Phenotypic plasticity and the effects of thermal fluctuations on specialists and generalists

Staffan Jacob, Léonard Dupont, Bart Haegeman, Mélanie Thierry, Julie Campana, Delphine Legrand, Julien Cote, Allan Raffard

To cite this version:

Staffan Jacob, Léonard Dupont, Bart Haegeman, Mélanie Thierry, Julie Campana, et al.. Phenotypic plasticity and the effects of thermal fluctuations on specialists and generalists. Proceedings of the Royal Society B: Biological Sciences, 2024, 291 (2025), 10.1098/rspb.2024.0256 . hal-04675396

HAL Id: hal-04675396
https://hal.inrae.fr/hal-04675396v1

Submitted on 11 Oct 2024

Phenotypic plasticity and the effects of thermal fluctuations on specialists and generalists

Staffan Jacob[1], Léonard Dupont[1], Bart Haegeman[2], Mélanie Thierry[1], Julie L. M. Campana[1], Delphine Legrand[1], Julien Cote[3], Allan Raffard[4*]

[1] Station d'Écologie Théorique et Expérimentale, UAR2029, CNRS, 09200, Moulis, France.

[2] CNRS/Sorbonne Université, UMR7621 Laboratoire d'Océanographie Microbienne, Banyuls-sur-Mer, France

[3] CNRS, UPS, IRD, Laboratoire Évolution et Diversité Biologique, UAR 5174, CNRS, UPS, IRD, 31062, Cedex 9 Toulouse, France

[4] Université catholique de Louvain, Earth and Life Institute, Biodiversity Research Centre, Louvain-la-Neuve, Belgium

[*] present address: Univ. Savoie Mont Blanc, INRAE, CARRTEL, 74200 Thonon-les-Bains, France

Corresponding author: *Staffan Jacob: staffan.jacob@sete.cnrs.fr*

Author contribution: Conceptualization: SJ, LD, BH, MT, DL, JC, AR; Data curation: SJ; Formal analysis: SJ, BH, MT, AR; Funding acquisition: SJ; Investigation: SJ, JLMC, AR; Methodology: SJ, AR; Project administration; SJ, DL; Resources: SJ, BH, MT, DL, AR; Software: SJ, BH, MT, AR; Validation: SJ; Visualization: SJ, BH, AR; Writing—original draft: SJ; Writing—review & editing: LD, BH, MT, JLMC, DL, JC, AR.

Data availability: The data supporting the findings of this study are available on Dryad: https://doi.org/10.5061/dryad.z612jm6k7

Acknowledgments

We thank Hervé Philippe for discussions about the concepts underlying this study. This work benefitted from financial support by the Agence Nationale de la Recherche for the projects CHOOSE (ANR-19-CE02-0016) and POLLUCLIM (ANR-19-CE02-0021-01), and is part of TULIP (Laboratory of Excellence Grant ANR-10 LABX-41). AR was supported by a F.R.S-FNRS research project (PDR T.0211.19) at the time of this study, and JCo by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 817779).

Type of article: Article

Counts: abstract 196; main text 3977; 3 figures

Keywords: Thermal tolerance, Phenotypic plasticity, Acclimation, Heat tolerance, Environmental fluctuations, protist

Abstract

Classical theories predict that relatively constant environments should generally favor specialists, while fluctuating environments should select for generalists. However, theoretical and empirical results have pointed out that generalist organisms might on the contrary perform poorly under fluctuations. In particular, if generalism is underlaid by phenotypic plasticity, performance of generalists should be modulated by the temporal characteristics of environmental fluctuations. Here, we used experiments in microcosms of *Tetrahymena thermophila* ciliates and a mathematical model to test whether the period or autocorrelation of thermal fluctuations mediate links between the level of generalism and the performance of organisms under fluctuations. In the experiment thermal fluctuations consistently impeded performance compared to constant conditions. However, the intensity of this effect depended on the level of generalism: while the more specialists strains performed better under fast or negatively autocorrelated fluctuations, plastic generalists performed better under slow or positively autocorrelated fluctuations. Our model suggests that these effects of fluctuations on organisms' performance may result from a time delay in the expression of plasticity, restricting its benefits to slow-enough fluctuations. This study points out the need to further investigate the temporal dynamics of phenotypic plasticity to better predict its fitness consequences under environmental fluctuations.

Introduction

Organisms inhabit environments that are constantly changing, leading to variations of selective pressures affecting their performance, and consequently their ecology and evolution [1]. Given the ubiquity of environmental fluctuations, understanding how organisms deal with such changing conditions has attracted much attention [2,3]. A common first step in this investigation process consists in quantifying tolerance curves, *i.e.,* variations in how organisms perform across a gradient of environmental conditions [4–6]. Tolerance curves allow to place organisms on a continuum ranging from specialists to generalists depending on the breadth of environmental conditions they manage to live in (*i.e.,* their niche width).

Theory classically predicts that constant environments should favor specialists, while organisms able to tolerate a broader range of environmental conditions should be selected in fluctuating environments [7–11]. However, empirical and theoretical studies have revealed that generalism is not always favored under environmental fluctuations [1,6,12–14]. This discrepancy suggests that evolutionary strategies providing fitness benefits under constant conditions may be neutral or even disadvantageous in fluctuating environments, and conversely [5,6,11,15]. For instance, Botero and colleagues [11] showed that the evolution of adaptive mechanisms underlying tolerance curves (*i.e.,* phenotypic plasticity, bet-hedging, adaptive tracking) should depend on the characteristics of environmental fluctuations, *i.e.,* the predictability and timescale of fluctuations relative to generation time. Especially, phenotypic plasticity, the ability of a given genotype to produce multiple phenotypes depending on the environment, is classically considered to underlie the degree of generalism or organisms, and requires environmental changes to evolve [11,16–19].

However, whether generalist organisms perform well in fluctuating environments should depend on the interaction between the characteristics of fluctuations and the mechanisms underlying generalism. In the case of phenotypic plasticity, implementing changes in trait expression most often takes time and notably depends on the speed at which underlying mechanisms occurs (*i.e.,* the rate of plasticity; [20–26]). Consequently, if generalism results from adaptive plastic changes occurring at a rate below that of environmental fluctuations, generalist organisms might attain only low performance under temporally fluctuating conditions [6,13,20,25–27]. Although considered in some theoretical works (*e.g.,* [19,20,28,29]), whether the rate of plastic changes could determine the effects of environmental fluctuations on how generalists and specialists perform remains experimentally unexplored [26].

