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Letter

Temporal dynamics and adaptiveness of thermal phenotypic plasticity in a ciliate

Léonard Dupont [1,*], Delphine Legrand[1], Mélanie Thierry[1,2], Staffan Jacob[1]

[1]Station d'Écologie Théorique et Expérimentale, UAR2029, CNRS, Moulis, France
[2]Centre de Recherche sur la Biodiversité et l'Environnement (CRBE), UMR5300 CNRS-IRD-TINP-UT3 Université Toulouse III – Paul Sabatier, Toulouse, France

Corresponding author: Station d'Écologie Théorique et Expérimentale, UAR2029, CNRS, 09200 Moulis, France. Email: leonard.dupont@normalesup.org

Abstract

Phenotypic plasticity is a widespread strategy used by organisms to cope with environmental fluctuations. Empirical studies have mostly focused on describing the amplitude of phenotypic change through reaction norms, which ignore the temporal dynamics of plasticity. Although the speed of plastic responses has recurrently been predicted to modulate their adaptiveness, it remains largely understudied. Here, we retraced the time course of plasticity across four traits in 12 isogenic strains of the ciliate *Tetrahymena thermophila* to test how the temporal dynamics of plasticity mediate its adaptiveness under fluctuations. We decomposed plastic responses into 3 parameters: a lag and a rate describing their temporal dimension and the canonical plastic capacity. All showed high intraspecific variability. We found the plastic capacity to be positively correlated to the rate of plasticity and not to the time required for plastic changes to be implemented. We then linked the dynamics of plasticity to how strains performed across a gradient of fluctuation periods. The temporal parameters of plasticity significantly explained performance in fluctuating conditions, more so than the plastic capacity alone. Interestingly, strains mounting morphological plasticity at a slower rate tended to be less sensitive to fluctuations. This study demonstrates that a better understanding of how organisms cope with environmental change requires us to consider and incorporate the temporal dynamics of plasticity in theories and experiments.

Keywords: rate of plasticity, plasticity capacity, environmental fluctuations, adaptive plasticity, microcosms

Introduction

Organisms are exposed to ever-changing environments in both space and time, which can affect their fitness (Laughlin & Messier, 2015; Ruokolainen et al., 2009). Coping with these spatio-temporal fluctuations can occur through phenotypic plasticity, a genotype's ability to produce different phenotypes depending on environmental conditions (Scheiner, 1993; West-Eberhard, 2003). Plasticity may allow organisms to buffer deleterious effects of environmental fluctuations that are predictable or fast relative to generation time (Botero et al., 2015; Chevin et al., 2010; Lande, 2014). The shift toward a new phenotype in response to changing conditions may occur irreversibly during ontogeny, as in the case of developmental plasticity (e.g., wing production in aphids [Braendle et al., 2006]), or back and forth throughout the lifetime of the individual in the case of reversible plastic responses (e.g., chromatic adaptation in cyanobacteria [Stomp et al., 2008]). In both cases, an organism's ability to implement phenotypic changes across a gradient of environmental conditions is commonly described using reaction norms measured at a fixed time following the onset of environmental change. The *plastic capacity* is classically quantified as the steepness of the slope of a linear reaction norm and is the canonical metric to quantify phenotypic plasticity (Einum et al., 2019; Pennekamp et al., 2014; Schlichting & Pigliucci, 1998; West-Eberhard, 2003).

When relying on sporadic phenotypic measurements, however, reaction norms miss a key component of phenotypic plasticity: its temporal dynamics (Burton et al., 2022; Dupont et al., 2024). Each of the steps preceding and underlying phenotypic changes takes some minimal amount of time: from detecting a change in local environment, then transducing it into interpretable cues to the implementation of phenotypic modifications per se (Dupont et al., 2024). A body of theoretical studies did emphasize the importance of the speed of plastic changes and its potential interactions with the plastic capacity (Gabriel, 2005; Lande, 2014; Padilla & Adolph, 1996; Siljestam & Östman, 2017). However, empirical investigations of the temporal aspect of plasticity remain scarce and mostly related to microbial growth rates or metabolic rates (Bertinetti & Torres-Dowdall, 2025; Einum & Burton, 2022; Fey et al., 2021; Nougué et al., 2016; Sandblom et al., 2014; Stomp et al., 2008; Turriago et al., 2023).

Yet, the temporal component of plastic responses is a likely cornerstone, as the adaptiveness of phenotypic plasticity should be modulated by the match between the speed of plasticity and that of environmental change. Indeed, the benefits of being highly plastic may be limited if only a small fraction of the plastic capacity can be expressed in due time (Burton et al., 2022; DeWitt et al., 1998; Dupont et al., 2024; Jacob et al., 2024; Padilla & Adolph, 1996; Siljestam & Östman, 2017), even more so if there are substantial production or maintenance costs for plasticity (DeWitt et

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al., 1998; Murren et al., 2015). Understanding the interactions between the plastic capacity, the speed of plasticity, and the rate of environmental change is therefore a pivotal step to understand the adaptiveness of plastic responses (Burton et al., 2022; Dupont et al., 2024; Vinton et al., 2022). For instance, a fast phenotypic response to environmental changes should reduce the amount of time spent in the new environment with a suboptimal phenotype. Consequently, it has been proposed that the rate of plasticity should be positively correlated with the plastic capacity (Burton et al., 2022; Dupont et al., 2024; Siljestam & Östman, 2017), although a recent meta-analysis detected the opposite pattern across ectotherms (Burton & Einum, 2025).

Likewise, we lack evidence testing if organisms expressing their plastic capacity quicker may be favored in faster-changing environments, despite recent results showing how thermal generalists through plasticity performed better when environmental fluctuations were slower (Jacob et al., 2024). In addition, we know little about the evolutionary constraints affecting parameters of plasticity's temporal dynamics, e.g., how fast can plasticity be, how do the speed and the capacity covary, or how costly a fast plasticity is (Burton et al., 2022; Dupont et al., 2024). Especially, the fact that lower-scale mechanisms (e.g., transcriptional changes underlying behavioral changes [Bukhari et al., 2017]) or costs could vary between traits makes it likely that the speed of plasticity itself will strongly differ across traits, across organisms, and within or across species (Burton et al., 2022; Dupont et al., 2024; Einum & Burton, 2022). These costs could lead to either positive or negative covariations between the speed of plasticity across multiple traits. The specialization of a genotype into a plastic strategy (e.g., fast morphological changes) could thus influence the temporal dynamics of other traits (Cote et al., 2022; Pennekamp et al., 2019; Ronce & Clobert, 2012; Stevens et al., 2014). Likewise, comparing the temporal aspects of plasticity could reflect the existence of alternative strategies, as for instance in the classical "change or leave" paradigm in face of environmental change (Edelaar et al., 2017; Thierry et al., 2025). In this case, we could expect some polymorphism between local changers responding quickly to local perturbations (e.g., fast plasticity for many metabolic or morphological traits) and others leaving the environment (e.g., displaying slower morphological changes, but hence faster dispersal plasticity [Campana et al., 2022; Jacob et al., 2019]). Whether this diversity in the temporal dynamics of multiple traits and their covariations could underlie organism performance in fluctuating conditions is an enticing but unanswered question.

