Phenotypic responses to temperature in the ciliate Tetrahymena thermophila

Weber de Melo, Vanessa; Lowe, Robert; Hurd, Paul J; Petchey, Owen L

Abstract: Understanding the effects of temperature on ecological and evolutionary processes is crucial for generating future climate adaptation scenarios. Using experimental evolution, we evolved the model ciliate Tetrahymena thermophila in an initially novel high temperature environment for more than 35 generations, closely monitoring population dynamics and morphological changes. We observed initially long lag phases in the high temperature environment that over about 26 generations reduced to no lag phase, a strong reduction in cell size and modifications in cell shape at high temperature. When exposing the adapted populations to their original temperature, most phenotypic traits returned to the observed levels in the ancestral populations, indicating phenotypic plasticity is an important component of this species thermal stress response. However, persistent changes in cell size were detected, indicating possible costs related to the adaptation process. Exploring the molecular basis of thermal adaptation will help clarify the mechanisms driving these phenotypic responses.

DOI: https://doi.org/10.1002/ece3.6486

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-200294
Journal Article
Accepted Version

Originally published at:
Weber de Melo, Vanessa; Lowe, Robert; Hurd, Paul J; Petchey, Owen L (2020). Phenotypic responses to temperature in the ciliate Tetrahymena thermophila. Ecology and Evolution, 10(14):7616-7626.
DOI: https://doi.org/10.1002/ece3.6486
Phenotypic responses to temperature in the ciliate *Tetrahymena thermophila*

Vanessa Weber de Melo¹*, Robert Lowe², Paul J. Hurd³, Owen L. Petchey¹

¹ Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland

² The Blizard Institute, Queen Mary University of London

³ School of Biological and Chemical Sciences, Queen Mary University of London

* Corresponding author

Running head: Phenotypic effects of temperature in *Tetrahymena*

Contact information:

Vanessa Weber de Melo

Department of Evolutionary Biology and Environmental Studies

University of Zurich

Winterthurerstrasse 190, 8057 Zurich

vanessawmelo@gmail.com
Abstract

Understanding the effects of temperature on ecological and evolutionary processes is crucial for generating future climate adaptation scenarios. Using experimental evolution, we evolved the model ciliate *Tetrahymena thermophila* in an initially novel high temperature environment for more than 35 generations, closely monitoring population dynamics and morphological changes. We observed initially long lag phases in the high temperature environment that over about 26 generations reduced to no lag phase, a strong reduction in cell size and modifications in cell shape at high temperature. When exposing the adapted populations to their original temperature, most phenotypic traits returned to the observed levels in the ancestral populations, indicating phenotypic plasticity is an important component of this species thermal stress response. However, persistent changes in cell size were detected, indicating possible costs related to the adaptation process. Exploring the molecular basis of thermal adaptation will help clarify the mechanisms driving these phenotypic responses.

Keywords

Temperature, experimental evolution, adaptation, morphology, phenotypic plasticity, *Tetrahymena*
1. Introduction

Temperature is one of the most important abiotic factors, influencing all levels of biological organization, from cell function to ecosystem dynamics (Johnston and Bennett 1996). Understanding how organisms respond and adapt to a novel temperature has, therefore, been the focus of multiple studies exploring physiological, ecological and evolutionary mechanisms of temperature response (Angilletta 2009; Clarke 2003). The current climate change crisis revived the interest in this research field, since understanding how populations will respond to new temperatures is of fundamental importance (Walther et al. 2002; Hoffmann and Sgrò 2011).

Microorganisms, like many other ectotherms, are particularly sensitive to the temperature of their environment as it directly affects their metabolism and many physiological processes (Pörtner et al. 2006). Since microorganisms play key functions in all ecosystems, understanding their responses to temperature is essential to forecasting the future of ecosystems (Singh et al. 2010). Besides their ecological importance, many of these organisms have short life cycles, large population sizes and are readily manipulated in the laboratory, offering many possibilities to experimentally study thermal adaptation over multiple generations (Elena and Lenski 2003; McDonald 2019).

The effect of temperature on the size of microorganisms is one of the most studied morphological responses to temperature. Most species display smaller cell sizes when grown at higher temperatures, a response known as
the temperature-size rule (Atkinson 1994). Besides cell size, many important phenotypic traits such as cell shape (Trueba et al. 1982) and swimming behavior are also affected by temperature (Schneider and Doetsch 1977; Beveridge, Petchey, and Humphries 2010).

Organisms can use different mechanisms to survive in a novel temperature. Many species display phenotypic plasticity, i.e. the same genotype can generate multiple phenotypes, in response to a change in their environment (see review Murren et al. 2015). Plastic responses can be further separated into two different types, developmental plasticity, i.e. traits that vary according to the environment during development, but are then irreversible during an organism’s life span; or traits that are context-dependent and show variation in the same individual, such as behavior or metabolic reactions, sometimes called phenotypic flexibility (Piersma and Drent 2003). Acclimation, i.e. the adjustment of physiological traits to environmental conditions, is one example of phenotypic flexibility (Wilson and Franklin 2002; Piersma and Drent 2003). Populations can also adapt to a novel temperature, which occurs when genetic changes lead to a population with higher fitness in the new environment. Temperature adaptation can lead to the evolution of specialization and have costs to an organism, such as reduced performance in the ancestral or other environments (Huey and Kingsolver 1989). These costs are predicted by theory and have been observed in previous experiments (Bennett and Lenski 2007; Jin and Agustí 2018). Phenotypic plasticity and adaptation are not mutually exclusive mechanisms; in fact it is likely that they
Several studies have experimentally examined thermal adaptation in microorganisms such as bacteria (Trueba et al. 1982; Hall, Neuhäuser, and Cotner 2008; Tenaillon et al. 2012; Sandberg et al. 2014), phytoplankton (Schlüter et al. 2014; Padfield et al. 2016) and yeast (Caspeta et al. 2014; Huang et al. 2018), but this topic has been little explored in protists. To better understand temperature adaptation in this group, we chose the ciliate protist *Tetrahymena thermophila* as our model system. This is one of the best-studied species of protists and it is able to grow in a wide range of temperatures (Laakso, Löytynoja, and Kaitala 2003).

