Effects of acclimation time and epigenetic mechanisms on growth of *Neurospora* in fluctuating environments

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Abstract

Reaction norms or tolerance curves are often used to predict how organisms deal with fluctuating environments. A potential drawback is that reaction norms measured in different constant environments may not capture all aspects of organismal responses to fluctuating environments. We examined growth of the filamentous fungus *Neurospora crassa* in fluctuating temperatures and tested if growth in fluctuating temperatures can be explained simply by growth in different constant temperatures or if more complex models are needed. In addition, as previous studies on fluctuating environments have revealed that past temperatures that organisms have experienced can affect their response to current temperature, we tested the roles of different epigenetic mechanisms in response to fluctuating environments using different mutants. We found that growth of *Neurospora* can be predicted in fluctuating temperatures to some extent if acclimation times are taken into account in the model. Interestingly, while fluctuating environments have been linked with epigenetic responses we found only some evidence of involvement of epigenetic mechanisms on tolerating fluctuating temperatures. Mutants which lacked H3K4 or H3K36 methylation had slightly impaired response to temperature fluctuations, in addition a mutant in the RNA interference pathway had altered acclimation times.

Introduction

Phenotypic plasticity, the ability of organisms to change or develop different phenotypes in response to environmental changes, is extremely common in nature [1]. We can describe phenotypic plasticity using reaction norms, where the phenotypic value of a particular trait is a function of an environmental variable. For example, the growth of a single genotype in different temperatures. Plasticity is important in evolutionary biology as plasticity can influence evolutionary dynamics of a population when it adapts to a new environment [2, 3]. Furthermore, there is evidence that natural selection can and does act on plasticity [4, 5]. Plasticity is also of interest in life-history theory [6], and sexual selection theory [7]. In addition, reaction norms are also important in plant and animal breeding [8, 9, 10]. In terms of other practical applications, reaction norms for temperature, also called thermal performance curves, have been used in predicting the effects of climate change on extinction risk in ectotherm populations [11, 12, 13, 14].

Reaction norms are a useful tool, but there exists a significant complication to their practical application. Reaction norms are often measured in the laboratory in constant environmental conditions. Obviously, in the wild most environments rarely stay constant for long: day and night cycles, seasons, and daily changes in weather all cause the environment to change constantly. This may be a significant drawback for the usefulness of reaction norms, as recently there have been reports that reaction norms measured in constant environments do not predict the performance of organisms in fluctuating environments [15, 16, 17, 18, 19]. There are a number of assumptions that need to be made if reaction norms measured in the lab are used to predict performance in natural environments [20], and Sinclair et al. [21] reviewed these complications for temperature reaction norms. Performance is not only dependent on the current temperature but also on temperatures experienced in the past [22, 17]; another important factor is how long the organism has experienced the current temperature, as acclimation to a given temperature may not be instantaneous [23]. The duration of exposure or the frequency of environmental fluctuations may also be important [20]. For example, the benefits of inducible plasticity could be diminished if response time for phenotypic adjustment is slower than a new alteration in the environment [24].

Mechanisms of phenotypic plasticity, especially at the level of gene expression, have been studied intensively, but little is known about the mechanisms that create lag effects in plasticity, or how memory of past temperature exposures is mediated. Expression of heat shock proteins has been investigated in several studies [25] but it is not clear if heat shock protein kinetics can explain all observations. Another possible mechanism, especially for the influence of past temperatures, are epigenetic mechanisms. These include chromatin modifications such as DNA methylation [26], and different types of histone modifications [27]. It is known that epigenetic mechanisms can mediate cell memory [26, 28, 29], plasticity [30, 31], and are required for certain parental effects [32] or between generation plasticity [33, 34]. If epigenetic mechanisms allow phenotypic plasticity they can contribute positively to tolerating fluctuating environments. However, as epigenetic mechanisms are known to act over generations, epigenetic mechanisms can also

create lag and are going to be selected against if environments fluctuate too rapidly.

This study has two aims: the first is to investigate if reaction norms measured in constant temperatures can be used to explain the growth rate of the filamentous fungus *Neurospora crassa* in fluctuating temperatures, and if not, why do reaction norms measured in constant environments fail? The second is to investigate the role of epigenetic mechanisms in fluctuating environments: does the absence of certain chromatin modifications change the shape of reaction norms in fluctuating environments, indicating that this specific mechanism is needed in fluctuating environment? We used two temperatures: 30 and 40 °C and different times of how long the cultures spent in each temperature, ranging from 30 to 720 minutes. We tested different models on how a reaction norm could be used to predict growth in a fluctuating environment, such as simply the mean of the growth in the two different environments or the sum of growth expected from the time spent in each environment. We also investigated how long it takes for the fungus to acclimate from one temperature to another, that is, to reach a constant growth rate in the new temperature. In order to investigate the importance of epigenetic mechanisms in response to fluctuating temperatures, we used a collection of deletion mutants that each lacked a certain epigenetic mechanism and grew these mutants in the different fluctuating environments.

We find that reaction norms measured in constant environments do not predict well growth rate in fluctuating environments if fluctuations happen often due to the time it takes for the fungus to acclimate to the new temperature. In a model where lag times are taken into account better predictions for growth rate are obtained. Epigenetic mechanisms, in particular histone modifications H3K36 methylation and H3K4 trimethylation, have some effects in fluctuating environments but in general epigenetic mechanisms do not seem as important as in constant environments in *N. crassa*.