Here, we used microcosms to quantify the temporal dynamics of plasticity and test how it relates to fitness under environmental fluctuations in populations of *Tetrahymena thermophila* ciliates (Figure 1). First, we tracked the time course of plastic responses following a thermal change by iteratively measuring phenotypic traits every 15 min for 6 hr (i.e., approximately one clonal generation) to quantify both the temporal dynamics of plasticity and the canonical plastic capacity (Figure 1A, experiment A). We studied two morphological traits (size, shape) and two movement traits (velocity, linearity) across 12 isolated and isogenic strains of *T. thermophila*. The size and shape of cells have previously been linked to thermal tolerance in protists (Wieczynski et al., 2021), while swimming speed has often been linked to dispersal in this species (Fronhofer & Altermatt, 2015; Pennekamp et al., 2019). High intraspecific variability of plasticity and fitness sensitivity along temperature had already been described in this species (Jacob & Legrand, 2021). We quantified the *lag time* (describing a latency before the onset of phenotypic change), the *plasticity rate* (the net speed at which plastic changes are implemented), and the *plastic capacity* (Dupont et al., 2024) (Figure 1A2). The lag and the rate combine into a delay before the full expression of the capacity, which we additionally quantified as the time needed to reach 95% of the plastic capacity: *t*95 (Figure 1A2). We first investigated how these different dimensions of plasticity covary with one another, within and among traits. We especially looked at the relationship between the plastic capacity and the speed of plastic responses (i.e., lag and rate of plasticity), as phenotypic changes of high magnitude are likely to be of restricted benefits if they are implemented at a slower rate than that of environmental change (Dupont et al., 2024). In parallel (experiment B), we compared the growth rates of the 12 strains under constant and fluctuating environments (Figure 1B) to test whether the temporal dynamics of plasticity were linked to their sensitivity to thermal fluctuations. We expected that the speed of plasticity would play a key role, especially under fast fluctuations. On the other hand, we predicted that the plastic capacity would be central to deal with slower fluctuations, regardless of its associated kinetics.

Methods

Study system

Tetrahymena thermophila is a 20–50 μm ciliate naturally living in freshwater ponds and streams (Doerder, 2019; Doerder & Brunk, 2012; Zufall et al., 2013). Here we used 12 strains (D1, D2, D3, D6, D8, D9, D10, D12, D13, D16, D17, and D19 [Derelle et al., 2023]) previously found to differ in their thermal tolerance curves (Jacob & Legrand, 2021; Jacob et al., 2018) and plasticity of morphological and movement traits (Jacob & Legrand, 2021; Jacob et al., 2016, 2018; Pennekamp et al., 2014, 2019). These strains are derived from those originally collected in the United States by Prof. Paul Doerder (Cleveland State University, USA) 20 years ago. They have been kept in lab cultures ever since. Based on the recent sequencing of their macronucleus (MAC) and mitochondrial genomes performed by our team (Derelle et al., 2024), we determined that the 12 strains used in this study are organized in five genetic clusters. We also detected incorrect assignments and/or contaminations compared to initial sampling performed by Paul Doerder, which likely occurred several years ago (Derelle et al., 2024) and limits our ability to reconstruct their geographical affiliation. Noteworthy, this recent study revealed that significant phenotypic variability occurs even between strains that show very low genetic differentiation, suggesting significant effects of non-genetic factors (Derelle et al., 2024). Since these isogenic strains reproduce clonally in laboratory conditions, changes in trait values between environmental conditions can be considered as caused by phenotypic plasticity. Cell maintenance and experiments were done in axenic liquid growth media (PPYE 0.3X: 0.3% Difco proteose peptone, 0.03% yeast extract). Mother cultures are kept at 23 °C. All manipulations were performed in sterile conditions under a laminar-flow hood.

Temporal dynamics of thermal plasticity

We separately exposed the 12 strains to either standard (23 °C) or stressful (35 °C) temperature. We iteratively measured morphological and movement traits every 15 min for 6 hr (i.e., approximately one clonal generation) to quantify the temporal dynamics of thermal plasticity.

For each strain, a 1-week-old cell preculture grown at 23 °C (close to asymptotic density) was used to inoculate 10 replicated populations spread between two 24-well plates: five replicates in a "control" plate staying at 23 °C (allowing to control for the tem-