In this study, we used experiments in microcosms and a mathematical model to explore how phenotypic plasticity affects the relationship between the degree of generalism and performance under thermal fluctuations. We performed experiments using 15 strains of the ciliate *Tetrahymena thermophila* that differ in their degree of thermal generalism and capacity of phenotypic plasticity for morphological and movement traits [30,31] (Figure 1). Cell morphology (cell size) was previously related to resource acquisition and metabolic rate in protists [32], while cell movement (velocity) tends to be associated with dispersal [33,34]. We first tested whether the strains' level of generalism correlated with morphological and movement plasticity. Then, each isolated strain was independently exposed to two fluctuation gradients: the first varied in the timescale of fluctuations (*i.e.,* period) and the second in the temporal autocorrelation of fluctuations (*i.e.,* as a proxy of predictability [2,18]; Figure 1A), with a timing of fluctuation centered on average generation time. Phenotypically-plastic generalists are expected to perform better under rapidly changing thermal conditions (*i.e.,* low period), and to be favored by positively autocorrelated fluctuations (*i.e.,* predictable) compared to less plastic genotypes. However, if phenotypic plasticity incurs time delays larger than the rate of environmental changes, fluctuations might become detrimental for plastic generalists [26]. Finally, we used a model to test whether effects of fluctuations on the performance of specialists and generalists can result from a rate of plasticity. We incorporated the temporal dynamics of the plastic response into a simple model of tolerance to fluctuations and compared the predictions to the effects of fluctuations on performance found in the experiment.

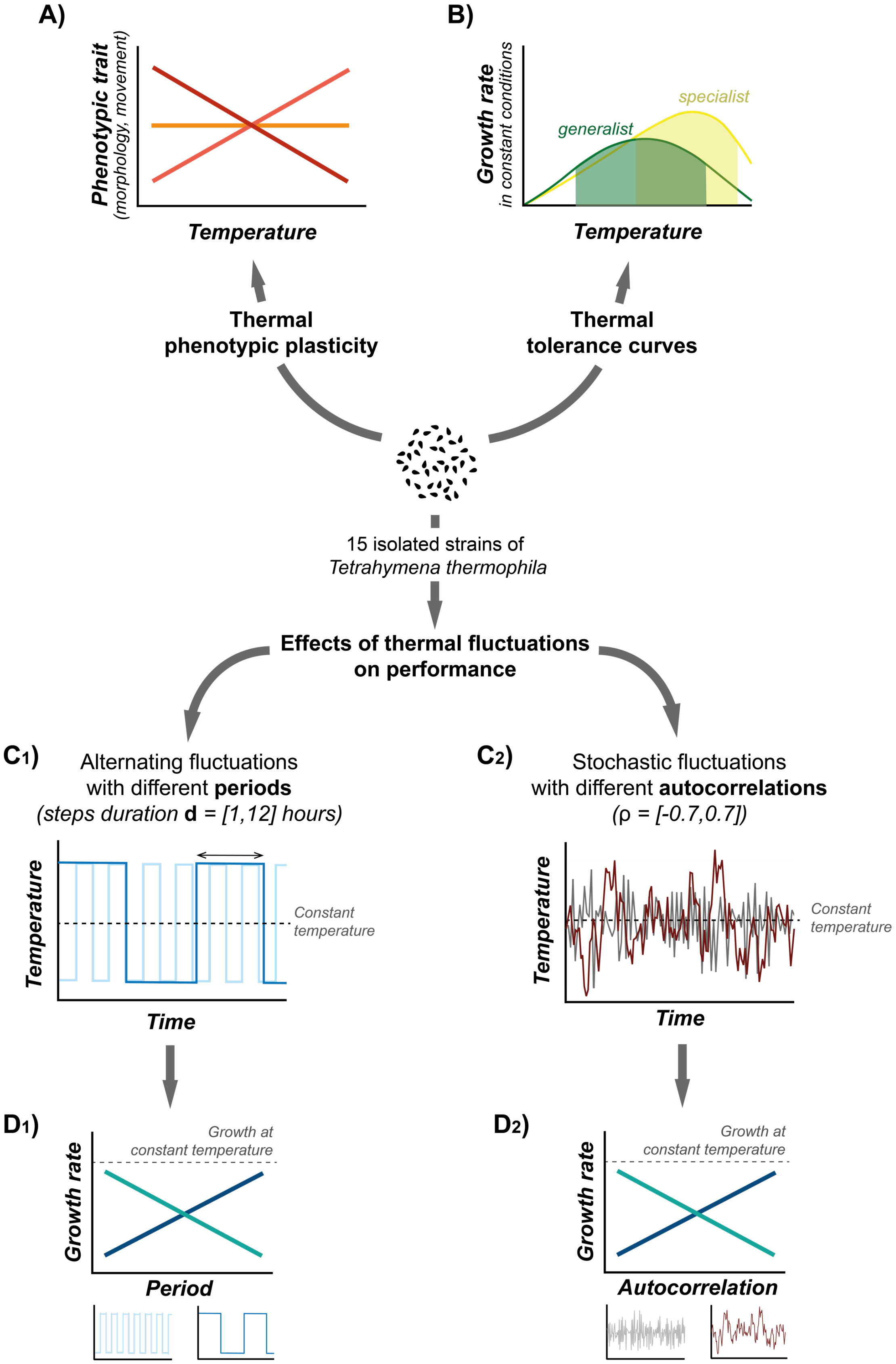
Methods

Study system

Tetrahymena thermophila is a 20 to 50µm ciliate naturally living in freshwater ponds and streams [35,36]. Previous studies provided evidence for differences between genotypes in thermal tolerance curves [30,31] and phenotypic plasticity of morphological and movements traits [30,31,37–39]. Moreover, thermal fluctuations are known in this species to affect population dynamics and the evolution of heat shock protein Hsp90 expression [40]. Here we used 15 strains originally sampled in the early 2000' from different locations in North America [41]. Isogenic strains reproduce clonally in laboratory conditions, meaning that for a given clonal strain, differences in trait values after two hours between replicated environmental conditions result from the expression of phenotypic plasticity [31,39,42]. Cells were maintained in axenic liquid growth media (0.6% Difco proteose peptone, 0.06% yeast extract) at 23°C, a classic laboratory maintenance condition for this species [43,44]. All manipulations were performed in sterile conditions under a laminar flow hood.

Figure 1: **Illustration of the key steps of the experimental design**. Using 15 isolated strains of *T. thermophila*, we quantified (A) morphological and movement plasticity following two hours of exposure to a gradient of thermal conditions to reconstruct thermal reaction norms for each trait and (B) tolerance curves across constant temperatures. Colors in A) and B) illustrate a diversity of possible forms of plasticity and tolerance curves expected based on previous studies [30,31] (see Figure S1). The same 15 isolated strains were separately exposed for two weeks to gradients of either period (C_1) or autocorrelation (C_2) of thermal fluctuations (period: from 1 to 12 hours; autocorrelation: from -0.7 to 0.7 with changes every 3 hours; average generation time across genotypes and

temperatures: 3 to 8 hours). During the two weeks of thermal fluctuations, population growth was measured to quantify the effects of fluctuation period (D_1) and autocorrelation (D_2) on population growth (*i.e.,* growth rate and maximal density).