Different studies have estimated the mutation rate of this species, which is an important feature for experimental evolution studies. *T. thermophila* has two nuclei, the micronucleus (MIC), which is diploid and has a germline function during sexual reproduction, and the macronucleus (MAC), which is polyploid (~45 N) and transcriptionally active. Brito et al. (2010) performed a mutation accumulation experiment and found high mutation rates in the MAC of this species, estimated at $U = 0.0333$ per haploid macronucleus genome per generation. Long et al. (2013), in a second mutation accumulation experiment, estimated a MIC mutation rate of $U = 0.0047$ (95% credible interval: 0.0015, 0.0125) per haploid MIC genome per generation and found no evidence that the mutation rate of the MAC is different than the MIC. This estimate is lower than in the previous experiment by Brito et al., but in the range of other...
eukaryotes. Together with large population sizes, *T. thermophila* is therefore a suitable model for experimental evolution.

In this study, we monitored the population dynamics and cell morphology of four replicate populations of *T. thermophila* exposed to $38 \degree C$, a highly stressful condition. With this, we tested whether populations can survive in a temperature near lethality without previous acclimation. Different mechanisms can be involved in this process, from phenotypic plasticity to adaptation to the new environment. When exposed to such a high temperature, we predict the growth rate of *T. thermophila* populations will have an immediate and strong decrease. This prediction is based on the observed reductions of population growth at temperatures above $37.5 \degree C$ (see Figure 1, which contains results from a pilot experiment described in the Methods section), and also because this temperature is close to the thermal limit of the species (Laakso, Löytynoja, and Kaitala 2003). Long-term exposure to $38 \degree C$ will likely result in growth rates similar to the ones in non-stressful temperatures, if populations are able to adapt to the novel temperature.

We hypothesize a short-term negative effect of temperature on cell size, as predicted by the temperature-size rule (Atkinson 1994) and established for many protists (Atkinson, Ciotti, and Montagnes 2003). The shape of protist cells can be affected by stressful environmental conditions (Kovács et al. 1999; Dias, Mortara, and Lima 2003), by the presence of predators (Kuhlmann and Heckmann 1985; Hammill, Petchey, and Anholt 2010) and is related to dispersal behavior (Pennekamp et al. 2014). Absent, however, are clear
hypotheses about how and why cell shape would change with temperature.

Nevertheless, we present the effects of temperature on cell shape, since exploratory analyses may yield insights into this understudied trait.

After many generations at this novel temperature, the populations were then returned to the control temperature, a benign environment. This enabled us to test if survival at high temperatures had any costs to the organisms. If there are costs associated to survival at high temperature, we expect to see reduced growth rates when adapted populations return to the control condition. Similar patterns should also be observed for the morphological traits, with reduced cell sizes when populations return to the control condition, since larger cells have a higher fitness. Opposite results would indicate the evolution of generalists, or the presence of a plastic response.
2. Materials and methods

2.1. Strain and culture conditions

All experiments were performed with the ciliate *Tetrahymena thermophila* (Figure 2A) strain 1630/1U cultured in axenic conditions in 2% proteose peptone medium. This strain was acquired from the Culture Collection of Algae and Protozoa and grown during many generations at 15 °C to acclimatize it to our laboratory conditions. We did not initiate the stock cultures from single cells; this strain likely contains very low genetic variability due to a long history of serial transfers (Ketola et al. 2004). *T. thermophila* only reproduced clonally in all experiments, since a single strain with one mating type was used.

The medium used in all experiments was prepared with proteose peptone from the same manufacturer batch, ensuring homogeneous conditions across all experimental replicates. The bottles and the medium used in the experiments were sterilized in an autoclave and all sampling procedures were performed in sterile conditions. Microbial contamination was regularly checked during the experiments by plating a sample of each culture on an agar plate incubated at 37 °C for 24 h. To ensure our treatments were reliable, we monitored every 15 minutes the actual temperature in the incubators used to grow the experimental populations. In all incubators, the mean daily temperature presented a standard deviation smaller than 0.28 °C.
2.2. Temperature range of *T. thermophila*

We performed a pilot experiment to identify the temperature range in which this *T. thermophila* strain is able to grow, exploring ten different temperatures from 20 to 42.5 °C, in intervals of 2.5 °C. Three replicate populations were grown in each temperature for a period of 13 days. The initial population density of all replicates was 500 cells/ml and the populations were monitored daily with videos to measure population density.

We observed cell division in all tested temperatures except at 42.5 °C (Figure 1). When grown in temperatures between 20 and 37.5 °C, populations immediately entered exponential growth and reached high and stable carrying capacities. At 40 °C, populations initially increased in density but collapsed after two days, indicating this temperature is close to the upper thermal limit of this strain.

To explore temperature adaptation in this species, we chose 20 °C as the control temperature of the experiment and 38 °C as the adaptation temperature. At 20 °C, populations grow very well and reach carrying capacity in a short period of time, while 38 °C is a stressful temperature that creates a strong selective pressure.

2.3. Experimental design

The temperature adaptation experiment was performed with populations of *T. thermophila* growing in axenic batch cultures in 2-liter bottles with 500 ml of medium. The cultures were placed in incubators with controlled temperature,
no light and in shakers to increase aeration. The experiment was initiated with four replicate populations from the same stock culture, giving rise to four separate evolving lineages. There were five consecutive batch cultures of each lineage that lasted a total of 41 days (Figure 2B).

All the batch cultures started at a low density (500 cells/ml) and once they reached carrying capacity new batch cultures were started from a small aliquot of the previous batch. The volume of this transferred aliquot depended on the population density of the culture at the end of the previous batch, to ensure that all new batch cultures started with the same cell density (500 cells/ml). Each batch had a different duration, since the time to reach carrying capacity changed during the experiment, in large part due to the temperature treatment. The shortest batch lasted five days, while the longest batch lasted 12 days.

In the first batch, the four replicate cultures were grown at 20 °C and in the second batch, the four replicates started the adaptation to 38 °C. Each culture in the second batch originated two cultures in the third batch, resulting in eight cultures still growing at 38 °C. In the fourth batch, one of each paired culture was moved back to 20 °C, while the other culture remained at 38 °C. In batch five, the cultures continued in the temperature experienced in the previous batch.