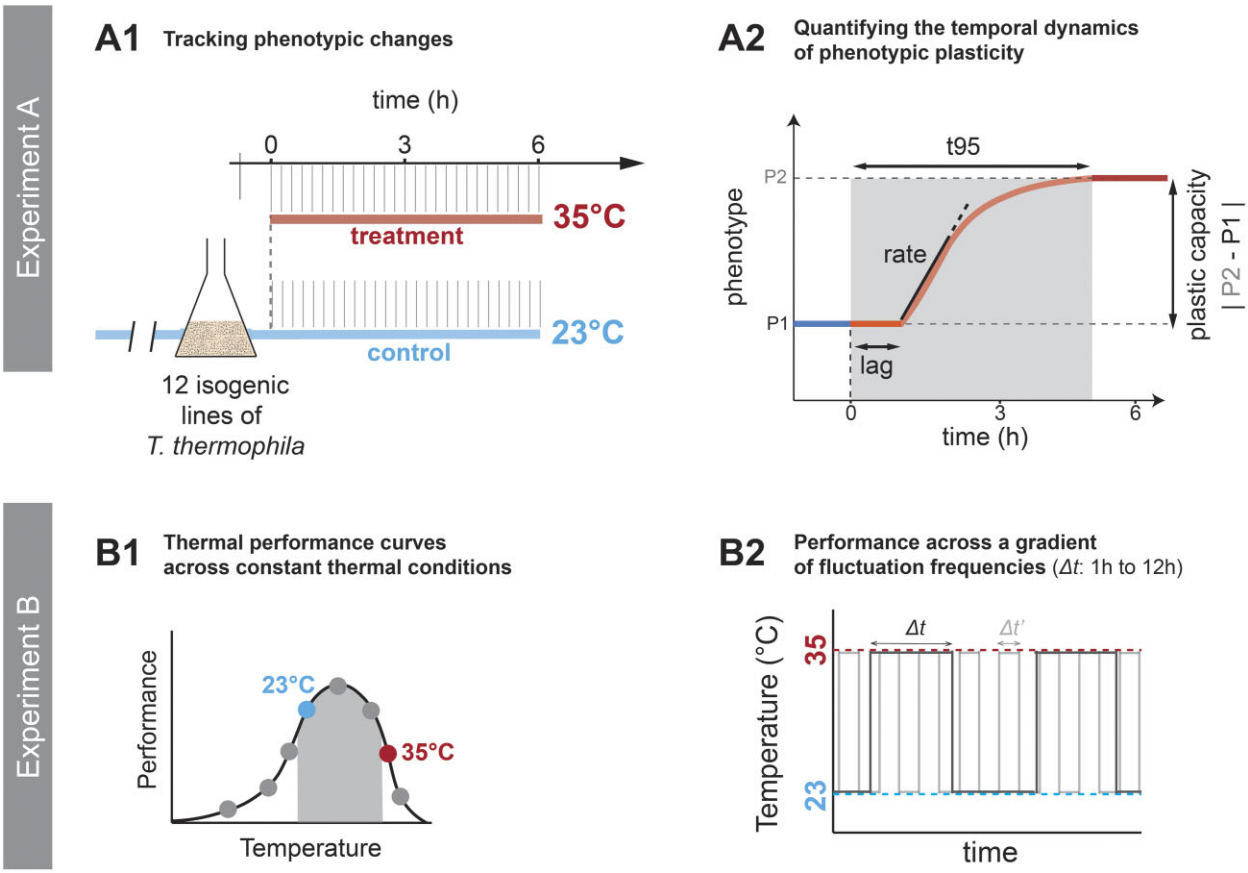

Figure 1. Quantifying the temporal dynamics of phenotypic plasticity and its role for performance under thermal fluctuations. (A1) Twelve isolated strains of *T. thermophila* were exposed to an acute change of thermal environment from 23 °C (rearing lab conditions) to 35 °C across five replicates. Phenotypic plasticity was tracked across four traits (size, shape, velocity, and linearity) at high temporal resolution (every 15 min, gray vertical bars) during 6 hr in control and treatment conditions. (A2) The data from (A1) were used to derive a lag, a rate, a capacity, and a time needed to reach 95% of this capacity (t95). These parameters were then used as strain and trait-specific characteristics of phenotypic plasticity. (B1) In a parallel experiment, population growth was quantified across a gradient of eight constant temperatures to reconstruct the thermal niche of each strain, showing the position of the experimental temperatures used hereafter within the niche. (B2) To assess the strains' sensitivity to the rate of environmental fluctuations, performance was quantified by measuring population growth for three weeks across a gradient of eight fluctuation periods (ranging from 1 to 12 hr), with temperatures oscillating between 23 °C and 35 °C.

poral dynamics of non-thermal plasticity) and five replicates in a "treatment" plate due to 35 °C. 200 ul of preculture were inoculated into 2 ml of PPYE 0.3X, which roughly corresponded to a dilution to the 10th of the asymptotic density. We then measured the initial phenotypic state, before immediately dispatching plates to either $T = 23$ °C (control plate, $N = 5$) or $T = 35$ °C (treatment plate, $N = 5$). From then on, we followed the temporal dynamics of plasticity in each replicated population every 15 min for 6 hr. For each timepoint, we pipetted a 10 µl sample (~30–100 clonal cells) from each well into a multichambered counting slide (Kima precision cell) and immediately recorded 15 s videos under dark-field microscopy. We used the BEMOVI R-package (Pennekamp et al., 2015) to measure cell morphology and movement (e.g., Jacob & Legrand, 2021). We quantified two morphological and two movement traits: cell size as the cell-surface area (μm^2), cell shape as the ratio of cell major/minor axis, cell velocity as the total distance travelled divided by the duration of the trajectory ($\mu m.min^{-1}$), and cell linearity as the ratio between the net distance travelled (in Euclidean space between start and end positions) and the effective distance moved as tracked by the system. Cell size and shape were previously linked to resource acquisition, metabolic rates, and thermal performance curves in protists (Wieczynski et al., 2021), while velocity has often been used as a proxy of dispersal in this species (Fronhofer et al., 2015; Pennekamp et al., 2019).

Deriving parameters from the dynamics of plasticity

Going back to the unfolding of a plastic response, the temporal dynamics of plasticity may be decomposed into several key parameters. In addition to the plateau at which the phenotype stabilizes, referred to as the plastic *capacity* (C), we here quantified the *lag* time (λ) before the expression of phenotypic change and the *rate* (r) of phenotypic change. In complement to the lag and the rate, we also computed t95 as the time needed to reach 95% of the plastic capacity. t95 was directly measured at the first timepoint when the trait had reached 0.95∗C. This metric was used as an aggregated measure of plasticity's speed and as a simple way to assess the speed of the plastic response. Following the framework proposed in Dupont et al. (2024), we extracted the lag, rate, and capacity of plasticity by fitting phenotypic changes through time ($P(t)$) with a logistic model, which was best at describing the diversity of shapes we observed in our experiment (Figure S2):

$$P(t) = \frac{C}{1 + e^{\frac{r(\lambda - t)}{C}}}. \quad (1)$$

As for classic reaction norms approaches where plasticity is quantified as the slope or delta of phenotypic change along environment, here we computed the difference of phenotypic values between 35 °C and control conditions (23 °C) at each timepoint (Figure S1) to control for non-thermal plasticity (due to, e.g., the inoculation, the initial dilution, circadian cycles). Extracting plasticity parameters that are comparable across the four traits is crucial to test for correlations between plasticity parameters and performance. For each trait and each strain, we thus divided values at each timestep by the maximal value observed across all strains and all replicates for the specific trait. In this way, any temporal dynamic in the dataset would reach a maximal value of 1 or lower (Figure S2). These scaled trait values were then used to fit the logistic model (1) using a non-linear least-square regression (*nls.multstart* function, R package *nls_multstart*) (Padfield & Matheson, 2018). The regression was randomly seeded 400 times per replicate within a parameter space constrained by the data of the replicate (e.g., the *rate* (r) had to be greater than or equal to 0 and could not exceed max(data) − min(data): a one-step change of maximal magnitude). Importantly, the fitting model used here makes the three parameters (C, λ, r) independent: A given capacity (C) may be reached using an infinite combination of rates and lags. A subset of phenotypic trajectories displayed non-monotonous responses, where the logistic plateau was of smaller duration than that of our experiment and the trait reverted back to its initial state (Figure S2). In these cases, the regression was performed on the first part of the trajectory (up to the plateau), before the inflexioninflection point of monotony reversal, as it is usually done for growth curves. Lag times, which are calculated as the time-to-midpoint in logistic regressions, were corrected into lags *stricto sensu* (Figure S3). When lags had converged to either 0 or to a value exceeding the time of the experiment, they were set as *NA* to avoid interpretation bias. Since the limit shape of the logistic model for $r = 0$ or $C = 0$ is of the linear form, cases of no plasticity were *a fortiori* included in the parameter space for model selection. Some R^2 of our model fit were below 0.3 (Figures S2 and S4) and corresponded to cases of low plasticity on average. In these instances, the constraint on lags was relaxed because the logistic inflexion was low (i.e., the temporal dynamics were of the linear form). In these cases, lags were set to *NA* as well. Finally, the intra-strain repeatability of lags, rates, and capacities was estimated using the *rpt* function (*rptR* package, Stoffel et al., 2017).