Growth along gradients of fluctuation period and autocorrelation

We quantified the influence of thermal fluctuations on the 15 isolated genotypes of *T. thermophila* by inoculating a small number of cells (~100) from each isolated strain into 250 µL of growth media in 96-well plates, and exposing them for two weeks to treatments of different fluctuation period and autocorrelation (Figure 1).

First, we quantified the role of fluctuation period by setting up a regime of alternating temperatures: 19 and 31°C, corresponding to the margins of 80% of the area under a Gaussian distribution representative of averaged thermal tolerance curve in this species [45]. We generated a gradient of fluctuation period from 1 to 12 hours (1, 2, 3, 4, 6, 8, 10, 12h; the average generation time of *T. thermophila* is ~3-8h in our experimental conditions [45] and depends on temperature; Fig. S1C). We performed three replicates per strain and fluctuating period. Second, we defined a gradient of fluctuation autocorrelation by generating time sequences where temperature changed every 3h, was distributed following a Gaussian distribution of mean 25°C, and was comprised between 11 and 39°C (considered as maximal viable margins). We defined two negatively autocorrelated fluctuation regimes (-0.7 and -0.4), one without temporal autocorrelation (autocorrelation = 0), and two positively autocorrelated regimes (0.4 and 0.7) by generating 10,000 time-series for each autocorrelation value, and selecting the series that best matched the requirements (mean, autocorrelation and variance of temperature through time). To avoid time series where parameters may change through the growth phase (*e.g.,* with lower mean value during earlier growth than at stationary phase [46]), we performed the selection of best matches by computing desired parameters for each 24h time window. As for the fluctuation period, we performed three replicates per strain and level of autocorrelation.

We quantified population growth rate, a classic proxy of fitness, through absorbance measurements at 450nm using a microplate reader (TECAN Infinite 200) twice a day until the stationary phase was reached. Growth curves were smoothed using General Additive Modelling (GAM; *gam* R-package [47]) to avoid any bias due to slight technical variability in absorbance measurements. For each strain and each fluctuation treatment, we computed performance using the *growth rate* measured as the maximum slope of population growth using the *gcfit* function (*grofit* R-package [48]) with spline fit. We additionally quantified the *maximal population density* reached at the plateau, which was highly correlated to growth rate (Pearson correlation: 0.893; df=583; t=47.91; p<0.001) and was therefore not included in the following analyses, thus focusing on the exponential phase.

Thermal tolerance curves

We additionally reconstructed thermal tolerance curves (Figure 1) by quantifying the growth rate of each isolated strain across a gradient of eight constant temperatures (11, 15, 19, 23, 27, 31, 35, 39°C; Figure S1), as done previously (*e.g.,* [30,31]). We quantified population growth through absorbance measurements as explained for growth under fluctuations: ~100 cells from each genotype into 250 µL of 96-well plates. For this part, we performed four replicates in different plates for each temperature, each being technically duplicated on each plate that were latter on averaged for analyses. We fitted the relationship between temperature and growth rate using GAMs, and computed the width of thermal tolerance curves as 90% of the area under the curve (other cutoffs leading to similar results [30]). We furthermore quantified the thermal optimum as the temperature corresponding to maximal growth rate, which did not significantly correlate with thermal niche width (Pearson correlation coefficient = -0.241; t = -0.894, p = 0.388).

Thermal plasticity

We quantified reaction norms of cell morphology and movement following [31], by exposing five replicates of one-week old cultures (close to asymptotic density) from each strain for two hours (less than the generation time) to five different temperatures: 11, 19, 25, 31 and 39°C (Figure 1). Immediately after the two-hour exposure, we recorded 20s videos of two samples of 10μl of cells placed in counting slides under dark-field microscopy to measure cell morphology and movement characteristics using the BEMOVI R-package [49]. We described cell morphology as cell *size* (measured as mean cell area in videos), a commonly measured trait known to be linked to resource acquisition and metabolic rate in protists [32]. In standard conditions, variability of cell size among strains in this species is not significantly correlated to population growth rate [50,51]. Cell movement was measured as *velocity*, defined as the total distance travelled by cells divided by the duration of the trajectory, a trait classically used to describe movement in microorganisms [33,34]. Averaged cell size and velocity across all cells of each experimental replicate (*i.e.,* two videos, see above) were then used to compute the morphological and movement thermal plasticity of each strain as the slope of the reaction norm of the scaled trait along temperature [31] (Figure S1). We summarized plasticity through linear slopes since quadratic relationships were not significant (morphology: temperature^2 x strain: $F_{14,171} = 1.192$; $p = 0.286$; temperature^2: $F_{1,171} = 0.018$; $p = 0.894$; movement: temperature^2 x strain: $F_{14,171} = 1.380$; $p = 0.168$; temperature^2: $F_{1,171} = 0.003$; $p = 0.987$). Slopes close to zero indicate flat reaction norms (and hence, no plasticity), while positive or negative values respectively denote increase and decrease in traits along temperature (Figure S1).

Statistical analyses

We first tested whether tolerance curve width correlated with the plasticity of morphological and movement traits using linear regressions (*lm* function, with 1/standard error of the reaction norm slope as weights). Then, we tested for the role of thermal tolerance width in response to thermal fluctuations using linear simple and mixed models, separately for periodic and autocorrelated fluctuations. First, we used the growth rate under fluctuations relative to constant temperature as dependent variable in models, allowing to quantify the effects of thermal fluctuations independently from differences of mean growth rate among genotypes using linear models. Second, we tested for effects of thermal tolerance width, thermal fluctuations (either period or autocorrelation) and their interaction on growth rate under fluctuations using linear mixed models with strain as a random factor. All analyses were performed using R (version 4.1.0; R Core Team 2021).

Model

We investigated how the rate of phenotypic plasticity affects growth in fluctuating environments by modifying the model of [19]. This model describes a population of N individuals experiencing a time-varying environment E, here temperature. Their phenotype P, the same for all individuals, varies as a deterministic function of the variation of the environment (*i.e.,* reversible plasticity).

The fitness landscape specifies the instantaneous growth rate $r(P,E)$ of the population with phenotype P at temperature E. Population growth is assumed density-independent:

$$\frac{dN}{dt} = r(P,E)N \qquad (1)$$

For a fixed temperature E, the growth rate is maximal at a specific phenotype $P = \varphi(E)$. Away from this optimal phenotype the growth rate decreases quadratically (see supplementary material).