Population sizes in this experiment were large, ranging from 374,000 cells at the beginning of each batch culture, when population density was 500 cells/ml, reaching population sizes of $187 \times 10^6$ at 38 °C and $433 \times 10^6$ at 20 °C.
Using the MAC mutation rate estimated by Brito et al (2010), we expect $2997 \times 10^3$ mutations in the shortest batch at 38 °C (8 generations), which is a significant proportion of the genome of this species, considering a MAC genome size of 104 Mbp. Hence we believe the duration of the experiment sufficient to allow adaptation to occur.

2.4. Video monitoring and processing

The cultures were monitored daily after the second or the third day of each batch, and every day in batch 5. These minor differences in the monitoring schedule compensated for minor differences in the timing of population dynamics. Monitoring included estimation of population abundances and multiple morphological measurements. On the monitoring days, each culture was sampled twice, since duplicate assessments provide more accurate estimates of the population abundance. Each sample consisted of 1 ml of culture. The samples were placed in counting chambers and the videos were taken on a stereomicroscope (Leica M205 C) mounted with a digital CMOS camera (Hamamatsu Orca C11440, Hamamatsu Photonics, Japan) with 1.57X magnification. When population density was high, samples were diluted with fresh medium before taking the videos. Each video comprised 125 frames in 5 seconds and monitored 40.26 μl of sample. The videos were processed using the R package BEMOVI version 1.0 (Pennekamp, Schtickzelle, and Petchey 2015), which extracts morphological information of all the moving cells in the field of view.
2.5. Data analysis

In total, 94,344 cells were measured, with an average of 513 ± 26.9 cells monitored per population per day. The number of detected cells was used to estimate population density throughout the experiment. We calculated the minimum number of generations (G) that took place in each batch culture with the equation $G = \frac{\ln(A_{\text{max}}/A_0)}{\ln(2)}$, where $A_0$ is the minimum population abundance, and $A_{\text{max}}$ is the maximum population abundance.

All statistical analyses were performed using R (R Core Team 2019). We used a Gompertz model (Zwietering et al. 1990) implemented in the R package growthrates version 0.8.1 (Petzoldt 2019) to estimate the maximum growth rate and the duration of the lag phase in each population per batch. The model fittings can be seen in Figure 4A. Despite different optimization strategies, this model presented low $R^2$ values for population 3 in batch 4 at 38 °C, indicating it could not properly model the growth dynamics of this population. We therefore calculated the maximum growth rate of this population as the log$_{10}$ difference in abundance between the maximum and the minimum population abundance, divided by the time period between these days. The lag phase of this population was set to zero, since it quickly entered exponential growth.

We used two morphological measurements in this study, the cell area and the cell shape (the ratio between the longer and the shorter axes of the cell). The morphological measurements were averaged per population per day and the coefficient of variation among individuals for cell size was calculated.
per population per day. We recorded information related to movement behaviour, such as swimming speed, but did not include this data in the present study. We acquired videos at room temperature, which differed from the growing temperature, and some samples required dilution due to high density. Therefore we were not confident that movement behavior recorded from the videos would reflect the effects of the temperature treatment of the experiment.

To investigate the effect of temperature on population dynamics and cell morphology, we performed two separate analyses, first on the populations at 38 °C and then on the populations that returned to the control temperature. We analyzed six different traits, the population growth rate, the lag phase, the mean cell size, its coefficients of variation and its variance, and the mean cell shape. For each of these six response variables, the average change relative to the control (batch 1) was calculated for each population in each batch. Relative change in the morphological traits was expressed as percent, and absolute differences were used for the lag phase and growth rate. We used the package MCMCglmm version 2.29 (Hadfield 2010) to fit linear mixed effects models to each of the six response variables, modeling the effect of temperature across all batches in each variable. All models included population lineage as a random effect and time as a fixed effect, and we used relatively uninformative priors (V = 1, nu = 0.002). We ran each model for 2,000,000 iterations, with a burn in of 30,000 iterations and storing every 1,000th iteration. We assessed model convergence with autocorrelation analyses and with trace plots using the package coda version 0.19-3 (Plummer et al. 2006).
3. Results

3.1. Population dynamics

All replicate populations grew well in the control temperature of 20 °C in the first batch (Figure 3A), immediately entering exponential phase and reaching carrying capacity in a few days. When populations started to grow at 38 °C, they entered a long lag phase and exponential growth started only after 7 days (Figure 4B and C). Similar dynamics were observed in batch 3, even though a few populations displayed a shorter lag phase. In batch 4, the populations that continued at 38 °C displayed a much reduced lag phase, similar to the populations that went back to the control temperature. Batch 5 had similar population dynamics to what was observed in the previous batch. The four batches in which *T. thermophila* populations experienced 38 °C comprise a minimum of 35 generations.

Temperature strongly affected the lag phase of *T. thermophila* cultures. In the first batch at 38 °C, lag phase was increased by 7.7 days [6.3; 8.9] (here and later in square brackets is the estimated 95% credible interval) in comparison to the control temperature, but it gradually decreased during the experiment. In the final batch, lag phase at 38 °C was not different from the lag phase at the control temperature (0.7 days [-0.6; 2.1]) (Figure 4C and Figure 5). Maximum growth rate slightly increased during the experiment, and we
found a significant effect of temperature, with an increase of $4.5 \ [0.4; 8.7]$ at the end of batch 5 (Figure 4B and Figure 5). Maximum population density decreased at $38 \, ^\circ\mathrm{C}$, but it remained lower throughout all batches (Figure 3A). In batch 4, the populations that moved back to $20 \, ^\circ\mathrm{C}$ displayed an immediate increase in maximum population density, similar to the maximum density observed in batch 1.

We observed a marked variation in population dynamics across the population replicates at $38 \, ^\circ\mathrm{C}$, especially in batches 2 and 3, while population dynamics at $20 \, ^\circ\mathrm{C}$ were much more similar. Some populations exhibited a much larger maximum growth rate in specific batches (Figure 4B), and there was also significant variation in lag phase duration, for example in batch 3, in which populations 1 and 2 presented a lag phase two times longer than populations 3 and 4 (Figure 4C).