Growth rates in constant and fluctuating conditions

In a distinct experiment, we measured the performance of the 12 strains both along a gradient of constant temperatures (to assemble their thermal niche and visualize the position of the experimental temperatures used for fluctuations [see below] within the niche) and along a gradient of environmental fluctuations (to detect their sensitivity to the rate of environmental change).

For the first part, we measured strain performance by quantifying population growth rate from a small number of cells (approx. 100) across a gradient of eight constant temperatures (Figure 1B1), including 23 °C and 35 °C. For the second part, we assessed how the growth rate of the 12 strains responded to fluctuating environments by exposing populations to a thermal environment fluctuating between $T = 23$ °C and $T = 35$ °C, with a gradient of fluctuation period from 1 to 12 hr (1, 2, 3, 4, 6, 8, 10, 12 hr) (Figures 1B2 and S7). In both experiments, 10 μl of cells were inoculated in 96-well plates filled with 250 μl growth media, with eight replicates per strain and condition (i.e., either temperature or fluctuation period). Population growth was quantified through absorbance measurements at 550 nm using a microplate reader (Tecan Infinite Spectrophotometer; e.g., Jacob & Legrand, 2021; Jacob et al., 2018), performed twice a day until populations ended their exponential growth (~7 days). From then on, we pursued daily measurements for 10 additional days until populations reached their maximal density. To avoid bias owing to noise in the absorbance measures, we smoothed the time data using a general additive model (*gam* package [Hastie 2018]), without any assumption regarding the shape of the curve. We then used the *grofit* package (*gcfit* function, R-package; Hastie, 2018) to fit a spline-based growth curve and compute the *growth rate* (μ) as the maximum slope of population growth through time. This growth rate (μ) was used as a strain-specific metric of fitness in each condition (i.e., either a given constant temperature or a given fluctuation period), as in previous studies (Campana et al., 2022; Jacob & Legrand, 2021; Jacob et al., 2017, 2018). For the fluctuation experiment (Figure 1B2), cells spent equal amounts of time at 23 °C and 35 °C conditions (half-half), the only changing factor being the fluctuation period. As such, the theoretical fitness under instantaneous and fully adaptive plasticity with no costs would be the average between 23 °C and 35 °C: $\bar{\mu} = \frac{\mu_{23°C} + \mu_{35°C}}{2}$. For each strain and each fluctuation period (P), we compared this theoretical case to the actual, realized growth rate (μ_P), resulting in the difference:

$$\Delta\mu_P = \mu_P - \bar{\mu}.$$

This fitness difference ($\Delta\mu_P$) was then taken as a quantitative descriptor of the strains' sensitivity to fluctuations for each period. As such, for strains expressing instantaneous plasticity with no costs, μ_P and $\bar{\mu}$ should be the same, leading to $\Delta\mu_P = 0$.

Statistical analyses

We first tested whether the parameters (lag, rate, capacity) of the temporal dynamics of plasticity were correlated, both within and between phenotypic traits. For each trait, we used the median of each of the three parameters across replicates to aggregate them in a single dynamic of phenotypic change. We computed a correlation matrix based on Pearson's *r* coefficient (*rcorr* and *corrplot* functions, R [R Core Team, 2021]). To assess the amount of parameter variance explained by the strain factor, we fitted analysis of variance models (*aov* calling *lm* in R) with a trait *parameter* (e.g., size *lag* or velocity *rate*) as the response variable and strain categories as the explanatory factor. The normalization procedure allowed us to compare the intraspecific variability of plasticity between traits but not the differences of plastic capacities across traits for each strain (but see Jacob & Legrand, 2021).

Finally, we ran a partial least squares (PLS) regression with "sensitivity to fluctuations" (the $\Delta\mu_P$ fitness difference between constant and fluctuating conditions) as a response variable, and all centered and reduced plasticity parameters as explanatory variables using *plsRglm* (*plsRglm* package, Bertrand & Maumy, 2023). PLS regression follows an algorithm searching for a linear combination of explanatory variables fitted successively under the orthogonality constraint. It allows unbiased coefficient estimation in the presence of multicollinearity of variables (Cassel et al., 1999). To account for intra-strain parameter variance (Table S1), we generated probability distributions of parameter values for each strain × trait (centered on the median; with the variance calculated using the five replicates from experiment A). We then bootstrapped the PLS (5,000 iterations) by independently drawing parameter values within each distribution, thus randomly associating these sampled values to replicates of the fitness experi-

ment (Figure 1B2). We considered coefficients as significant when 95% of their bootstrapped values were higher or lower than 0. All these steps were conducted using *bootplsglm* from the *plsRglm* R-package. All analyses were performed on R (version 4.2.3 [R Core Team, 2021]) and Python (version 3.7.3 [Van Rossum & Drake, 2009]).

The data that support the findings will be made freely available on Zenodo upon publication.

Results

The temporal dynamics of plasticity are trait-dependent and intraspecifically diverse

Using iterative phenotypic measurements, we retraced and quantified the temporal dynamics of plasticity for cell size, shape, swimming velocity, and swimming linearity using 12 *T. thermophila* isogenic strains (Figures 2A–D, S1 and S2). The estimated lag, rate, and capacity showed moderate to high repeatability between replicates (mean repeatability across traits: Rpt lag = 0.388, Rpt rate = 0.511, Rpt capacity = 0.832; Table S1). We used average parameter values per strain and trait in the following analyses, except for the PLS (see the *Methods* section). We first examined how the plasticity lag, rate, and t95 varied across strains and traits. We found that rates and t95 significantly differ between traits (rate: $F_{(4,55)} = 23.64$, $p < 0.001$; t95: $F_{(4,55)} = 8.25$, $p < 0.001$) but not the lag ($F_{(4,55)} = 2.89$, $p = 0.052$). Velocity was the fastest changing phenotype, and morphological plasticity was significantly slower than movement plasticity (Figure 2E and F, Table S2). Finally, significant intraspecific variability was found for all characteristics of plasticity, except the lag of linearity (Figure 2, Table S3).