We incorporated into this model a rate of plasticity, specifying the dynamical response of the phenotype to the thermal fluctuations:

$$\frac{dP}{dt} = -\frac{1}{\tau_P}(P - \psi(E)) \qquad (2)$$

where $\psi(E)$ is the target phenotype at a constant temperature E, and τ_P is the time-lag of the plasticity. The function $\psi(E)$ determines the reaction norm, and together with the fitness landscape $r(P,E)$ the thermal performance curve $r(\psi(E),E)$. In particular, for generalists the reaction norm $\psi(E)$ is close to the optimal phenotype $\varphi(E)$, leading to a wide thermal niche (see supplementary material).

For simplicity, we considered periodic thermal fluctuations with a sine wave:

$$E(t) = c_E + a_E \sin(2\pi \frac{t}{\tau_E}) \qquad (3)$$

where c_E is the mean temperature, a_E the amplitude and τ_E the period of the fluctuations. By combining equations (1-3) and averaging the instantaneous growth rate r over time, we obtained the long-term population growth rate R (see supplementary material):

$$R = c_R - a_R \frac{(1-a_\psi)^2+(2\pi\frac{\tau_P}{\tau_E})^2}{1+(2\pi\frac{\tau_P}{\tau_E})^2} a_E^2 \qquad (4)$$

where c_R is the growth rate in the constant environment c_E, and a_R is a positive constant independent of the degree of generalism a_ψ.

We used equation (4) to construct tolerance curves for generalists and specialists, characterized by a high and low degree of plasticity $a\psi$, respectively. This allowed us to investigate how the rate of plasticity affects the relationship between fluctuation period τ_E and growth rate R in generalists and specialists.

The model predicts an increase of R along τ_E both for generalists and specialists. Since the experimental results showed that the relationship between growth rate and fluctuation period can also decrease in specialists (Figure 2), we added to the model a mechanism that can generate such a pattern: we assumed that the transmission of the temperature to the individuals is buffered by a thermal inertia. The simplest model for thermal transmission with inertia is:

$$\frac{dE'}{dt} = -\frac{1}{\tau_I}(E' - E) \qquad (5)$$

where E is the requested temperature (e.g., the temperature set on the incubator), E' is the body temperature of the individuals, and τ_I is the time-lag of the thermal transmission. The transmitted fluctuations E' can differ substantially from the intended fluctuations E if τ_I is comparable or larger than τ_E. For the sine-wave fluctuations considered above, the reduction in amplitude from the requested E to the transmitted E' is given by:

$$\frac{a_{E'}}{a_E} = \frac{1}{\sqrt{1 + \left(2\pi\frac{\tau_I}{\tau_E}\right)^2}}$$

The long-term population growth rate becomes:

$$R = c_R - a_R \frac{(1-a_\psi)^2+(2\pi\frac{\tau_P}{\tau_E})^2}{1+(2\pi\frac{\tau_P}{\tau_E})^2} \frac{a_E^2}{1+\left(2\pi\frac{\tau_I}{\tau_E}\right)^2} \qquad (6)$$

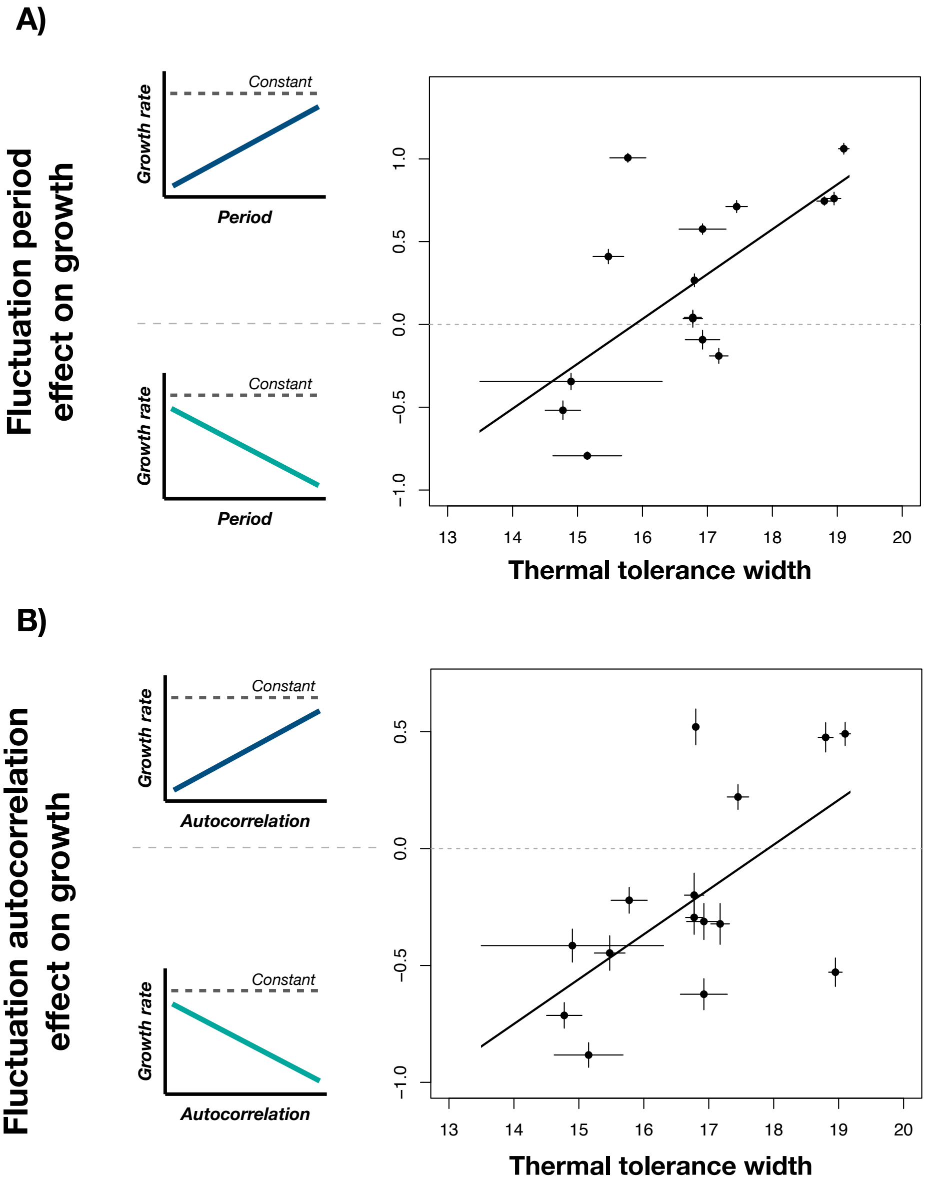
Results

We first quantified the width of thermal tolerance curves and the plastic capacity of 15 strains of the ciliate *T. thermophila* (Figure S1). We refer to strains with broader tolerance curves as the most generalists, and to those with narrower thermal tolerance as the most specialists. The level of thermal generalism of strains was positively correlated with cell size plasticity (estimate ± SE = 0.170 ± 0.053; df = 1,13; t = 3.222; p = 0.007), but not with the plasticity of cell velocity (-0.044 ± 0.063; df = 1,13; t = -0.688; p = 0.503). The most generalist strains showed higher size plasticity with positive reaction norm slopes (*i.e.,* cells became larger with increasing temperature), while most specialists strains appeared less plastic or even showed negative slopes (Figure S2). Note that strains' plasticity did not significantly correlate with thermal optimum (-0.090; t = -0.327, p = 0.749).