3.2. Cell morphology

Temperature had a strong and long-lasting effect on cell size and shape. There was an immediate decrease of $27.3 \, \% \ [9.3; 47.5]$ in cell area when populations moved to the higher temperature (Figure 3B and Figure 5). This size reduction was maintained throughout the entire experiment and populations displayed a cell area reduction of $30.2 \, \% \ [11.2; 49.8]$ at the final batch. When populations moved back to $20 \, ^\circ\mathrm{C}$ after many generations growing at $38 \, ^\circ\mathrm{C}$, cell size recovered and the mean cell area increased, but populations
To explore the variation in cell sizes during the experiment, we modeled both the variance and the coefficient of variation (CV) of cell size in response to temperature, which showed slightly different patterns, since the CV is scaled by the sample mean. The CV of cell area significantly increased at 38 °C through the entire experiment (32.9 % [5.75; 56.6] in batch 5) (Figure 3D and Figure 5), while the variance in cell size was not different than the control (-17.4 % [-39.5; 4.79] in batch 5) (Figure 3C and Figure 5). Taken together, they indicate a slightly wider range of cell sizes when populations first moved to the novel temperature, but a reduction in variation towards the end of the experiment (Figure 5).

Similar patterns were observed in cell shape, as can be seen in the mean cell shape of the populations during the adaptation experiment (Figure 3E and Figure 5). Cells became rounder as they adapted to 38 °C (16.5 % [14.1; 19.0] rounder in batch 5), and cell shape returned to more elongated formats when populations moved back to 20 °C (only 5.2 % [0.1; 10.6] rounder in batch 5).

4. Discussion

The results of this experiment clarify phenotypic and population responses of *T. thermophila* exposed to a high temperature environment for
more than 35 generations. Immediate effects of high temperature included a 
large increase in the lag phase. There was, however, no effect on the growth 
rate of the populations. The long lag phases in the first batch at 38 °C returned 
to control levels after around 26 generations, while growth rate at 38 °C slightly 
increased during the experiment. These results provide further evidence to the 
importance of lag phase in microbial population dynamics (Bertrand 2019) and 
its importance during responses to environmental change.

Phenotypic effects were observed in the morphology of *T. thermophila*, 
with a prevalence of smaller and rounder cells at high temperature. Previous 
experiments using *Tetrahymena* species have also observed reductions in cell 
size at high temperatures (James and Read 1957; DeLong et al. 2017), a 
pattern also present in other ciliates (e.g., Weisse et al. 2002), but the duration 
of these experiments was much shorter, comprising only a small number of 
generations. Our study shows that cell size is immediately reduced at high 
temperatures and remains lower as populations evolve. Despite this general 
trend, one of the replicate populations displayed a much smaller cell size 
reduction (population 4, Figure 3B), indicating that larger cells are also a viable 
phenotype at high temperatures. Furthermore, cell size showed an increased 
variation at the high temperature environment, while at 20 °C cells had a more 
uniform morphology. Stressful conditions often lead to a higher phenotype 
variability (Hoffmann and Hercus 2000), but this pattern is another indication 
that more than one phenotype is viable in this environmental condition.
Different hypotheses try to clarify the mechanisms through which temperature affects body size. Although most of them were developed to explain the plastic response of the temperature-size rule, these hypotheses are also applicable for long-term adaptive responses to temperature. One hypothesis relates smaller cells at high temperatures to higher metabolic rates and therefore higher oxygen demands. Since oxygen diffusion is reduced as temperature increases, a reduction in cell size compensates for that (Atkinson, Morley, and Hughes 2006). Another possible explanation is based on growth rate being more affected by temperature than development rate, which would lead to organisms dividing at a younger age and thus being smaller (Zuo et al. 2012). A third mechanism is based on body size optimization between the organisms demand and the expected resource supply in a given temperature (DeLong 2012). These hypotheses are not mutually exclusive and all are relevant to our study system. Each of these hypotheses received support from theoretical models and experimental data, but their importance for the observed patterns is still under debate.

Little is known about the effect of temperature on cell shape in *T. thermophila* and in other ciliates. DeLong et al. (2017) grew *T. thermophila* in three different temperatures, 20, 26 and 32 °C, and although variation in cell shape was observed, no clear pattern related to temperature was found. A few studies exposed populations of *T. thermophila* to different stressful conditions and have also observed rounder cells (Dias, Mortara, and Lima 2003; Nilsson 2005). Taken together, these experiments indicate that round cells could be
related to harsh environmental conditions in general, and not only to high
temperature. The rounder cells could also be connected to malfunction of
cytoskeleton proteins at high temperature, since these proteins have an
important role in maintaining cell shape (Williams 2004). Investigations of gene
and protein functions in high temperature environments would help clarify these
mechanisms.

Possible costs related to thermal adaptation were estimated by
analyzing the performance of the populations that returned to the control
temperature after more than 18 generations at 38 °C. No significant reduction
was observed at the growth rate in batches 4 and 5, and cell shape returned to
the control levels, indicating little costs related to the high temperature
adaptation. Cell size, however, remained smaller even after many generations
back in the control temperature (Figure 5), and populations displayed a small
increase in lag phase during batch 4 at 20 °C (Figure 5). Cell size is an
important trait for the fitness of unicellular organisms (Monds et al. 2014)
including *T. thermophila* (H. Long and Zufall 2015) and the observed pattern
may indicate the occurrence of costs when populations adapt to a new
temperature. Evidence for costs related to thermal adaptation have been
described in previous experiments investigating different microorganisms.

Bennett and Lenski (2007) found fitness trade-offs in *E. coli* populations
adapted to 20 °C in comparison to the ancestral populations adapted to 40 °C.
Baker et al. (2018) described trade-offs in growth rate during adaption to supra-
optimal temperatures in a dinoflagellate, and Duncan et al. (2011), using
another ciliate, *Paramecium caudatum*, observed trade-offs when populations were adapted to a specific temperature and became specialists. The dynamics of cell size found in this study indicate that costs may also take place in our study system, but longer experiments are needed to confirm the relevance of this, since trade-offs might be transient and only present while populations are still adapting to the new environment.