Materials and methods

Neurospora strains

We previously obtained a set of deletion mutants from the *Neurospora* knockout mutant collection [35] from Fungal Genetics Stock Center (FGSC) [36] and backcrossed these mutants to the wild type strain 2489 [31]. Backcrossing was done in order to make sure that the genetic background of the mutants is the same, and they differ only in the deleted gene from the control. Mutants used in this study are listed in table 1, all strains were of mating type A. Procedures used for backcrossing and confirming the presence of the deletions by PCR can be found in Kronholm et al. [31]. In comparison to Kronholm et al. [31] we did not include some mutants that grew very poorly in general. In total the experiment contained 20 mutants and the wild type strain, 21 strains in total.

Environmental fluctuations

We used programmable growth chambers (MTM-313 Plant Growth Chamber, HiPoint Corp., Taiwan) to change the temperature periodically. We used fluctuations with an amplitude of 10 °C from 30 °C to 40 °C. In the first experiment, we had five different fluctuation regimes. In each fluctuation regime temperature changed in a stepwise manner (Supplementary figure S1), so that during one cycle cultures spent half of the time in 30 °C and half of time in 40 °C. Thus the mean temperature for each treatment was 35 °C and the total time spent in 30 and 40 °C the same. The different fluctuation regimes were such that cultures spent either 30, 60, 90, 120, or 150 min in one temperature during one cycle, thus fluctuations had periods of 1, 2, 3, 4, or 5 h. In the second experiment we increased the time of the fluctuations and used three different regimes where cultures spent 240, 480, or 720 min in one temperature during one cycle, thus periods were, 8, 16, and 24 h. Throughout this study, when discussing the different fluctuation regimes we will refer to the time cultures spent in one temperature as duration of a step.

In a preliminary experiment we measured the rate of temperature equilibration between growth chamber air and the agar tubes used to measured growth rates of the strains. We inserted a temperature probe into the agar of a growth tube and monitored the temperature in the growth tube and the air temperature in the growth chamber during heating and cooling. It took about twelve minutes for the growth chamber air temperature to rise from 30 to nearly 40 °C, while it took approximately 20 minutes for temperature in the agar to do so (Supplementary figure S2). During heating the growth chamber first raised the temperature nearly to the set point, but to prevent overshooting the set temperature, raising the temperature the last remaining degree took most of the elapsed time. Cooling was more efficient and it took approximately six minutes for the agar temperature to drop from 40 to 30 °C. Therefore the actual times the cultures spent in 40 and 30 °C were shorter than the nominal temperature settings, and cycles of 30 min at each temperature were the shortest fluctuations possible that could be achieved with our system.

Growth measurements

We grew the strains on Vogel's standard growth medium [37] with 1.5% agar in disposable 25 ml pipettes prepared by the method of White and Woodward [38]. At the start of each experiment a strain was inoculated at the other end of the pipette and the growth of a strain was followed by marking the position of the mycelial front twice a day. Growth was followed for a period of 104 h, and growth rate was obtained as the slope of the regression line for time against distance. Detailed description of the growth measurements can be found in Kronholm et al. [31].

During the first experiment we grew the 21 strains in the 5 different fluctuation regimes with 5 replicates each. Giving 525 growth assays in total. In the second experiment we grew 20 strains in 3 fluctuation regimes with 5 replicates, giving 300 growth assays in total, strain *nst-7* was missing from the second experiment. The growth chamber we used had three different compartments that could be controlled independently, and with two chambers we had six

different compartments available. To control for growth chamber compartment effects, we ran the different replicates in different compartments. The treatments were randomly distributed across growth chamber compartments and different strains of a single replicate were always randomised within a compartment.

Temperature shift experiments

We performed separate temperature shift experiments to measure more accurately how the growth rate changes, and if there was a lag time in growth rate profile during which acclimation to a new temperature took place, when *Neurospora* experienced a change in temperature. For the temperature shift experiments we used two genotypes: the wild type control genotype 2489 and the *qde-2* mutant, each genotype was replicated 10 times giving 40 growth assays in total. We included *qde-2* in these experiments as our previous results suggested that it is involved in temperature responses [31]. All mutants could not be included in these experiments for logistical reasons. We initially inoculated cultures and grew them in either constant 30 or 40 °C for 24 h to allow the cultures to acclimate to the current temperature. Then we swapped the cultures between the two different incubators, those cultures that had been initially growing in 30 °C were moved to 40 °C and vice versa. As the growth chambers were constantly at the same temperature and the cultures were shifted, there was no time lag associated with heating or cooling the growth chamber, only the heat transfer to agar medium itself. After the shift we recorded the growth of the cultures every hour for 8 hours to allow fine grain monitoring of changes in growth rate. For analysis we only calculated growth rates for two hour intervals as recording one hour of growth in the tubes was difficult as the marks were very close to each other and thus the data measured every two hours provided a reasonable smoothing of measurement noise.

Data analysis

Analysis of growth rates in fluctuating environments

We used a linear mixed model to investigate the effects of the different fluctuation regimes on the growth of the different strains. We first fitted the growth chamber effect to the data and removed the growth chamber effect by subtracting the effect from the raw data. Because the strains and treatments were randomly distributed, removing the average effects of growth chambers has no risk of removing strain or treatment effects. We encoded the different step durations as factors to allow us to fit the same model for all strains in the case that some of the responses were non-linear. The model was

$$y_{ij} = \mu + F_i + G_j + F_i \times G_j + \epsilon \tag{1}$$

where μ is the intercept, F_i is the ith step duration, G_j is the jth genotype, ϵ is the residual, and y_{ij} is the growth rate of the jth genotype in step duration i. The duration of the step was fitted as a fixed effect and genotype as a random effect.