We then separately exposed the 15 strains to two independent gradients of period and temporal autocorrelation of thermal fluctuations (Figure 1). Fluctuations impeded the growth of all strains compared to constant conditions: when averaged across all period and autocorrelation levels, growth rates were respectively reduced by 66.5 ± 1.6% (mean ± SE; period), and by 59.1 ± 2.5% (autocorrelation; Figure S3). This averaged sensitivity of strains to thermal fluctuations did not significantly correlate with their degree of generalism (periodic fluctuations: -0.004 ± 0.009; df = 1,358; t = -0.484; p = 0.629; autocorrelated fluctuations: -0.013 ± 0.014; df = 1,223; t = -0.952; p = 0.342).

However, the extent to which the period and autocorrelation of fluctuations impeded performance significantly depended on the degree of generalism (tolerance curve width * period: $F_{1,343} = 38.234$; $p < 0.001$; tolerance curve width * autocorrelation: $F_{1,208} = 9.912$; p = 0.002; Figure 2). Specifically, the effect of the fluctuation period on performance reversed along the degree of thermal specialization (Figure 2A). The most specialist strains (*i.e.,* with narrowest thermal tolerance) performed better under fast fluctuations compared to slower ones (*i.e.,* negative effect of fluctuation period on growth rate; Figure 2A). Conversely, the most generalist strains performed better under slow fluctuations (*i.e.,* positive effect of period on growth rate; Figure 2A). Similarly, the effect of autocorrelation depended on thermal tolerance width: the most specialist strains showed higher growth rates under negatively autocorrelated fluctuations compared to positively autocorrelated ones, while the most generalist ones performed better under positively autocorrelated fluctuations (Figure 2B). As expected from the correlation between thermal generalism and morphological plasticity, equivalent analyses using phenotypic plasticity of cell size instead of thermal tolerance width as explanatory variable gave similar results (fluctuation period * morphological plasticity: $F_{1,356} = 24.048$; $p < 0.001$; fluctuation autocorrelation * morphological plasticity: $F_{1,221} = 6.114$; p = 0.014). Finally, the interactions between fluctuation period or autocorrelation and thermal optimum had non-significant effects on growth rate (thermal optimum * period: $F_{1,343} = 0.074$; p = 0.787; thermal optimum * autocorrelation: $F_{1,208} = 2.070$; p = 0.152).

Figure 2: **Relationships between tolerance width and effects of fluctuation period (A) and autocorrelation (B) on performance (*i.e.,* growth rate)**. Each point in the main plots corresponds to a strain, with bars showing standard error. Values on the y-axis are the effect of fluctuation period or autocorrelation on growth, measured as the slope of the relationship between fluctuations and growth (either positive or negative, as illustrated by the schematic relationships on the left of the axes; see also Figure S3). They are computed as a *Z*-score effect size derived from the linear relationship between growth rate and the fluctuation gradients [52]: positive values indicate that strains are more affected by fast fluctuations compared to slow ones, or by negatively autocorrelated fluctuations compared to positively autocorrelated, while negative values show that strains are more affected by slow or positively autocorrelated fluctuations.

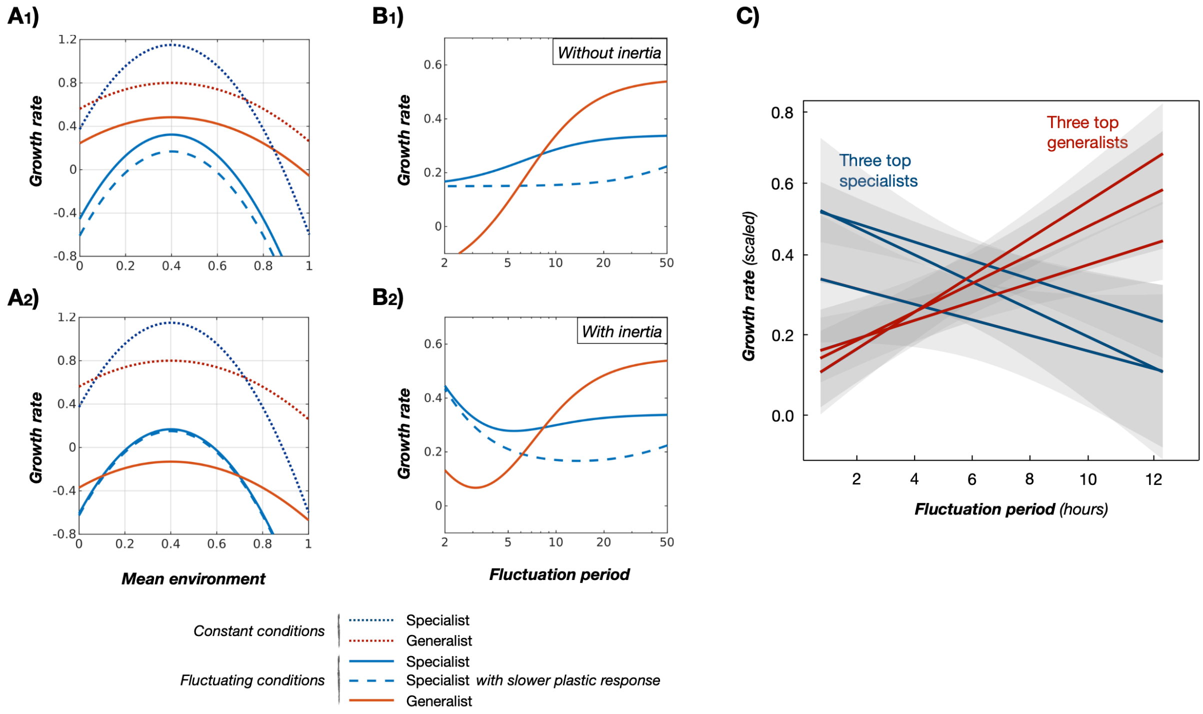


To explicitly test the potential role of the speed of plasticity in how organisms responded to environmental fluctuations, we used a simple model that included a rate in the adaptive plastic response underlying tolerance curves. We restricted the model to a simple gradient of fluctuation period, since period and autocorrelation of fluctuations led to similar effects in the experiment and gave similar results in the model (Figure 2). In the model, generalists have steeper reaction norms (*i.e.,* higher plasticity capacity) and therefore reached wider tolerance curves but with a reduced maximal performance compared to specialists (here due to a cost of plasticity; Figure 3A_1). This leads to a classical specialist-generalist trade-off [53], already known in the experimental system we used in this study [30].