Phenotypic plasticity can also play an important role in thermal adaptation, as observed in experiments with bacteria (Shi and Xia 2003) and zooplankton (Yampolsky, Schaer, and Ebert 2013). A plastic response likely also explains some of the patterns in this study, for example the immediate recover of cell shape when populations return to 20 °C in batches 4 and 5 (Figure 5). The long lag phases observed in the batch 2 (Figure 4C), the first batch exposed to the novel temperature, probably also represents a plastic response in the form of acclimation to the new environment. However, populations also displayed longer lag phases in batch 3, and acclimation is not sufficient to explain this pattern, since populations had already been exposed to this novel temperature for multiple generations. Developmental plasticity and adaptive responses are possible mechanisms generating the observed temperature response. Analysis of the molecular basis of this response could clarify the role of plasticity and would also help understanding the mechanisms behind the phenotypic changes during thermal adaptation. Further studies comparing the phenotypic plasticity in the ancestral and in the evolved lineages
would also be important to advance our understanding of the relative roles of phenotypic plasticity and genetic variation during temperature adaptation.

One important feature of our study, caused by time and resource constraints, was the use of the ancestral populations grown at 20 °C in batch 1 as the control (Figure 2), instead of maintaining populations at 20 °C during the entire experiment, or keeping individuals from batch 1 in suspended animation (which is technically difficult for *T. thermophila*) to compare with individuals from later batches in a common garden setting. When comparing the ancestral populations in batch 1 (20 °C) and the evolved populations in batch 5 (20 °C), as we did, two things differ: 1) prior exposure to 38 °C (our treatment) and amount of time in the experimental conditions (i.e. batch). Although we cannot rule out the possibility, we find it unlikely that the amount of time in experimental conditions could account for the observed differences because: i) individuals in batch 1 had already experienced many generations in conditions similar to the experimental conditions; ii) low variation in population dynamics and morphological traits, relative to treatment effects; iii) comparison with previous 20 °C populations from the pilot experiment (not shown).

It would be interesting and relevant to investigate if different *T. thermophila* strains display growth and morphological responses to temperature similar to the ones we observed in this experiment. A previous study in the ciliate *Euplotes vannus* observed significant variation in the growth rate thermal dependence across four strains of this species (Walton et al. 1995), and in *T. thermophila* there is extensive variation of dispersal propensity.
across genotypes (Pennekamp et al. 2014). Patterns of temperature adaptation in natural populations of *T. thermophila* or other ciliates have received little attention so far. Krenek, Petzoldt, and Berendonk (2012) explored this question in *Paramecium caudatum* populations sampled in a latitudinal range across Europe, but found no indication of local adaptation in this species, while Gächter and Weisse found support for local adaptation to temperature studying the freshwater ciliate *Meseres corlissi* (Gächter and Weisse 2006). Our study only investigates a single strain of *T. thermophila*, but the results provide evidence that generalists are present in this species as well, since adaptation to a higher temperature had little effect on growth at the ancestral temperature. Studying natural populations of this species would be an interesting comparison to the results obtained in these laboratory experiments and would help better understand the process of thermal adaptation in microorganisms.

**Acknowledgments**

We thank Yves Choffat for his assistance in the laboratory experiments and Andrea Tabi for feedback on this manuscript. We thank Dr. Aaron J. Bell for providing the image of *T. thermophila*. This work was funded by the University Research Priority Program ‘Evolution in Action’ and by the University Research Priority Program ‘Global Change and Biodiversity’ of the University of Zurich.
Author contributions

VWM, RL, PH and OP conceived the study. VWM performed the experiments and analyzed the data, with input from all authors. VWM and OP wrote the manuscript.

Data accessibility

Population abundances and morphological trait data are available on Dryad Digital Repository (https://doi.org/10.5061/dryad.v15dv41tb). Code for all figures and statistical analyses is accessible at Github (https://github.com/vanessawmelo/Temperature_response_Tetrahymena) with the identifier (10.5281/zenodo.3871397).

Competing interests

The authors declare no competing interests.
References

Angilletta, Michael J. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press.

http://www.oxfordscholarship.com/view/10.1093/acprof:oso/9780198570875.0875.001.1/acprof-9780198570875.

Atkinson, David. 1994. “Temperature and Organism Size - a Biological Law for Ectotherms?” In *Advances in Ecological Research*, 25:1–58. Academic Press. https://doi.org/10.1016/S0065-2504(08)60212-3.

Atkinson, David, Benjamin J Ciotti, and David J S Montagnes. 2003. “Protists Decrease in Size Linearly with Temperature: Ca. 2.5% °C⁻¹.” *Proceedings of the Royal Society B: Biological Sciences* 270 (1533): 2605–11. https://doi.org/10.1098/rspb.2003.2538.

Atkinson, David, Simon A. Morley, and Roger N. Hughes. 2006. “From Cells to Colonies: At What Levels of Body Organization Does the ‘Temperature-Size Rule’ Apply?” *Evolution & Development* 8 (2): 202–14. https://doi.org/10.1111/j.1525-142X.2006.00090.x.

Baker, Kirralee G., Dale T. Radford, Christian Evenhuis, Unnikrishnan Kuzhiumparam, Peter J. Ralph, and Martina A. Doblin. 2018. “Thermal Niche Evolution of Functional Traits in a Tropical Marine Phototroph.” *Journal of Phycology* 54 (6): 799–810. https://doi.org/10.1111/jpy.12759.
Bennett, Albert F., and Richard E. Lenski. 2007. “An Experimental Test of Evolutionary Trade-Offs during Temperature Adaptation.” *Proceedings of the National Academy of Sciences* 104 (Supplement 1): 8649–54. https://doi.org/10.1073/pnas.0702117104.

Bertrand, Robert L. 2019. “Lag Phase Is a Dynamic, Organized, Adaptive, and Evolvable Period That Prepares Bacteria for Cell Division.” *Journal of Bacteriology* 201 (7): e00697-18. https://doi.org/10.1128/JB.00697-18.

Beveridge, Oliver S., Owen L. Petchey, and Stuart Humphries. 2010. “Mechanisms of Temperature-Dependent Swimming: The Importance of Physics, Physiology and Body Size in Determining Protist Swimming Speed.” *Journal of Experimental Biology* 213 (24): 4223–31. https://doi.org/10.1242/jeb.045435.