The parameters of plasticity dynamics covary within and across phenotypic traits

We then explored the patterns of covariation of the plasticity lag, rate, t95, and capacity within and between phenotypic traits. Within traits, there was a significant positive correlation between the plastic capacity and the rate, except for cell size (Figure 3A). Hence, expressing greater phenotypic change was associated with shifting phenotypes at a faster rate. There was a tendency for the capacity to also correlate positively with the lag (Figure 3B), while lags and rates were not correlated (Figure 3C). Still, the overall time to reach the plastic capacity (*t95*) was not correlated to the plastic capacity (Figure 3D), implying that there was no particular link between the time to reach the asymptote and the amplitude of the response. As expected, t95 correlated negatively with the rate (Figure 3E; $R^2 = 0.45$, $p < .001$) and positively with the lag (Figure 3F; $R^2 = 0.42$, $p < .001$).

Across traits, we found that size and shape capacities were positively correlated, as were capacities between velocity and linearity (Figure S5). There was no significant correlation between morphological and movement plastic capacities (Figure S5). However, there was a significant negative correlation between size and shape capacities and the rate of linearity, and a tendency for a negative correlation with the rate of velocity (Figure S5). Overall, this suggested that the capacity for morphological plasticity traded off with the rate of movement plasticity.

The temporal dynamics of plasticity explains performance under fluctuations

To decouple the explanatory role of parameters describing the unfolding of phenotypic plasticity, we ran a PLS regression. We ranked the predictors according to their regression estimate, and found that the most important parameters accounting for the strains' sensitivity to fluctuations were related to the speed of morphological plasticity, especially the shape lag and the size rate (Figure 4, Table S4). Plastic capacities for size and shape also had significant effects. On the other hand, the temporal dynamics of movement plasticity were little related to strain fitness under fluctuating conditions. To complement this analysis, we ran a parallel PLS regression with lags and rates combined into *t95*. Once again, the temporal parameters of size and shape plasticity were strong predictors of the strains' sensitivity to fluctuations (Figure S8). Interestingly, lower rates (*resp*. higher t95) were related to better performance under fluctuating temperatures (Table S4). This meant that strains with slower plasticity in experiment A coped better with environmental fluctuations in experiment B.

Discussion

The benefits of adaptive phenotypic plasticity should depend on the match between the expressed phenotype and the encountered environment (Chevin et al., 2010; Lande, 2014). However, the adaptiveness of plasticity should not only be dependent on the fitness of the induced phenotype (Hendry, 2016; Scheiner, 1993; Snell-Rood et al., 2018), but be modulated by the interaction between the speed of environmental change and the rate at which the plastic capacity is implemented (Burton et al., 2022; Dupont et al., 2024; Ketola & Kristensen, 2017; Padilla & Adolph, 1996; Siljestam & Östman, 2017; Vinton et al., 2022). Here, we revealed a high intraspecific diversity of plasticity's temporal dynamics, comparable to that of the already known variability in the plastic capacity (Campana et al., 2022; Jacob & Legrand, 2021; Jacob et al., 2019; Matesanz & Ramírez-Valiente, 2019; Morel-Journel et al., 2020; Sentis et al., 2019). We furthermore found that the temporal dynamics of plasticity, especially the speed of morphological plasticity, were related to how organisms performed under thermal fluctuations. This all suggests that morphological plasticity could be a pivotal driver of performance in our model, although direct causal links between cell size or shape and fitness benefits still need to be delineated (Campana et al., 2022; Ghalambor et al., 2007; Hendry, 2016; van Kleunen & Fischer, 2005). Still, these results provide the first empirical support for the temporal dynamics of plasticity playing a key role in the adaptiveness of plastic responses in fluctuating environments.

Theoretical studies have broached the role of rates in the evolution and adaptiveness of plasticity (Gabriel, 2005; Gabriel et al., 2005; Lynch & Gabriel, 1987; Padilla & Adolph, 1996; Siljestam & Östman, 2017). For instance, Siljestam and Östman (2017) explained how the plasticity rate may constrain the evolution of the plastic capacity. Although they relied on fixed, non-evolving rates for reasons of model complexity, they ran simulations using different fixed rate values, which allowed them to assess how this parameter may constrain the evolution of the plastic capacity. They suggested that a higher phenotypic rate would reduce the mismatch between realized and optimal phenotype under fluctuating conditions, and thus allow for the evolution of higher plastic capacities. In addition, spending energy to maintain or produce high plastic capacities (Auld et al., 2009; Callahan et al., 2008; DeWitt et al., 1998; Murren et al., 2015) may turn out to be too costly if only a fraction of the possible phenotypic change can be expressed in due time (Siljestam & Östman, 2017). In light of such models, we expected selection for a high plastic capacity to be associated with the existence of fast plasticity. Accordingly, we found that the rate of plasticity shared a recurrent positive correlation with the plas-