In slowly fluctuating environments, specialists were more affected by fluctuations compared to constant environments than generalists: because their tolerance curve is narrower, excursions from their optimal environment led to stronger fitness reductions (Figure 3A_1). Without a rate of plasticity (*i.e.,* in a case of immediate plasticity), the fluctuation period had no effect at all on generalist growth rates (Figure 3). When the rapidity of environmental fluctuations increased, the rate of plasticity constrained the expression of adaptive plasticity to lesser degrees, leading generalists to suffer stronger fitness reduction compared to specialists (Figure 3A_2). This trend reproduced what we observed in the experiment, where generalists suffered more from fast fluctuations than slower ones (Figure 3C).

It however did not reproduce the pattern of decreasing growth rates along increasing fluctuation period, as observed for specialists in the experiment. One simple mechanism that might generate such a pattern is environmental inertia, which would buffer to some degree the effects of rapid fluctuations on organisms. Implementing this simple mechanism in the model (see Methods) resulted in a negative relationship between growth rate and fluctuation period in specialists for fast fluctuations, while the positive relationship in generalists remained unchanged (Figure 3B_2). Interestingly, this decrease in specialists was even steeper if we considered that, in addition to their smaller degree of plasticity, specialists also had a slower plastic response than generalists (*i.e.,* slow rate of plasticity, as expected if plasticity rate and capacity correlate [26]; Figure 3B$_2$).

Figure 3: **The differential effects of environmental fluctuations on the performance of specialists and generalists depend on the underlying rate of phenotypic plasticity**. In the mathematical model, tolerance curves of specialists and generalists along the gradient of mean environments (*i.e.,* either constant temperatures or means of temporally fluctuating conditions) are modified by environmental fluctuations. **A$_1$)** while the maximal performance of specialists was higher than for generalists in a constant environment (respectively dotted blue and red lines), generalists reached higher maximal performance in environments fluctuating relatively slowly (high period: $\tau_E = 20$, solid red and blue curves); **A$_2$)** in rapidly fluctuating environments ($\tau_E = 2$), generalists suffered stronger fitness reduction due to not fast enough plastic response compared to specialists; **B$_1$)** In slowly fluctuating environments (i.e., long fluctuation period), specialists were more affected by fluctuations than generalists because deviations from their optimal environment lead to stronger fitness reductions. When fluctuations became faster (i.e., low period), phenotypic plasticity was not fast enough to follow environmental fluctuations, which is particularly detrimental for generalists. In a case of immediate plasticity (*i.e.,* infinite rate), there was no effect of the fluctuation period on growth rate. **B$_2$)** When environmental fluctuations are to some extent buffered as in the case of a thermal inertia, very fast fluctuations had small effects on fitness compared to constant conditions. When fluctuation period increased, generalists again showed increasing fitness as without inertia. However, specialists now showed decreasing fitness with increasing fluctuation period, as observed in the experiment (see panel C). This negative relationship was steeper if we assumed that specialists also showed slower plastic response than generalists (dashed blue line). **C)** Illustration of growth rate variations along fluctuation period in the three most specialist (red) and generalist (blue) genotypes (see Figure S3 for all strains). As expected with a lag time of phenotypic plasticity, generalists performed better under slow fluctuations compared to fast ones.



Discussion

Although fluctuating environmental conditions are a necessary condition for plastic generalists to evolve [7–11], some theoretical and empirical studies showed that generalists able to live in a wide range of relatively stable conditions might in contrast perform badly under fluctuations [1,6,54]. In this study, we experimentally showed that while thermal fluctuations always decreased performance relative to constant conditions, the magnitude and direction of their effects depended on the interaction between the width of tolerance curves and the characteristics of fluctuations. In particular, the most specialist strains performed better under fast or negatively autocorrelated fluctuations, while the most generalist strains performed better under slower or positively autocorrelated fluctuations. Using a mathematical model, we showed that a time delay in the expression of phenotypic plasticity can generate such effects of fluctuations on organisms' performance.

Strains with broader thermal tolerance curves showed higher morphological plasticity with positive reaction norm slopes (cells became larger with increasing temperature). On the contrary, the most specialist strains appeared less morphologically plastic, or became smaller with increasing temperatures. Our results thus provide correlative support for the hypothesis that the cell-size plasticity in response to temperature may underlie part of the ability to tolerate broad thermal conditions. Incidentally, the link between temperature sensitivity and body size is at the core of the metabolic theory in ecology [55]. Body size is also commonly related to demography and species interactions [55–57], including in protists [32,57,58]. However, whether cell size plasticity is adaptive, neutral or maladaptive in *T. thermophila* and other ciliates, and whether and how it might affect species interactions are still unsolved questions [31]. Answering them would especially require establishing causal relationships between cell size, thermal tolerance and how organisms perform under a diversity of environmental fluctuation scenarios.

If generalism is achieved through phenotypic plasticity as suggested in this study, the performance of generalists should depend on the characteristics of fluctuations, and especially their rapidity [1,6,54]. In the experiment, the most generalist strains suffered more from fast fluctuations than from slow ones, the reverse being true for specialists. This pattern might have resulted from the existence of a rate of plasticity [25,26]. Changes of performance metrics across a given environmental gradient (*i.e.,* tolerance curves) indeed often depend on acute plastic responses and acclimation mechanisms, either adaptive or not [1,59]. These plastic responses to changes in environmental conditions likely takes some amount of time [1,21–23,25–27]. This rate of phenotypic change may for instance depend on the rapidity of underlying mechanisms, such as transcriptional or hormonal changes, that precede variations in the phenotypic traits of interest [25,26]. Our model accordingly suggested that a time delay in the expression of phenotypic plasticity may restrict its benefits to slow-enough fluctuations.

An organism with a broad tolerance curve under a range of constant conditions might thus perform badly under too-rapid fluctuations if the underlying mechanisms involve significant time delays relative to the speed of environmental changes [6,13,25–27]. These results point out that considering the rate at which phenotypic plasticity takes place, together with the rate of environmental changes, is key to understand the conditions under which phenotypic plasticity is expected to be favored [25,26].

Interestingly, generalists performed better under positively autocorrelated fluctuations compared to negatively autocorrelated ones, and the reverse for specialists. These results therefore match with the general expectation that plasticity should be beneficial in predictable environments (*sensus* positively autocorrelated), as recently demonstrated experimentally [18]. Yet, positively autocorrelated fluctuations do not only translate into environmental predictability: they are also associated with a reduced degree of environmental change through time, which somehow leads to the perception of slower fluctuations than non-autocorrelated or negatively autocorrelated fluctuations. In our experiment, generalists performed better in both slow fluctuations and positively autocorrelated ones. Our results thus confirm that is that the predictability of environmental fluctuations is probably not an intrinsic property of the environmental fluctuations alone, but should rather be understood relative to the considered organisms, and especially to their rate of phenotypic plasticity [25,26].