The mixed model was fitted with the "lmer" function in R [39] and statistical testing performed with the "lmerTest" package [40]. The lmerTest package implements F-tests using type III sums of squares with Satterthwaite correction for degrees of freedom. For comparing reaction norm shape for individual genotypes to the control we performed a pairwise ANOVA where were compared each of the mutants to the control strain and tested if the genotype by step duration interaction was significant. Correction for multiple testing was done using the Bonferroni-Holm method [41].

Growth modeling based on temperature shift experiments

In order to model growth in fluctuating environments we used the empirical data obtained from the temperature shift experiments to obtain the acclimation times and tested if we could predict growth in fluctuating environments based on this data. First, we fitted non-linear regression curves to the temperature shift data separately for each direction of temperature change using the model

$$y(t) = \beta_0 - \beta_1 2^{-t/\beta_2} + \epsilon \tag{2}$$

where y is the growth rate, β_0 is the asymptote or the final growth rate after acclimation, β_1 is the amount of growth rate to be gained (i.e. $\beta_0 - \beta_1$ is the growth rate immediately after temperature change), β_2 is the time taken to gain half the amount remaining to be gained, t is the time elapsed since temperature change, and ϵ is the residual [42]. This yielded a set of parameters β_0, β_1, and β_2 estimated for each direction of temperature change. Fitting this form of negative exponential function as the model has the advantage that all the parameters have a biological interpretation. When fitting the model to the shift from 30 to 40 °C data we excluded data points from constant 30 °C and assumed that growth rate drops faster than the resolution in our data collection. For fitting the model we used the "nls" function in R [39].

After obtaining numerical estimates for equation 2 for both acclimation directions, we defined two lag functions giving the instantaneous growth rate as a function of time t elapsed since temperature change as

$$l_i(t) = \beta_{0,i} - \beta_{1,i} \times 2^{-t/\beta_{2,i}} \tag{3}$$

where $i = 1$ for a change from 30 to 40 °C and $i = 2$ for a change from 40 to 30 °C. Then we used growth rate models to predict growth in fluctuating environments. In our initial model, growth rate r_p in a fluctuating environment where step duration was p hours during one cycle was

$$r_p = \frac{\int_0^p l_1(t)\,\mathrm{d}t + \int_0^p l_2(t)\,\mathrm{d}t}{2p} \tag{4}$$

where the numerator gives the amount for growth (in mm) that occurs during one temperature cycle, $l_1(t)$ and $l_2(t)$

are the lag functions obtained from fitting equation 2 to the different temperature shifts and using the β parameters obtained empirically. We integrate over the time spent in given temperature to obtain the predicted growth that occurs after temperature shift, the denominator gives the total time (in h) for one full temperature cycle to obtain the growth rate as mm/h.

As our initial lag model did not work very well for fast fluctuations (see results) we later refined our inital model to account for partial acclimation. In the fast fluctuations the predicted lag times are much longer than the periods of temperature fluctuations. Therefore acclimation happens only partially before the temperature changes and consequently next acclimation does not begin from the growth rate the culture would eventually reach in constant temperature. We modified the model so that we update the parameter β_1, so setting the amount of growth rate to be gained in the next cycle to reflect the growth rate reached in the previous temperature.

The refined model followed the following algorithm: a culture spent p hours first in 30 °C and we calculated the distance grown, then we calculated the current growth rate, r^*, at time p. If $r^* < \beta_0$ of the next lag function ($\beta_{0,2}$ during first cycle), then we set $\beta^*_{1,2} = \beta_{0,2} - r^*$. Otherwise $\beta^*_{1,2} = \beta_{1,2}$. We then calculated the distance grown using the updated l_2 function. After p hours of growth in the next temperature, current growth rate was again calculated and it was checked if $r^* < \beta_{0,1}$, then we set $\beta^*_{1,1} = \beta_{0,1} - r^*$, and to $\beta^*_{1,1} = \beta_{1,1}$ otherwise. We then calculated the distance grown using the updated l_1 function. The empirical measurements for the different strains in the fluctuating environments were done over 103 hours, so the number of temperature cycles in the model, n, was determined by how many full cycles of $2p$ could fit into 103 hours. The growth rate was obtained from

$$r_p = \frac{\sum_{k=1}^{n} \left(\int_0^p l_{1,k}(t)\,\mathrm{d}t + \int_0^p l_{2,k}(t)\,\mathrm{d}t \right)}{2np} \tag{5}$$

where the $\beta_{1,i}$ parameters were updated for each step k as described above.

We compared how well the simulation models fit the observed data by calculating mean squared deviations from the observed data for each model. We can use mean squared deviations (MSD) for comparing the relative ranks of the different models, but MSD does not tell us how well the model fits the data in an absolute sense. We chose to use MSD because traditional measures of model fit, like R^2 or AIC, cannot be calculated for our simulated models.

Results

Effect of temperature fluctuations on growth

At constant 30 °C the control genotype 2489 grows at 4.4 mm/h and at constant 40 °C at 2.4 mm/h [31]. The control strain has its optimum at 35 °C (Figure 1A). In our initial experiments we observed that the growth of the control strain

in fluctuating environment with step duration from 30 to 150 min was mainly determined by its growth in constant 40 °C (Figure 1B), as growth rate of 2489 in all of the temperature regimes was very close to 2.4 mm/h and is only slightly higher. There was a tendency for the growth to get faster when the period of the fluctuations was increased. Under the assumption that acclimation to 30 and 40 °C would be instantaneous and growth rate would change immediately to corresponding growth rate at constant temperature, the growth rate in fluctuating temperature per hour would be average of the growth rates in constant 30 and 40 °C because under all fluctuation regimes the cultures spend equal amount of time in 30 and 40 °C. This naive expected growth rate would be 3.38 mm/h, the observed growth rates in fluctuating temperatures are well below this rate (Figure 1B).