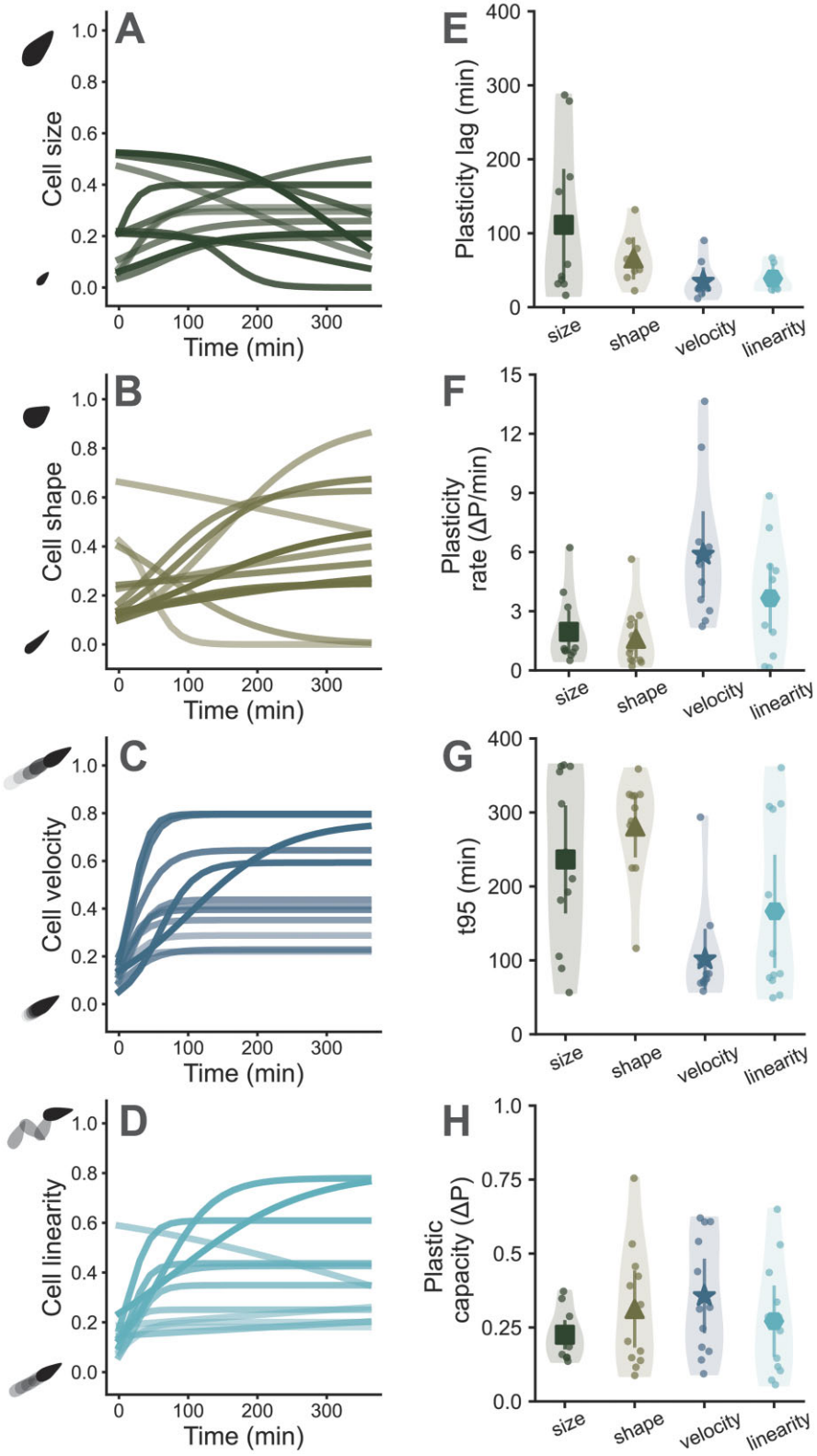

Figure 2. Temporal dynamics of thermal phenotypic plasticity of four phenotypic traits and their intraspecific diversity. (A–D) For each phenotypic trait (A: cell size; B: cell shape; C: movement velocity; D: movement linearity), curves represent changes of trait values across time following a thermal change (values of the best logistic model for each of the 12 strains are shown). We parameterized these temporal dynamics through a logistic regression yielding a lag time, a rate, and a capacity. In addition, we computed the time to reach 95% of the capacity (t95) as a simpler metric of the overall response speed. All parameter distributions are detailed throughout panels (E)–(H). (E–H) Violin plots of the lag, rate, t95, and capacity distributions for all four phenotypic traits. Larger markers show median values of each distribution, and small points show values of each strain. Error bars show 95% confidence intervals. Plasticity rates were used as absolute values to describe the speed of phenotypic change regardless of its direction. Note that panel (H) illustrates only differences of intraspecific variability between traits, since the plastic capacity values cannot be comparable between traits that have different units.

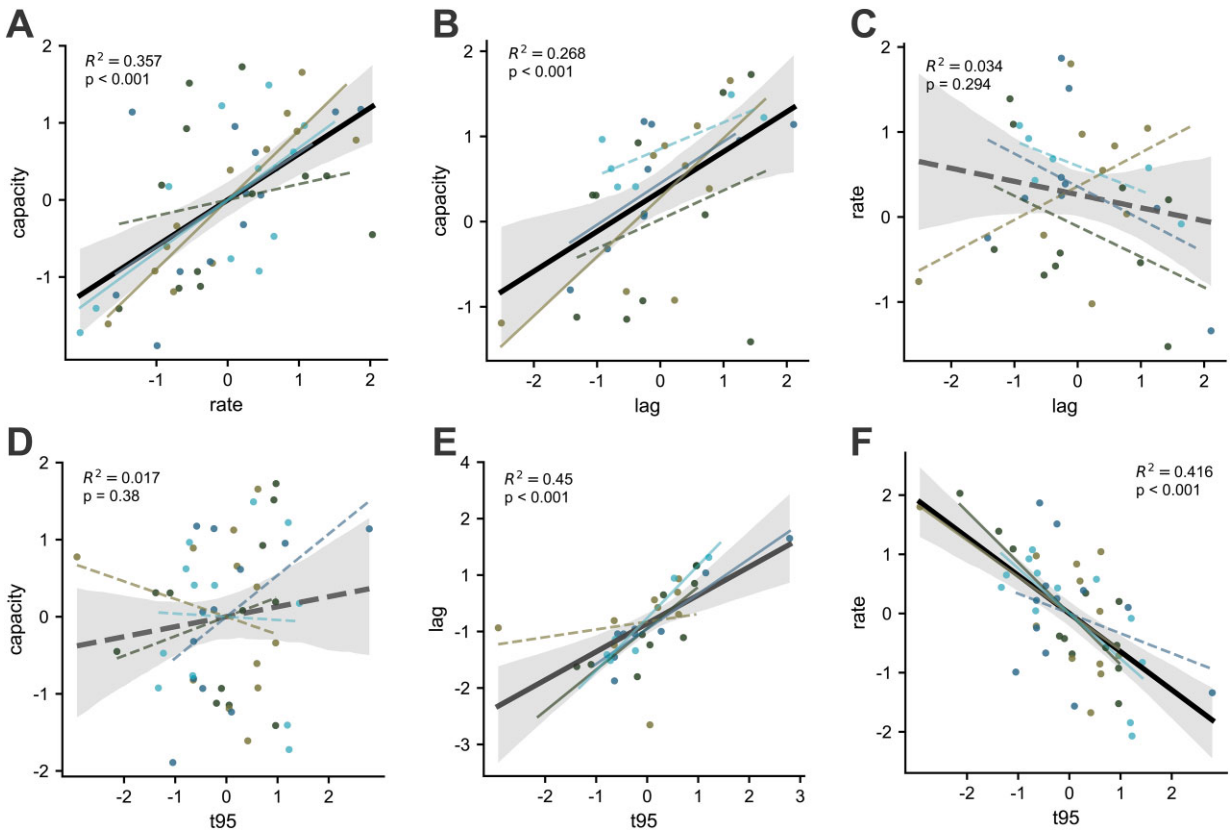

Figure 3. Covariations between parameters of plasticity's temporal dynamics. Pairwise linear relationships between lag, rate, t95, and capacity. Parameter values were Z-scored within each trait prior to regression. For all parameter pairs (A–F), we present the global linear model ($N = 48$, black line with 95% confidence interval) along each within-trait regression ($N = 12$, colored lines). Each point corresponds to a strain and a trait. Solid line regressions indicate a significant linear relationship (sig. level: $p < .05$), contrarily to dashed ones. Overall, the capacity was positively correlated to both the rate (A) and the lag (B), while the latter two parameters shared no particular relationship (C). Although there was a positive trend, t95 was not significantly correlated to the plastic capacity (D). As expected, it was however positively correlated to the lag (E) and negatively correlated to the rate (F). All pairwise correlations within and across traits can be found in Figure S6.