To conclude, our study revealed that the effect of fluctuations on performance depended on the width of thermal tolerance curves: plastic generalists performing better under slow or positively autocorrelated fluctuations became poor performers under fast and negatively autocorrelated fluctuations. As reminded by our model, such dependence to fluctuations may result from the temporal dynamics of phenotypic plasticity. The speed of plasticity might thus play a major role in organisms’ response to environmental fluctuations. A better understanding of the relationship between classical measures of generalism and the response of organisms to environmental fluctuations would thus require investigating the temporal dynamics of plasticity [25,26]. Whether phenotypic plasticity and the associated width of tolerance curves are adaptive strategies to face environmental fluctuations [5,6,15] is likely to depend on the interplay between the characteristics of fluctuations and the speed of phenotypic plasticity [25,26]. Exploring further into the mechanisms that underlie tolerance curves and the timing of phenotypic plasticity is therefore of key importance to understand the response of organisms to the different types of environmental fluctuations they face.

References

1. Schulte PM, Healy TM, Fangue NA. 2011 Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integrative and Comparative Biology* **51**, 691–702. (doi:10.1093/icb/icr097)
2. Ruokolainen L, Lindén A, Kaitala V, Fowler MS. 2009 Ecological and evolutionary dynamics under coloured environmental variation. *Trends in Ecology & Evolution* **24**, 555–563. (doi:10.1016/j.tree.2009.04.009)
3. Laughlin DC, Messier J. 2015 Fitness of multidimensional phenotypes in dynamic adaptive landscapes. *Trends in Ecology & Evolution* **30**, 487–496. (doi:10.1016/j.tree.2015.06.003)
4. Angilletta MJ. 2006 Estimating and comparing thermal performance curves. *Journal of Thermal Biology* **31**, 541–545. (doi:10.1016/j.jtherbio.2006.06.002)
5. Chevin L-M, Lande R, Mace GM. 2010 Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS Biology* **8**, e1000357. (doi:10.1371/journal.pbio.1000357)
6. Ketola T, Kristensen T. 2017 Experimental approaches for testing if tolerance curves are useful for predicting fitness in fluctuating environments. *Frontiers in Ecology and Evolution* **5**, 129.
7. Levins R. 1968 *Evolution in Changing Environments: Some Theoretical Explorations*. Princeton University Press. Princeton NJ.
8. DeWitt T, Scheiner, SM. 2004 *Phenotypic plasticity: functional and conceptual approaches*. Oxford University Press.
9. Duncan AB, Fellous S, Quillery E, Kaltz O. 2011 Adaptation of Paramecium caudatum to variable conditions of temperature stress. *Research in Microbiology* **162**, 939–944. (doi:10.1016/j.resmic.2011.04.012)
10. Condon C, Cooper BS, Yeaman S, Angilletta MJ. 2014 Temporal variation favors the evolution of generalists in experimental populations of </i>Drosophila melanogaster</i>. *Evolution* **68**, 720–728. (doi:10.1111/evo.12296)
11. Botero C, Weissing F, Wright J, Rudenstein D. 2015 Evolutionary tipping points in the capacity to adapt to environmental change. *Proceedings of the National Academy of Sciences* **112**, 184–189.

12. Hughes BS, Cullum AJ, Bennett AF. 2007 An Experimental Evolutionary Study on Adaptation to Temporally Fluctuating pH in *Escherichia coli*. *Physiological and Biochemical Zoology* **80**, 406–421. (doi:10.1086/518353)

13. Ketola T, Saarinen K. 2015 Experimental evolution in fluctuating environments: tolerance measurements at constant temperatures incorrectly predict the ability to tolerate fluctuating temperatures. *Journal of Evolutionary Biology* **28**, 800–806. (doi:10.1111/jeb.12606)

14. Schmalensee L, Gunnarsdottir K, Näslund J, Gottard K, Lehmann P. 2021 Thermal performance under constant temperatures can accurately predict insect development times across naturally variable microclimates. *Ecology Letters* **24**, 1633–1645.

15. Kristensen TN, Hoffmann AA, Overgaard J, Sørensen JG, Hallas R, Loeschcke V. 2008 Costs and benefits of cold acclimation in field-released *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 216–221. (doi:10.1073/pnas.0708074105)

16. Gavrilets S, Scheiner SM. 1993 The genetics of phenotypic plasticity. V. Evolution of reaction norm shape. *Journal of Evolutionary Biology* **6**, 31–48.

17. Lande R. 2009 Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology* **22**, 1435–1446.

18. Leung C, Rescan M, Grulois D, Chevin L. 2020 Reduced phenotypic plasticity evolves in less predictable environments. *Ecology Letters* **23**, 1664–1672. (doi:10.1111/ele.13598)

19. Lande R. 2014 Evolution of phenotypic plasticity and environmental tolerance of a labile quantitative character in a fluctuating environment. *Journal of Evolutionary Biology* **27**, 866–875.

20. Padilla DK, Adolph SC. 1996 Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. *Evolutionary Ecology* **10**, 105–117.

21. DeWitt T, Sih A, Wilson D. 1998 Costs and limits of phenotypic plasticity. *Trends in ecology & evolution* **13**, 77–81.

22. Callahan HS, Maughan H, Steiner UK. 2008 Phenotypic Plasticity, Costs of Phenotypes, and Costs of Plasticity. *Annals of the New York Academy of Sciences* **1133**, 44–66. (doi:10.1196/annals.1438.008)

23. Auld JR, Agrawal AA, Relyea RA. 2010 Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proc. R. Soc. B.* **277**, 503–511. (doi:10.1098/rspb.2009.1355)

24. Kremer C, Fey S, Arellano A, Vasseur D. 2018 Gradual plasticity alters population dynamics in variable environments: thermal acclimation in the green alga Chlamydomonas reinhartdii. *Proceedings of the Royal Society B: Biological Sciences* **285**, 20171942.

25. Burton T, Ratikainen II, Einum S. 2022 Environmental change and the rate of phenotypic plasticity. *Global Change Biology* , gcb.16291. (doi:10.1111/gcb.16291)

26. Dupont L, Thierry M, Zinger L, Legrand D, Jacob S. 2023 Beyond reaction norms: the temporal dynamics of phenotypic plasticity. *Trends in Ecology & Evolution* , S0169534723002252. (doi:10.1016/j.tree.2023.08.014)

27. Fey SB, Kremer CT, Layden TJ, Vasseur DA. 2021 Resolving the consequences of gradual phenotypic plasticity for populations in variable environments. *Ecol Monogr* **91**. (doi:10.1002/ecm.1478)

28. Gabriel W. 2005 How stress selects for reversible phenotypic plasticity. *Journal of Evolutionary Biology* **18**, 873–883.