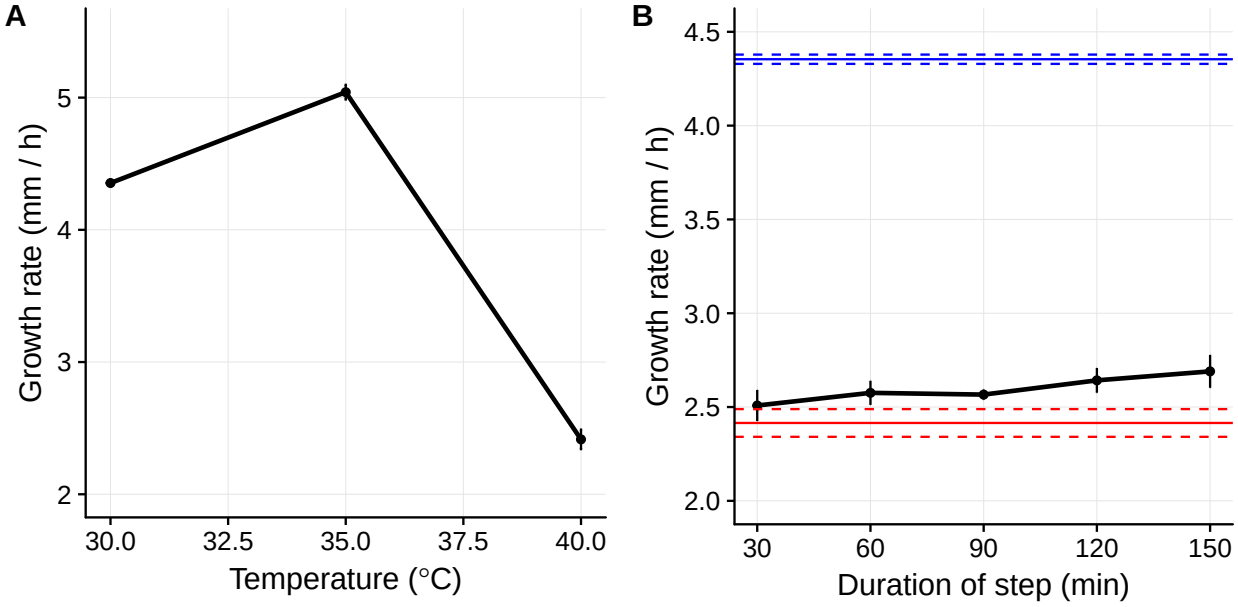

Figure 1: A) Reaction norm for the genotype 2489 in constant temperatures, n = 12 for each point. Data from Kronholm et al. [31]. B) Reaction norm for the genotype 2489 in different step durations when temperature fluctuates between 30 and 40 °C, n = 5 for each point. Horizontal blue and red lines show growth rate at constant 30 and 40 °C, respectively. Error bars and dashed lines show means ± SE.

Estimating acclimation times

Since growth in environments that experienced short term fluctuations in temperature could not be explained by the naive model of instant acclimation, and was more similar to growth in constant 40 °C, we suspected that there was a lag time in growth rate changes, when the cultures were acclimating from one temperature to another. To estimate how fast growth rates changed we performed temperature shift experiments, where we shifted cultures from 30 to 40 °C and vice versa and monitored how growth rate changed. Acclimation times were estimated for two different genotypes, the control genotype 2489 and the *qde-2* mutant. When cultures were shifted from 30 to 40 °C growth rate initially dropped to around 2 mm/h and then recovered to around 2.6 mm/h (Figure 2A). This took around 6 hours,

there was an increase in the growth rate around 8 hours that but later the growth rate seems to stabilise to slightly lower value. The origin of this transient increase is unknown, it shows up earlier also in the *qde-2* mutant, so it probably cannot be attributed to a random fluctuation in the growth chamber conditions. However, the overall pattern of low initial growth rate after the switch and subsequent recovery was clear. When cultures were shifted from 40 to 30 °C growth rate recovered to the growth rate at constant 30 °C but this took up to 8 hours (Figure 2B). These data show that temperature acclimation in *Neurospora* is not instantaneous but can take up to 6 to 8 hours. Furthermore, acclimation is not symmetric, as the direction of environmental change produces a different sort of response, with recovery from high temperature taking longer than acclimation to high temperature. Acclimation times were much longer than time it took for growth chambers to equilibrate to a certain temperature (Supplementary figure S2), so technical reasons cannot explain asymmetries in acclimation. Control and *qde-2* differed in their responses, in particular the *qde-2* mutant recovers slower from high temperature than the control strain (Figure 2B).