tic capacity across traits. The corollary was the absence of correlation between plastic capacity and overall time needed to express it (t95): Since larger phenotypic changes occurred at a faster rate, they did not need more time to be mounted. How the speed and capacity covary more widely between contexts or species, and how this may affect plasticity's adaptiveness are key questions for future investigation, especially in face of diverging conclusions in this pattern (Burton & Einum, 2025). This covariation may be bounded, as organisms inhabiting more variable environments do not always evolve greater plastic capacities (Gunderson & Stillman, 2015; Kelly et al., 2017; MacLean et al., 2019; Pereira et al., 2017). Such trends could notably result from the existence of limits in the speed of plasticity for some traits. Assessing what these limits are, if they are caused by purely mechanistic constraints or energetical costs (e.g., production costs of fast plasticity), could prove useful in our understanding of plasticity and of its evolution.

Interestingly, the speed of plasticity (i.e., lag, rate, and t95) reached levels of intraspecific diversity comparable to those of the plastic capacity within this species (Jacob & Legrand, 2021; Morel-Journel et al., 2020). One recent meta-analysis has documented the elevated diversity of plasticity rates across taxa (Einum & Burton, 2022). Here, the high inter-strain variance found across all plasticity parameters suggests that the temporal components of plasticity may evolve too (Bertinetti & Torres-Dowdall, 2025), providing genetic variability for selection to act on, as previously advanced (Burton et al., 2022). Such intraspecific variation in the speed of plasticity could also be a necessary condition for the evolution of the plastic capacity (Ketola & Kristensen, 2017; Schulte et al., 2011). Investigating what constrains the evolution of plasticity rates (e.g., costs, mechanistic limitations) and how it explains discrepancies in our ability to predict the occurrence of plastic strategies in fluctuating environments (Basan et al., 2020; Burton et al., 2022; Dupont et al., 2024; Siljestam & Östman, 2017) is an interesting direction for future research. In addition, while rates significantly differed between plastic traits, this was not the case for lag times (Figure 2). This suggests that the different steps of a plastic response (i.e., assessing the environment [*lag*], then mounting plasticity [*rate*]) (Dupont et al., 2024; Hill et al., 2024) may sustained by distinct mechanisms and could thus evolve independently. Investigating these aspects may call upon the experimental evolution of lags and rates by exposing populations to long-term fluctuations differing in their period and amplitude—as previously done for the plastic capacity (e.g., Leung et al., 2020; Rescan et al., 2020).

The existence of covariations between morphological and movement traits in *Tetrahymena* had already been highlighted (Jacob et al., 2019; Junker et al., 2021; Nelsen & Debault, 1978; Pennekamp et al., 2019; Thierry et al., 2025). For instance, Junker and colleagues showed how linear-swimming disperser strains tended to have longer ciliæ and a higher aspect ratio (Junker et al., 2021). In addition, some studies have discussed the existence

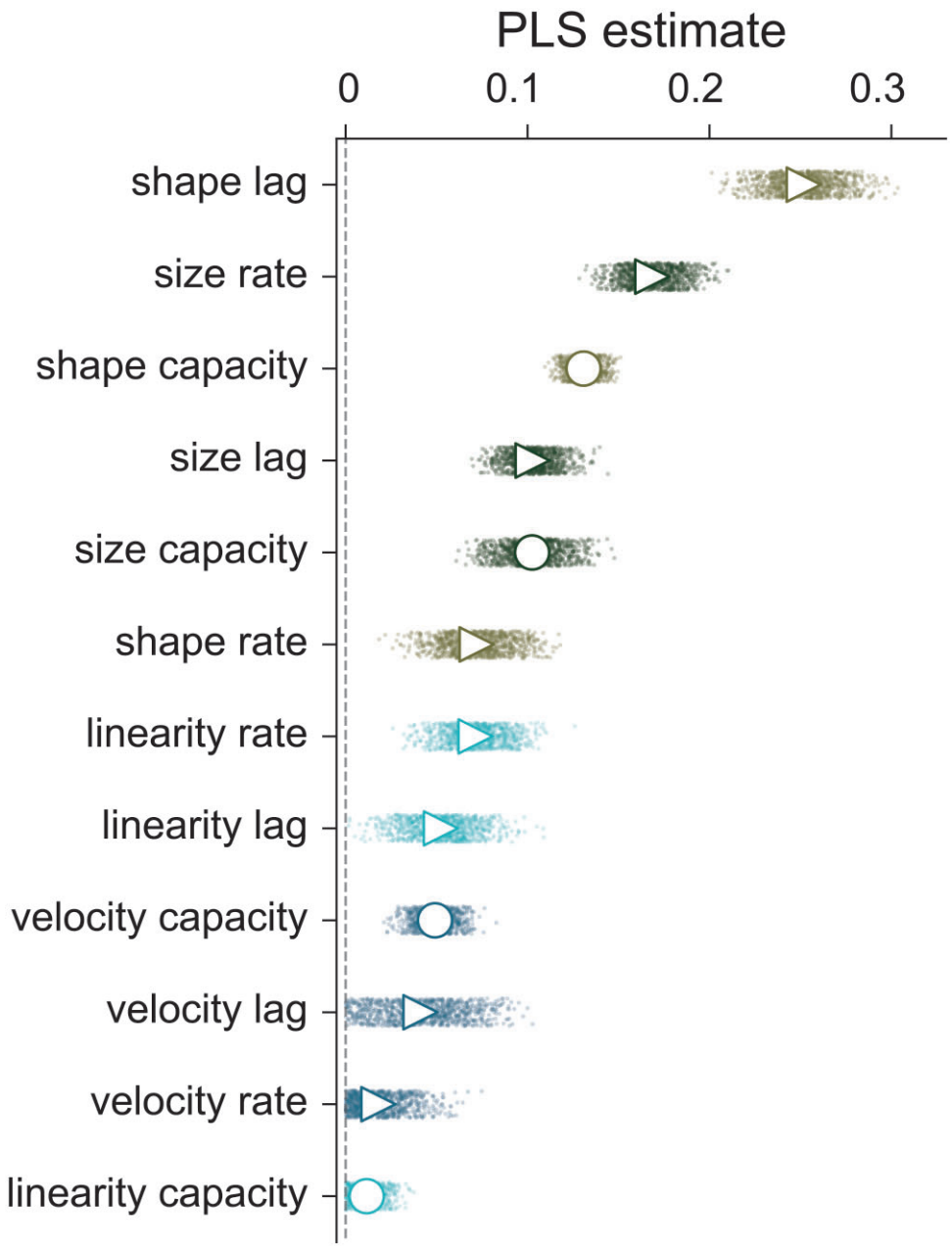

Figure 4. The speed of morphological plasticity is the main predictor of performance in fluctuating thermal environments. A partial least squares (PLS) regression was performed with all parameters of plasticity's temporal dynamics as predictors, and the sensitivity to fluctuations as the response variable. Average PLS estimates are represented as absolute values ranked in descending order, with circles for capacities and triangles for temporal parameters (lag or rate). The regression was bootstrapped 5,000 times to account for intra-strain variance of plasticity parameters in experiment A (see the *Methods* section). Individual bootstrap results are shown as smaller points in the background. Corroborating results were found when running the PLS regression with t95 instead of the lag and the rate (Figure S8). Full statistics are available in Table S4.