Brito, Patrícia H., Elsa Guilherme, Helena Soares, and Isabel Gordo. 2010. “Mutation Accumulation in *Tetrahymena*.” *BMC Evolutionary Biology* 10 (1): 354.

Caspeta, Luis, Yun Chen, Payam Ghiaci, Amir Feizi, Steen Buskov, Björn M. Hallström, Dina Petranovic, and Jens Nielsen. 2014. “Altered Sterol Composition Renders Yeast Thermotolerant.” *Science* 346 (6205): 75–78. https://doi.org/10.1126/science.1258137.

Clarke, Andrew. 2003. “Costs and Consequences of Evolutionary Temperature Adaptation.” *Trends in Ecology & Evolution* 18 (11): 573–81. https://doi.org/10.1016/j.tree.2003.08.007.
Davis, Margaret B., and Ruth G. Shaw. 2001. “Range Shifts and Adaptive Responses to Quaternary Climate Change.” Science 292 (5517): 673–79. https://doi.org/10.1126/science.292.5517.673.

DeLong, John P. 2012. “Experimental Demonstration of a ‘Rate-Size’ Trade-off Governing Body Size Optimization.” Evolutionary Ecology Research 14: 343–352.

DeLong, John P., Chad E. Brassil, Emma K. Erickson, Valery E. Forbes, Etsuko N. Moriyama, and Wayne R. Riekhof. 2017. “Dynamic Thermal Reaction Norms and Body Size Oscillations Challenge Explanations of the Temperature-Size Rule.” Evolutionary Ecology Research 18: 293–303.

Dias, Nicolina, Renato A. Mortara, and Nelson Lima. 2003. “Morphological and Physiological Changes in Tetrahymena Pyriformis for the in Vitro Cytotoxicity Assessment of Triton X-100.” Toxicology in Vitro 17 (3): 357–66. https://doi.org/10.1016/S0887-2333(03)00023-7.

Duncan, Alison B., Simon Fellous, Elsa Quillery, and Oliver Kaltz. 2011. “Adaptation of Paramecium Caudatum to Variable Conditions of Temperature Stress.” Research in Microbiology 162 (9): 939–44. https://doi.org/10.1016/j.resmic.2011.04.012.

Elena, Santiago F., and Richard E. Lenski. 2003. “Evolution Experiments with Microorganisms: The Dynamics and Genetic Bases of Adaptation.” Nature Reviews Genetics 4 (6): 457–69. https://doi.org/10.1038/nrg1088.
Gächter, E, and T Weisse. 2006. “Local Adaptation among Geographically Distant Clones of the Cosmopolitan Freshwater Ciliate Meseres Corlissi. I. Temperature Response.” Aquatic Microbial Ecology 45 (December): 291–300. https://doi.org/10.3354/ame045291.

Gienapp, Philip, Celine Teplitsky, Jussi S. Alho, James A. Mills, and Juha Merilä. 2008. “Climate Change and Evolution: Disentangling Environmental and Genetic Responses.” Molecular Ecology 17 (1): 167–78. https://doi.org/10.1111/j.1365-294X.2007.03413.x.

Hadfield, Jarrod D. 2010. “MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package.” Journal of Statistical Software 33 (1): 1–22. https://doi.org/10.18637/jss.v033.i02.

Hall, Edward K., Claudia Neuhauser, and James B. Cotner. 2008. “Toward a Mechanistic Understanding of How Natural Bacterial Communities Respond to Changes in Temperature in Aquatic Ecosystems.” The ISME Journal 2 (5): 471–81. https://doi.org/10.1038/ismej.2008.9.

Hammill, Edd, Owen L. Petchey, and Bradley R. Anholt. 2010. “Predator Functional Response Changed by Induced Defenses in Prey.” The American Naturalist 176 (6): 723–31. https://doi.org/10.1086/657040.

Hoffmann, Ary A., and Miriam J. Hercus. 2000. “Environmental Stress as an Evolutionary Force.” BioScience 50 (3): 217–26. https://doi.org/10.1641/0006-3568(2000)050[0217:ESAEF]2.3.CO;2.
Hoffmann, Ary A., and Carla M. Sgrò. 2011. “Climate Change and Evolutionary Adaptation.” *Nature* 470 (7335): 479–85. https://doi.org/10.1038/nature09670.

Huang, Chih-Jen, Mei-Yeh Lu, Ya-Wen Chang, and Wen-Hsiung Li. 2018. “Experimental Evolution of Yeast for High-Temperature Tolerance.” *Molecular Biology and Evolution* 35 (8): 1823–39. https://doi.org/10.1093/molbev/msy077.

Huey, Raymond B, and Joel G Kingsolver. 1989. “Evolution of Thermal Sensitivity of Ectotherm Performance.” *Trends in Ecology & Evolution* 4 (5): 131–35. https://doi.org/10.1016/0169-5347(89)90211-5.

James, Thomas W., and C. P. Read. 1957. “The Effect of Incubation Temperature on the Cell Size of *Tetrahymena Pyriformis*.” *Experimental Cell Research* 13 (3): 510–16. https://doi.org/10.1016/0014-4827(57)90080-0.

Jin, Peng, and Susana Agustí. 2018. “Fast Adaptation of Tropical Diatoms to Increased Warming with Trade-Offs.” *Scientific Reports* 8 (1): 17771. https://doi.org/10.1038/s41598-018-36091-y.

Johnston, Ian A., and Albert F. Bennett. 1996. *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge, UK: Cambridge University Press. https://www.cambridge.org/ch/academic/subjects/life-sciences/zoology/animals-and-temperature-phenotypic-and-evolutionary-adaptation.
Ketola, Tarmo, Jouni Laakso, Veijo Kaitala, and Susanna Airaksinen. 2004. “Evolution of Hsp90 Expression in Tetrahymena Thermophila (Protozoa, Ciliata) Populations Exposed to Thermally Variable Environments.” Evolution 58 (4): 741–748.