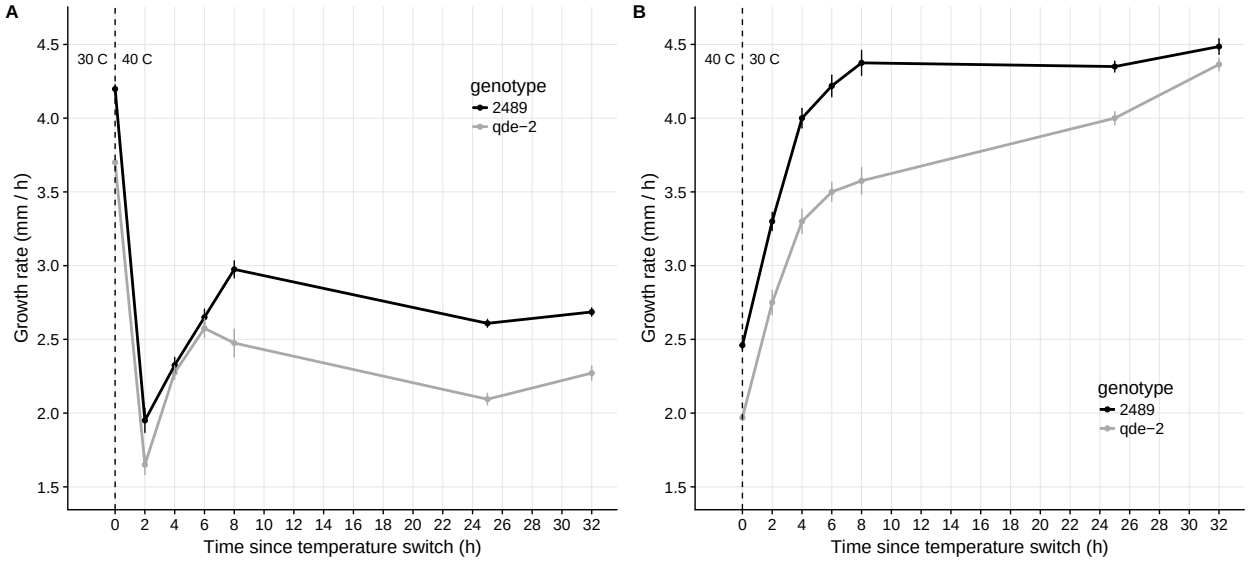

Figure 2: Changes in growth rate after temperature change. Lines and error bars show means ± SE. A) Temperature changes from 30 to 40 °C. B) Temperature changes from 40 to 30 °C.

Modeling growth in fluctuating environments

The long lag times provide a plausible mechanistic explanation for why the growth in rapidly fluctuating environments was close to the growth in constant 40 °C. We tested this by fitting a growth model based on the observed lag times. We first fitted non-linear regressions to the lag data to estimate empirical parameters for the lag functions (Equation 3, Table 2). Then we used those lag functions to predict the growth rates in fluctuating environments with different step durations in 30 and 40 °C. In the initial lag model we calculated predicted growth rates by integrating over the times the culture spent in the different temperatures. Comparing the predictions from the initial lag model to the entire

observed data set shows that the initial lag model worked poorly (Figure 3), particular in short step durations. Probable reason for failure of the initial model is that in short step durations there was not enough time to acclimate completely and reach the expected growth rate under constant conditions. We then refined the initial model to account for partial acclimation during the short time intervals, the refined model fits the data much better (Figure 3). To assess how well each model fits the data, we calculated mean squared deviations for each predicted value from the observed data as a measure of model fit for the two different lag models, naive expectation of no lag, and for growth in constant 40 °C. The model with no lag had the highest deviation of 0.4, then the initial lag model had a deviation of 0.31 and growth at constant 40 °C also had a deviation of 0.31, the refined model had a much smaller deviation of 0.02. All the other models are clearly inferior compared to the refined lag model. These results indicate that growth rate of *Neurospora* in the fluctuating environments can be predicted to a reasonable degree if the lag functions are known.

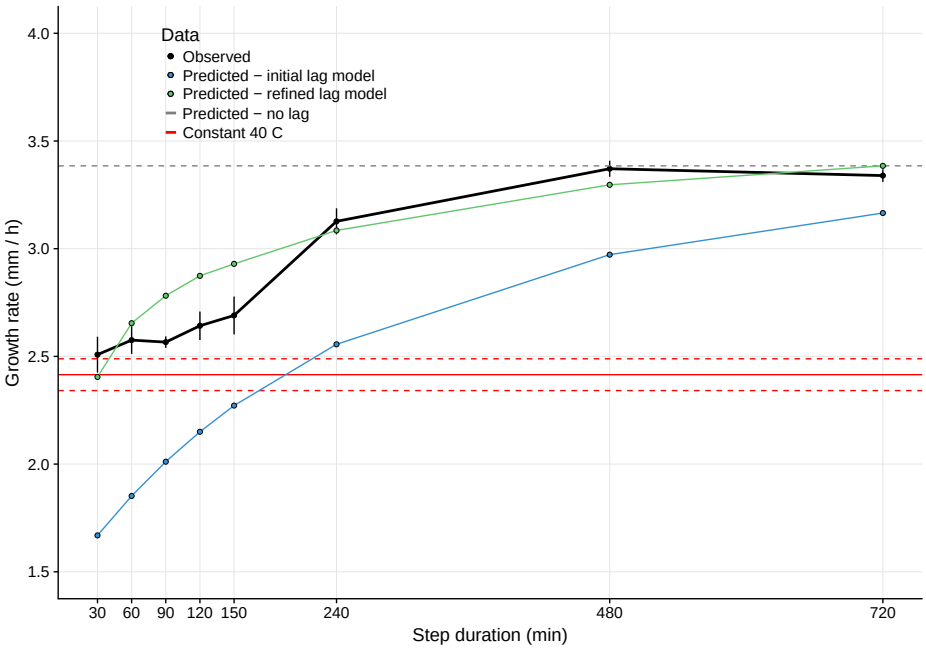

Figure 3: Comparison of how well different growth models with lag fit to the observed fluctuation data of genotype 2489. Red line is the growth in constant 40 °C, dashed lines and error bars show mean ± SE. Grey dashed line is predicted growth rate if there were no lag.