of covariations between plastic capacities leading to syndromes of plasticity (Mitchell & Houslay, 2021). Here, the plastic capacities for shape and size were correlated with each other and negatively correlated to the rate of linearity. Such correlations suggest that some covariations between plastic traits may happen beyond the classical capacity metric. This interdependence could be indirect and result from lower-scale molecular processes, tying the temporal dynamics of these traits' plasticity together and affecting their co-expression (Dupont et al., 2024; Leung et al., 2022). Production costs may also interconnect these plastic responses through energetical tradeoffs, e.g., it may be very costly to express fast plasticity across all traits (Auld et al., 2009; Callahan et al., 2008; DeWitt et al., 1998; Hendry, 2016; Murren et al., 2015). Whether some environmental contexts favor different covariations or syndromes of plastic dynamics across organisms or contexts seems like an exciting prospect for future research (Laughlin & Messier, 2015).

Our study also revealed that movement plasticity was faster than morphological plasticity (Table S2). These results echo back to works showcasing behavioral changes as examples of fast plasticity in face of local changes (e.g., Bukhari et al., 2017; Snell-Rood, 2013; Tinbergen, 1951). Plastic strategies are known to encompass a variety of timescales, ranging from fast and reversible changes to developmental plasticity and transgenerational plasticity (Snell-Rood et al., 2018). Describing differences in timing

between morphological and behavioral plastic responses triggers a series of mechanistic questions at a more detailed level. In a motile unicellular organism, such a difference of timing between movement and morphological plasticity could be explained by the beating speed of cilia (Junker et al., 2021) being mechanistically quicker to adjust than cytoskeleton-based traits (Dziadosz, 1981), such as the shape or biovolume. However, rates may also reflect past selection and temporal hierarchy in how organisms react to a local change. For example, cells may start to plastically change their movement (e.g., to forage, explore, and maybe prepare for dispersal [Cote et al., 2017; Jacob et al., 2020]) before expressing morphological plasticity later on. The occurrence of transient plastic responses in our dataset (Figure S2) suggests that such temporal sequences of plastic responses could exist in *T. thermophila*. Exploring the temporal overlapping of traits with distinct functions or consequences on fitness could be of high interest.

One major finding of this study is that the temporal dimensions of plasticity (i.e., lag and rate) are important drivers of performance under thermal fluctuations, equalling or even exceeding the importance of the capacity of plasticity (Figure 4), as previously proposed (Dupont et al., 2024; Jacob et al., 2024; Ketola & Kristensen, 2017; Ketola & Saarinen, 2015). In particular, our analysis indicated that the speed of morphological change had strong effects on the strains' sensitivity to fluctuating environments. This result was robust to a coarser-grained approach relying on t95 values (Figure S8). Interestingly, the capacities for size and shape plasticity also had notable effects, emphasizing that the speed and capacity are intertwined properties of a plastic response (Burton et al., 2022; Padilla & Adolph, 1996), which should not be considered independently. Across species, a previous study had shown that smaller and more elongated protists tended to have higher intrinsic growth rates, and that species with more elongated cell shape tended to tolerate a wider range of temperatures (Wieczynski et al., 2021). In *T. thermophila*, the capacity for morphological plasticity has recently been described as a candidate mediating the relationship between thermal generalism and performance under thermal fluctuations (Jacob et al., 2024). This suggests that morphological plasticity could be the main driver of performance in our model, although direct causal links between cell size or shape and fitness benefits still need to be deciphered. Interestingly, strains expressing slower morphological plasticity (i.e., with lower rates, higher t95) were less sensitive to thermal fluctuations. At first, these results may seem contradictory with classical expectations (e.g., Burton et al., 2022; DeWitt et al., 1998; Dupont et al., 2024; Lande, 2014). However, the fact that our faster fluctuation treatment (a 1 hr period) was still slow relative to plastic changes, or the potential existence of costs for fast plasticity could underlie this trend. Measuring the temporal dynamics of plasticity and fitness under fluctuations in a single experiment could help ascertain these hypotheses. Overall, these results are strong incentive to integrate the temporal dynamics of morphological plasticity in future work aimed at understanding how organisms react and adapt to changing environments.

Understanding how organisms respond to environmental fluctuations through the means of phenotypic plasticity is a long-lasting challenge in ecology and evolutionary research. Critical to tackle this question is to estimate the adaptiveness of plastic responses, which may not only depend on the plastic capacity, but also on the speed of plasticity relative to that of environmental change (Burton et al., 2022; Dupont et al., 2024). Our experimental results shed light on the intraspecific diversity of plasticity's temporal dynamics and emphasize the importance of considering the speed of phenotypic change as a key driver of performance under fluctuating conditions. More pragmatically perhaps, measuring these parameters is often challenging and comes with numerous methodological choices. In particular, iteratively sampling organisms' phenotypes may confront us with observations that are otherwise overlooked, such as traits changing through time in control populations (Figure S1) or transient plasticity (Figure S2). Using two metrics from a logistic regression (lag and rate) to describe the speed of plasticity requires a high-enough sampling frequency, which may prove incompatible with some fast-changing traits depending on the studied organism. Alternatively, computing the overall time to reach the asymptotic phenotypic value (t95) can help interrogate the importance of the net speed of plastic changes. On the other hand, looking at the lag before the onset of plasticity or at the net rate of phenotypic change may provide fine-grained information about, e.g., the mechanistic processes underlying changes in the trait of interest.

Supplementary material

Supplementary material is available online at *Evolution Letters* .

Data and code availability

Data and code for the following work can be accessed on Zenodo (doi: 10.5281/zenodo.14699691).

Author contributions

L.D. and S.J. designed the experiments; L.D., D.L., M.T., and S.J. carried out the experiments; L.D. analyzed the data with input from all authors; L.D. wrote the first version of the manuscript; all authors contributed to the final version of the manuscript.

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Conflict of interest

The authors declare no source of competing interest.

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