29. Siljestam M, Östman Ö. 2017 The combined effects of temporal autocorrelation and the costs of plasticity on the evolution of plasticity. *Journal of Evolutionary Biology* **30**, 1361–1371.

30. Jacob S *et al.* 2018 Habitat choice meets thermal specialization: competition with

specialists may drive sub-optimal habitat preferences in generalists. *Proceedings of the National Academy of Sciences* **115**, 11988–11993.

31. Jacob S, Legrand D. 2021 Phenotypic plasticity can reverse the relative extent of intra- and interspecific variability across a thermal gradient. *Proc. R. Soc. B.* **288**, 20210428. (doi:10.1098/rspb.2021.0428)
32. Wieczynski DJ, Singla P, Doan A, Singleton A, Han Z-Y, Votzke S, Yammine A, Gibert JP. 2021 Linking species traits and demography to explain complex temperature responses across levels of organization. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2104863118. (doi:10.1073/pnas.2104863118)
33. Fronhofer EA, Altermatt F. 2015 Eco-evolutionary feedbacks during experimental range expansions. *Nature Communications* **6**, 6844. (doi:10.1038/ncomms7844)
34. Pennekamp F, Clobert J, Schtickzelle N. 2018 The interplay between movement, dispersal and morphology in Tetrahymena ciliates. *PeerJ Preprints* , 6:e26540v1.
35. Doerder F, Brunk C. 2012 Natural populations and inbred strains of Tetrahymena. In *Tetrahymena thermophila*, pp. 277–300. Elsevier.
36. Doerder FP. 2018 Barcodes Reveal 48 New Species of *Tetrahymena* , *Dexiostoma* , and *Glaucoma* : Phylogeny, Ecology, and Biogeography of New and Established Species. *Journal of Eukaryotic Microbiology* (doi:10.1111/jeu.12642)
37. Pennekamp F, Mitchell KA, Chaine A, Schtickzelle N. 2014 Dispersal propensity in Tetrahymena thermophila ciliates - a reaction norm perspective. *Evolution* **68**, 2319–2330. (doi:10.1111/evo.12428)
38. Jacob S, Wehi P, Clobert J, Legrand D, Schtickzelle N, Huet M, Chaine A. 2016 Cooperation-mediated plasticity in dispersal and colonization. *Evolution* **70**, 2336–2345. (doi:10.1111/evo.13028)
39. Cayuela H, Jacob S, Schtickzelle N, Verdonck R, Philippe H, Laporte M, Huet M, Bernatchez L, Legrand D. 2022 Transgenerational plasticity of dispersal-related traits in a ciliate: genotype-dependency and fitness consequences. *Oikos*
40. Ketola T, Laakso J, Kaitala V, Airaksinen S. 2004 Evolution of Hsp90 expression in Tetrahymena thermophila (Protozoa, Ciliata) populations exposed to thermally variable environments. *Evolution* **58**, 741–748.
41. Derelle R, Verdonck R, Jacob S, Huet M, Akerman I, Philippe H, Legrand D. 2023 The macronuclear genomic landscape within *Tetrahymena thermophila*. (doi:10.1101/2023.10.02.560512)
42. Junker AD, Jacob S, Philippe H, Legrand D, Pearson CG. 2021 Plastic cell morphology changes during dispersal. *iScience* **24**, 102915. (doi:10.1016/j.isci.2021.102915)
43. Ruehle MD, Orias E, Pearson CG. 2016 Tetrahymena as a Unicellular Model Eukaryote: Genetic and Genomic Tools. *Genetics* **203**, 649–665. (doi:10.1534/genetics.114.169748)
44. Altermatt F *et al.* 2015 Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. *Methods in Ecology and Evolution* **6**, 218–231. (doi:10.1111/2041-210X.12312)
45. Jacob S, Legrand D, Chaine A, Bonte D, Schtickzelle N, Huet M, Clobert J. 2017 Gene flow favours local adaptation under habitat choice in ciliate microcosms. *Nature Ecology and Evolution* **1**, 1407–1410.
46. Burton T, Lakka H, Einum S. 2020 Measuring phenotypes in fluctuating environments. *Functional Ecology* **34**, 606–615.
47. Hastie T. 2018 gam: Generalized Additive Models. *R package version 1.16* (doi:https://CRAN.R-project.org/package=gam)
48. Kahm M, Hasenbrink G, Lichtenberg-Frate H, Ludwig J, Kschischo M. 2010 grofit: Fitting Biological Growth Curves with R. *Journal of Statistical Software* **33**, 1–21.

49. Pennekamp F, Schtickzelle N, Petchey OL. 2015 BEMOVI, software for extracting behavior and morphology from videos, illustrated with analyses of microbes. *Ecology and Evolution* **5**, 2584–2595. (doi:10.1002/ece3.1529)

50. Fjerdingstad EJ, Schtickzelle N, Manhes P, Gutierrez A, Clobert J. 2007 Evolution of dispersal and life history strategies – Tetrahymena ciliates. *BMC Evolutionary Biology* **7**, 133. (doi:10.1186/1471-2148-7-133)

51. Jacob S, Chaine A, Huet M, Clobert J, Legrand D. 2019 Variability in dispersal syndromes is a key driver of metapopulation dynamics in experimental microcosms. *The American Naturalist* **194**.

52. Nakagawa S, Cuthill I. 2007 Effect size, confidence inter- val and statistical significance: a practical guide for biologists. *Biological Reviews* **82**, 591–605.

53. Abrams PA. 2006 The prerequisites for and likelihood of generalist-specialist coexistence. *The American Naturalist* **167**, 329–342.

54. Sinclair BJ *et al.* 2016 Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters* **19**, 1372–1385. (doi:10.1111/ele.12686)

55. Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004 Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789. (doi:10.1890/03-9000)

56. Gibert JP, Allen RL, Hruska RJ, DeLong JP. 2017 The ecological consequences of environmentally induced phenotypic changes. *Ecol Lett* **20**, 997–1003. (doi:10.1111/ele.12797)

57. Hatton IA, Dobson AP, Storch D, Galbraith ED, Loreau M. 2019 Linking scaling laws across eukaryotes. *Proceedings of the National Academy of Sciences* **116**, 21616–21622. (doi:10.1073/pnas.1900492116)

58. Tabi A *et al.* 2020 Species multidimensional effects explain idiosyncratic responses of communities to environmental change. *Nat Ecol Evol* **4**, 1036–1043. (doi:10.1038/s41559-020-1206-6)

59. Schaum C-E, Buckling A, Smirnoff N, Yvon-Durocher G. 2022 Evolution of thermal tolerance and phenotypic plasticity under rapid and slow temperature fluctuations. *Proc. R. Soc. B.* **289**, 20220834. (doi:10.1098/rspb.2022.0834)