Effects of epigenetic mechanisms in fluctuating environment

Next we tested if epigenetic mechanisms were important for growth in fluctuating environments. We combined the data from our initial experiment with short step durations and a subsequent experiment with longer step durations. Growth rate of most strains is around 2.51 mm/h with step duration of 30 min and increases to 2.70 mm/h when step duration increases to 150 min. With longer step durations growth rate further increases to around 3.34 mm/h (Figure 4). In a mixed model the effect of step duration was significant (F = 383.14, numerator df = 7, denumerator df = 137.7,

$p < 2.2 \times 10^{-16}$), showing that different step durations affected the growth of the strains in fluctuating environments. Reaction norms for growth in fluctuating environments of the genotypes show that in general there was a small increase in growth rate for short step durations until the 150 minute step, and then growth rate increased faster as the strains had more time to acclimate in the 240 and 480 minute steps but growth rate plateaued after that (Figure 4). We also tested whether the small increase in growthrate observed for the short step durations was significant by analyzing the steps from 30 to 150 minutes separately. In this model the effect of duration of the step was significant (F = 18.69, numerator df = 4, denumerator df = 489.01, p = 2.62×10^{-14}), indicating that while the effect of duration of the step in short timescales may be small, it is likely a real effect.

There were differences in reaction norm elevation among the different genotypes, as shown by a significant genotype effect (χ^2 = 387.62, df = 1, p = 2.73×10^{-86}). Differences in reaction norm elevation indicate that some mutants grew slower overall (Figure 4), some of the mutants are undoudtedly impaired in their normal cellular metabolism and grow slower overall as a consequence. However, we are interested in differences in reaction norm shape, which indicates that responses to the environment are different in the different strains. There was some evidence that reaction norm shapes were different as indicated by a significant step duration $\times$ genotype interaction (χ^2 = 17.18, df = 1, p = 3.4×10^{-5}). As some strains respond to the environment differently, this suggests that epigenetic mechanisms may play a role in growth in fluctuating temperatures. In order to identify which mutants react differently than than the control strain, we performed a pairwise ANOVA where we tested each mutant separately (Table 3). Next we will look at each of the mutant classes, that correspond to a specific mechanism, in more detail.

DNA methylation

Reaction norms for growth rate in fluctuating environments of strains with deficiencies in DNA methylation are shown in Figure 4A. The *dim-2* mutant lacks a DNA methyltransferase enzyme but shows no difference to the control. Indicating that DNA methylation is not important for temperature response in *Neurospora*. The *dmm-2* mutant is deficient in containing DNA methylation to heterochromatic regions, and as a consequence DNA methylation spreads to normally unmethylated regions in this mutant. It grows slower than the control but responds to different step durations the same way as the control. These results are consistent with our previous results for temperature in constant environments [31].

Histone methylation

We tested three different mutants with deficiencies in histone methylation, *set-1* which lacks H3K4 trimethylation, *set-2* which is impaired in H3K36 methylation, and *set-7* which lacks H3K27 trimethylation. The *set-7* mutant was not different from the control (Figure 4B), but both *set-1* and *set-2* had lowered reaction norm elevation. Furthermore both *set-1* and *set-2* differed from the control in reaction norm shape (Table 3). Both genotypes have reached their

