions.

Functionally, the activation energy for acute movement speed response, which is 0.2 - 0.4 eV lower than the activation energy for respiration, leads us to draw a parallel between the thermal adaptation process of autotrophs and heterotrophs. In autotrophs the energy acquisition process of photosynthesis

has a lower activation energy than the energy expenditure process (respiration), and this mismatch imposes adaptation strategies based on the up- or down-regulation of the two processes (see e.g. [13]). In heterotrophs, energy acquisition is largely mediated through movement, because of its effect on prey-encounter rate and prey-capture. It is interesting to note that – albeit for completely different reasons – similar temperature dependencies describe the energy intake and energy consumption of both primary producers and of ciliate consumers. While previous studies have already emphasized some of the possible ecological implications of the temperature dependence of animal movement (see e.g. [8]), our study indicates that complex adaptations of thermal responses –which depend on the time scale and on the phenotypic trait considered– can already be observed also in axenic populations of one single organism. In future studies, our mechanistic micro-level approach could be generalized to study the multiple drivers of phenotypic adaptation in multi-species microcosms.

Adaptation was incredibly rapid in our experiments, with cells changing their movement speed and volume over just a few days in our 'long-term' temperature response data. Changes in cell volume probably accounted for most of the other observed phenotypic changes in respiration rate and movement speed. The reduction of cell size with increasing temperature is a well-described phenomenon in protists [26], for which various possible explanations have been put forward, ranging from temperature-induced limitations in oxygen supply and demand [27, 28], to developmental changes in the allocation of metabolic resources directed to maintenance and growth [29, 30], to cell size optimization in relation to the available resources at a given temperature [31] (summarized in [10]). In our experiments, the match between cell size variation and other variables such as respiration rate and *per capita* growth suggests that temperature-size relations in tetrahymena are primarily metabolic, while oxygen availability is likely not a directly limiting factor in our experiments, given the small size of tetrahymena, the rates of oxygen consumption that we measured, and the fact that we worked with cultures in their exponential growth phase, before high cell density could likely deplete oxygen and nutrients (see SI section S.III.C for additional arguments in support of this point).

Adaptation to different nutrient concentrations did not have a major effect on cell size, respiration, or movement speed in our experiments. Possibly, however, this was simply a consequence of having relatively limited variation in nutrient concentration (variation only spanning a factor of 4 from 50% to

200% medium concentration), combined with otherwise non-limiting growth conditions (exponential growth phase throughout). Previous studies [15, 14] have suggested that nutrient concentrations become a limiting factor for population growth when the metabolic requirements of organisms are high (e.g. at high temperature) and nutrient concentrations are low. Our results show some agreement with these predictions, for instance in figure 2(b), where cells adapted at 25 °C and 50% medium concentration show a reduced growth rate.

Our study highlights the multiple phenotypic changes associated with the adaptation of microorganisms to changes in their environment. Short-term, acute responses to changes in the environment are characterised by rapid and marked changes in metabolic activity and movement speed. Long-term exposure to a new environment results instead in a mitigation of the acute responses, with both metabolic rate and movement speed converging to similar values, independently of the environment. We propose an inchoate theoretical framework to understand these phenotypic changes in which short-term responses are directly driven by the physical parameters of the environment such as temperature and viscosity, while long-term adaptation is 'biological' in nature and involves changes that maximize biomass production in their new environment. Organisms can achieve this by modulating their behaviour (e.g. movement), their physiology, and their development, and the way how they do so is, to some extent, predictable. Future research could aim to refine our methods to develop a fully quantitative model of phenotypic changes, also including other variables such as digestion time and the interplay between the mechanics of ciliary propulsion and cell morphology that we did not measure directly in our experiments. Perhaps then, the same arguments we have introduced here on the interplay between physical constraints and biological optimisation could be refined to derive better predictions on individual organisms' and ecological communities' future adaptations to changes in their environment.

5 Data availability

The data and analysis code used for this work are available at `https://github.com/pernafrost/Tetrahymena`.

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