Kovács, Péter, Hargita Hegyesi, Laszlo Köhidai, P. Nemes, and György Barabás. 1999. “Effect of C2 Ceramide on the Inositol Phospholipid Metabolism (Uptake of 32P, 3H-Serine and 3H-Palmitic Acid) and Apoptosis-Related Morphological Changes in Tetrahymena.” Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 122 (2): 215–24. https://doi.org/10.1016/S0742-8413(98)10103-2.

Krenek, Sascha, Thomas Petzoldt, and Thomas U. Berendonk. 2012. “Coping with Temperature at the Warm Edge – Patterns of Thermal Adaptation in the Microbial Eukaryote Paramecium Caudatum.” PLOS ONE 7 (3): e30598. https://doi.org/10.1371/journal.pone.0030598.

Kuhlmann, Hans-Werner, and Klaus Heckmann. 1985. “Interspecific Morphogens Regulating Prey-Predator Relationships in Protozoa.” Science 227 (4692): 1347–49. https://doi.org/10.1126/science.227.4692.1347.

Laakso, Jouni, Katja Löytynoja, and Veijo Kaitala. 2003. “Environmental Noise and Population Dynamics of the Ciliated Protozoa Tetrahymena Thermophila in Aquatic Microcosms.” Oikos 102 (3): 663–671.
Long, Hong-An, Tiago Paixao, Ricardo B. R. Azevedo, and Rebecca A. Zufall. 2013. “Accumulation of Spontaneous Mutations in the Ciliate Tetrahymena Thermophila.” *Genetics* 195 (2): 527–40. https://doi.org/10.1534/genetics.113.153536.

Long, Hong-An, and Rebecca A. Zufall. 2015. “Mutational Robustness of Morphological Traits in the Ciliate Tetrahymena Thermophila.” *The Journal of Eukaryotic Microbiology* 62 (2): 249–54. https://doi.org/10.1111/jeu.12174.

McDonald, Michael J. 2019. “Microbial Experimental Evolution – a Proving Ground for Evolutionary Theory and a Tool for Discovery.” *EMBO Reports* 20 (8): e46992. https://doi.org/10.15252/embr.201846992.

Monds, Russell D., Timothy K. Lee, Alexandre Colavin, Tristan Ursell, Selwyn Quan, Tim F. Cooper, and Kerwyn Casey Huang. 2014. “Systematic Perturbation of Cytoskeletal Function Reveals a Linear Scaling Relationship between Cell Geometry and Fitness.” *Cell Reports* 9 (4): 1528–37. https://doi.org/10.1016/j.celrep.2014.10.040.

Murren, Courtney J., Josh R. Auld, Hillary Callahan, Cameron K. Ghalambor, Corey A. Handelsman, Mary A. Heskel, Joel G. Kingsolver, et al. 2015. “Constraints on the Evolution of Phenotypic Plasticity: Limits and Costs of Phenotype and Plasticity.” *Heredity* 115 (4): 293–301. https://doi.org/10.1038/hdy.2015.8.
Nilsson, Jytte R. 2005. “Ethanol Affects Endocytosis and Proliferation of Tetrahymena Pyriformis GL and Promotes Encystment.” *Acta Protozoologica* 44: 293–99.

Padfield, Daniel, Genevieve Yvon Durocher, Angus Buckling, Simon Jennings, and Gabriel Yvon Durocher. 2016. “Rapid Evolution of Metabolic Traits Explains Thermal Adaptation in Phytoplankton.” *Ecology Letters* 19 (2): 133–42. https://doi.org/10.1111/ele.12545.

Pennekamp, Frank, Katherine A. Mitchell, Alexis Chaine, and Nicolas Schtickzelle. 2014. “Dispersal Propensity in Tetrahymena Thermophila Ciliates - a Reaction Norm Perspective.” *Evolution* 68 (8): 2319–30. https://doi.org/10.1111/evo.12428.

Pennekamp, Frank, Nicolas Schtickzelle, and Owen L. Petchey. 2015. “BEMOVI, Software for Extracting Behavior and Morphology from Videos, Illustrated with Analyses of Microbes.” *Ecology and Evolution* 5 (13): 2584–95. https://doi.org/10.1002/ece3.1529.

Petzoldt, Thomas. 2019. *Growthrates: Estimate Growth Rates from Experimental Data.* (version 0.8.1). https://CRAN.R-project.org/package=growthrates.

Piersma, Theunis, and Jan Drent. 2003. “Phenotypic Flexibility and the Evolution of Organismal Design.” *Trends in Ecology & Evolution* 18 (5): 228–33. https://doi.org/10.1016/S0169-5347(03)00036-3.
Plummer, Martyn, Nicky Best, Kate Cowles, and Karen Vines. 2006. “CODA: Convergence Diagnosis and Output Analysis for MCMC.” *R News* 6 (1): 7–11.

Pörtner, Hans O., Albert F. Bennett, Francisco Bozinovic, Andrew Clarke, Marco A. Lardies, Magnus Lucassen, Bernd Pelster, Fritz Schiemer, and Jonathon H. Stillman. 2006. “Trade-Offs in Thermal Adaptation: The Need for a Molecular to Ecological Integration.” *Physiological and Biochemical Zoology* 79 (2): 295–313. https://doi.org/10.1086/499986.

R Core Team. 2019. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Sandberg, Troy E., Margit Pedersen, Ryan A. LaCroix, Ali Ebrahim, Mads Bonde, Markus J. Herrgard, Bernhard O. Palsson, Morten Sommer, and Adam M. Feist. 2014. “Evolution of *Escherichia Coli* to 42 °C and Subsequent Genetic Engineering Reveals Adaptive Mechanisms and Novel Mutations.” *Molecular Biology and Evolution* 31 (10): 2647–62. https://doi.org/10.1093/molbev/msu209.

Schlüter, Lothar, Kai T. Lohbeck, Magdalena A. Gutowska, Joachim P. Gröger, Ulf Riebesell, and Thorsten B. H. Reusch. 2014. “Adaptation of a Globally Important Coccolithophore to Ocean Warming and Acidification.” *Nature Climate Change* 4 (11): 1024–30. https://doi.org/10.1038/nclimate2379.
Schneider, W R, and Raymond N Doetsch. 1977. “Temperature Effects on Bacterial Movement.” Applied and Environmental Microbiology 34 (6): 695–700.