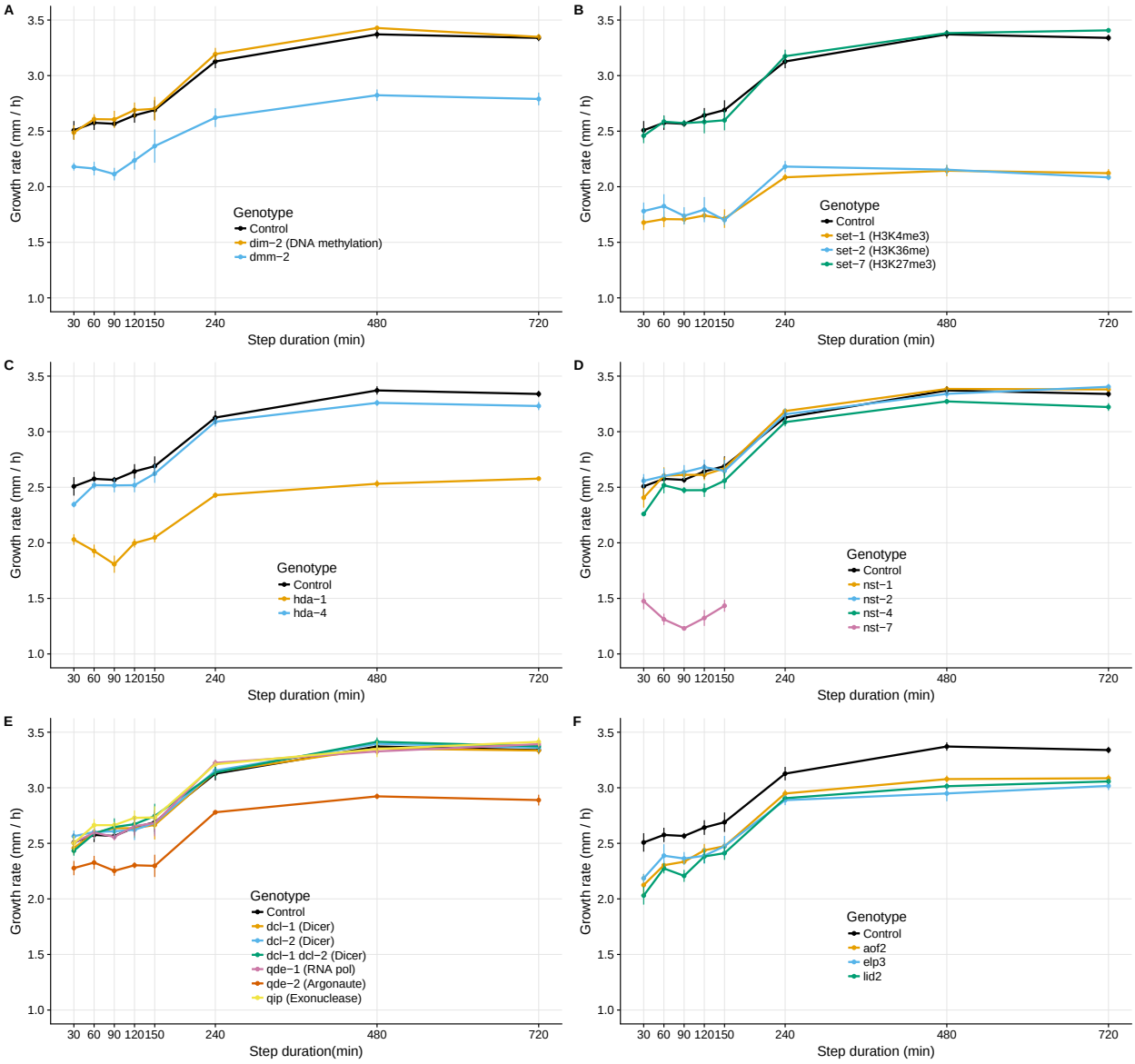

Figure 4: Reaction norms of different classes of mutants. Lines and error bars show means ± SE. A) DNA methylation mutants. B) Histone methylation mutants. C) Histone deacetylation class I mutants. D) Histone deacetylation class III mutants. E) RNA interference mutants. F) Histone demethylation and acetylation mutants.

maximal growth rate already at the 240 min step, and during the short fluctuations they don't increase their growth rate as much as the control (Figure 4B). These results are also consistent with our previous findings that *set-1* and *set-2* contribute to phenotypic plasticity in response to temperature in constant environments [31]. However, as *set-1* and *set-2* grew slower than the control, we evaluated if changes in reaction norm shape could conceivably be the result of these strains just reaching their steady-state growth rate faster if they have to acclimate less than the control even if they are acclimating at the same rate. We calculated the expected growth rate under the assumption of no lag from their growth rates at constant temperature, data obtained from Kronholm et al. [31]. For *set-1* its growth rate in 720 min step was 2.12 mm/h, which is slightly below its no lag expectation of 2.32 mm/h. For *set-2* growth rate in 720 min step was 2.08 mm/h and this was exactly at the no lag expectation of 2.08 mm/h. Thus, for *set-2* it is possible that differences in reaction norm shape reflect just lower acclimation requirement. However, we cannot exclude other possibilities as both mutants also do not seem to increase their growth rate as much as the control in short step durations (Figure 4B).

Histone deacetylation

We tested two mutants of the histone deacetylase class I genes: *hda-1* and *hda-4*, and four mutants of the histone deacetylase class III genes: *nst-1*, *nst-2*, *nst-4*, and *nst-7*. The *hda* mutants grew slower overall than the control (Figure 4C, Table 3), but there were no significant differences in reaction norm shape. For the *nst* mutants, *nst-1* and *nst-2* did not differ from the control, *nst-4* had slightly lower elevation than the control but same shape, and *nst-7* grew very poorly overall indicating severe problems in normal cellular functioning (Figure 4D, Table 3).

RNA interference

Neurospora produces a diverse set of different small RNAs, we tested mutants in the RNA interference pathway; including the two Dicer ribonuclease genes *dcl-1*, *dcl-2* and their double mutant *dcl-1 dcl-2*, *qde-1*, *qde-2*, and *qip*. All other mutants except *qde-2* did not differ from the control (Figure 4E). The *qde-2* mutant grew overall slower than the control, but its reaction norm shape was not different from the control (Table 3). In short step duration, it looks like *qde-2* does not increase its growth rate like the control, but this effect was not significant. In the temperature shift experiments *qde-2* acclimated slower than the control, but our power to detect subtle shape changes in reaction norms may not be enough in this case.

Histone demethylation and acetylation

The ELP3 protein is an inferred histone acetyl transferase, while LID2 and AOF2 are inferred histone demethylases. All three mutant strains have a lower growth rate than the control (Figure 4F), but they have the same reaction norm shape as the control (Table 3). This was also observed in a previous experiment in constant temperatures [31].

Discussion

Growth in fluctuating environments

We investigated stepwise fluctuations in temperature and could show that for *N. crassa* fast fluctuating environment slowed growth down more than expected under from growth rates under constant temperatures, and this was mostly due to the time lag it took to recover from hot temperature. If fluctuations were faster than recovery times the fungus never reached its higher growth rate in the cooler temperature. Once the duration of fluctuation steps increased beyond recovery time growth in fluctuating environment sped up to or near the levels one would expect based on reaction norms in obtained in constant temperatures.