Shi, Bihong, and Xuhua Xia. 2003. “Morphological Changes of Pseudomonas Pseudoalcaligenes in Response to Temperature Selection.” Current Microbiology 46 (2): 120–23. https://doi.org/10.1007/s00284-002-3824-4.

Singh, Brajesh K., Richard D. Bardgett, Pete Smith, and Dave S. Reay. 2010. “Microorganisms and Climate Change: Terrestrial Feedbacks and Mitigation Options.” Nature Reviews Microbiology 8 (11): 779–90. https://doi.org/10.1038/nrmicro2439.

Tenaillon, Olivier, Alejandra Rodriguez-Verdugo, Rebecca L. Gaut, Pamela McDonald, Albert F. Bennett, Anthony D. Long, and Brandon S. Gaut. 2012. “The Molecular Diversity of Adaptive Convergence.” Science 335 (6067): 457–61. https://doi.org/10.1126/science.1212986.

Trueba, Frank J., Edwin A. van Spronsen, Jan Traas, and Conrad L. Woldringh. 1982. “Effects of Temperature on the Size and Shape of Escherichia Coli Cells.” Archives of Microbiology 131 (3): 235–40. https://doi.org/10.1007/BF00405885.

Walther, Gian-Reto, Eric Post, Peter Convey, Annette Menzel, Camille Parmesan, Trevor J. C. Beebee, Jean-Marc Fromentin, Ove Hoegh-Guldberg, and Franz Bairlein. 2002. “Ecological Responses to Recent
Climate Change.” *Nature* 416 (6879): 389–95.

https://doi.org/10.1038/416389a.

Walton, B. Michael, Michael A. Gates, Anne Kloos, and JoAnne Fisher. 1995. “Intraspecific Variability in the Thermal Dependence of Locomotion, Population Growth, and Mating in the Ciliated Protist *Euplotes Vannus*.” *Physiological Zoology* 68 (1): 98–113.

https://doi.org/10.1086/physzool.68.1.30163920.

Weisse, Thomas, Peter Stadler, Eva S. Lindström, Susan A. Kimmance, and David J. S. Montagnes. 2002. “Interactive Effect of Temperature and Food Concentration on Growth Rate: A Test Case Using the Small Freshwater Ciliate *Urotricha Farcta*.” *Limnology and Oceanography* 47 (5): 1447–55. https://doi.org/10.4319/lo.2002.47.5.1447.

Williams, Norman E. 2004. “The Epiplasm Gene EPCI Influences Cell Shape and Cortical Pattern in *Tetrahymena Thermophila*.” *Journal of Eukaryotic Microbiology* 51 (2): 201–6. https://doi.org/10.1111/j.1550-7408.2004.tb00546.x.

Wilson, Robbie S., and Craig E. Franklin. 2002. “Testing the Beneficial Acclimation Hypothesis.” *Trends in Ecology & Evolution* 17 (2): 66–70.

https://doi.org/10.1016/S0169-5347(01)02384-9.

Yampolsky, Lev Y., Tobias M. M. Schaer, and Dieter Ebert. 2013. “Adaptive Phenotypic Plasticity and Local Adaptation for Temperature Tolerance in Freshwater Zooplankton.” *Proceedings of the Royal Society B:*
Zuo, Wenyun, Melanie E. Moses, Geoffrey B. West, Chen Hou, and James H. Brown. 2012. “A General Model for Effects of Temperature on Ectotherm Ontogenetic Growth and Development.” *Proceedings of the Royal Society B: Biological Sciences* 279 (1734): 1840–46.

https://doi.org/10.1098/rspb.2011.2000.

Zwietering, Marcel H., Ida Jongenburger, Frank M. Rombouts, and Klaas van 't Riet. 1990. “Modeling of the Bacterial Growth Curve.” *Applied and Environmental Microbiology* 56 (6): 1875–81.

https://doi.org/10.1128/AEM.56.6.1875-1881.1990.
Figure 1 Population dynamics of *T. thermophila* strain 1630/1U growing in ten different temperatures. Each line represents one replicate population and the colors indicate the temperature in which the population was grown.
Figure 2 Image of the ciliate *Tetrahymena thermophila* (A) and experimental design of the temperature experiment (B). In the schematic of the experiment, each bottle represents one replicate batch culture, and the colors indicate the temperature in which the culture was grown. (A) Image credit: Dr. Aaron J. Bell
Figure 3 Population dynamics and morphological traits of each *T. thermophila* population during the temperature experiment. Population density (A), mean cell size (B), variance of cell size (C), coefficient of variation of cell size (D) and mean cell shape (E) are shown for each population and for each batch separately. Minimum number of generations that took place in each batch is shown in boxes in plot A. Error bars indicate standard errors of means for cell size and cell shape (B and E). The colors indicate the temperature in which the population was grown, and the shade represents the population replicate. Dashed lines mark the range of observed values at the control temperature (20 °C) in the first batch of the experiment.
Figure 4 Growth dynamics of *T. thermophila* during the temperature experiment. Gompertz model was used to estimate demographic parameters.

(A) Points show population abundances and lines show the fitting of the model. No model fit is shown for population 3 in batch 4 at 38 °C since growth
parameters were manually calculated (see methods). Maximum growth rate (B) and lag phase (C) of each *T. thermophila* population per batch culture. In all plots, the colors indicate the temperature in which the population was grown, and the shades represent the population replicate. The lag phases in batch 1 are estimated as smaller than $1 \times 10^{-5}$ and are not visible in the plot.

Figure 5 Change in population dynamics and morphological traits of *T. thermophila* populations. Change in maximum growth rate, lag phase, cell size, variance of cell size, coefficient of variation (CV) of cell size and cell shape are shown, for each population and for each batch separately, using the control
cultures at 20 °C in batch 1 as a reference. Maximum growth rate difference is expressed in day\(^{-1}\) and lag phase difference is expressed in days, while all other values are expressed as percent difference. The lines represent the fitted mixed effects models and the shaded areas represent the 95 % credible interval (see methods for details). The colors indicate the temperature in which the population was grown, and the shades represent the population replicate. The dashed lines mark no change in comparison to the control.