There has been lot of interest in predicting performance in fluctuating environments based on reaction norms measured in constant environments [16, 17, 18, 19, 43, 21]. Our results show that prediction works in some circumstances: intuitively, if fluctuations are much slower than the time it takes to acclimate to a new temperature, then performance in constant environments predicts performance in a fluctuating environment. In addition, if fluctuations happen faster than acclimation time, then performance in fluctuating environments can be predicted to some degree if acclimation times, in our case lag functions, are known. However, there are some complications with this in our system that are likely to be present in other systems as well. Importantly, the lag functions we estimated for *N. crassa* were not symmetric and the shape of the functions is likely to change when the organism has only partially acclimated to a new temperature and the temperature changes again. This complication is reflected in our results, our refined lag model gave the worst predictions in step durations of 60 - 150 min where some acclimation had happened but which was not enough time to completely acclimate. With longer step durations predictions of the model were much better, and was nearly completely in line with observed data. These results reflect what has been found in bacteria, as experimental evolution under slow fluctuations caused evolution of a tolerance curve that indicated adaptation to fluctuations, whereas fast fluctuations did not [44, 18, 20].

In a recent modeling study Kingsolver and Woods [43] introduced a general model to predict performance in fluctuating environment specifically accounting for time-dependent effects. Time-dependent effects in this model were framed in terms of heat shock protein production and degradation. It was assumed that both production and degradation happen at similar rates, this assumption is likely to be violated in several cases, as highlighted by our data in that acclimation responses were asymmetric. More empirical estimates of acclimation responses are needed so that these assumptions can be relaxed in future work on performance curves.

Role of epigenetic mechanisms in fluctuating environments

Our results suggest that in general epigenetic mechanisms do not have large effects in responding to fluctuating environments. This is in contrast to our results where we investigated the role of epigenetic mechanism in phenotypic plasticity in constant environments, where much stronger effecs were observed [31]. One reason for this may be that the time frame of environmental fluctuations investigated here may be faster than the normal timescale of epigenetic regulation. The longest period investigated here was 24 h. Transcriptional response to environmental changes can be rapid [45, 46] and occur within minutes, and in yeasts the half-life of most proteins is around ten hours, but with considerable heterogeneity among different proteins [47]. While there is evidence that some chromatin state changes occur in a circadian manner [48], and thus there was potential for epigenetic changes to occur during the fluctuations, not much is known about the time scale of epigenetic regulation in general. Since the mutants did not show clear sensitivity to different durations of fluctuations this suggest that lag functions are not epigenetically modified, either.

Of the epigenetic effects we did observe, one was that of *set-1* mutant, which is deficient in histone 3 lysine 4 trimethylation, and has improper expression of certain genes [49]. The H3K4me3 modification has been associated with 5'-regions of actively transcribed genes [50, 51], and thus the absence of this histone mark may interfere with the proper activation of correct transcription response required for acclimation. The H3K4me3 mark is required for proper expression of the circadian clock in *Neurospora* [49], and the clock is affected by temperature [52]. The temperature changes used in this study were large enough to reset the clock [52]. However, in previous experiments mutants affecting the circadian period did not show any differences in growth rate [53]. Thus it is possible that temperature related effects of *set-1* are not mediated through the clock.

The histone mark histone 3 lysine 36 methylation, which is absent in the *set-2* mutant [54], may also be important in fluctuating environments. The role of H3K36me is likely to keep transcriptionally active regions of the chromosome in open conformation [55] and the mark is present in actively transcribed regions [56, 57]. We have previously observed that *set-2* is important in temperatures above 25 °C [31]. If the absence of H3K36 methylation slows or otherwise interferes with rates of transcription at high temperatures this can explain the observed results that the *set-2* mutant also affects growth in fluctuating environments.

It may be surprising that we did not observe significant effect for the *qde-2* mutant in fluctuating environments, while in constant temperatures we find evidence that *Neurospora* ARGONAUTE homolog encoded by *qde-2* is important in response to temperature [31]. We also observed some indications of this in the temperature shift experiments, where the *qde-2* mutant acclimated slower than the control strain. However, it may be that this difference is not large enough to be detected when we let the strains grow in fluctuating environments. QDE-2 protein is responsible for processing of diverse types of RNAs, such as siRNAs [58], microRNA-like RNAs [59] in *Neurospora* and small RNAs have been linked to temperature responses in different organisms [60, 31, 61]. Small RNAs have the potential to

act as thermoregulatory molecules as they can adopt different conformations based on temperature, and especially in prokaryotes there are examples of RNAs acting as thermosensors [62].

Conclusions

Predicting performance in fluctuating environments depends on the timescale of environmetal fluctuations and acclimation functions of the organism in question. Increasing the complexity of the models allows taking lags into account but empirical data are needed to parametrize the models. An added complication is that acclimation seems to be asymmetric and the shape of acclimation functions is likely to change when the temperature range of acclimation changes. Overall, these results warrant caution when using reaction norms to predict performance in fluctuating environments. One cannot simply use data measured in constant environments and extrapolate to fluctuating environments, but by measuring acclimation functions with careful experiments, reasonably accurate models for growth in fluctuating environments can be constructed. Such experiments can also give insight into the mechanisms of acclimation. Surprisingly, we did not find strong effects for epigenetic mechanisms that were specific to fluctuations per se, and it seems that average temperature or, slower variations what we explored in this study, are more important for physiological responses to temperature that are dependent on epigenetic mechanisms.

Data accessibility

Data and R scripts for analysis will be deposited in Dryad Digital Repository upon acceptance.

Authors' contributions

I.K. and T.K. conceived the study and designed the experiments, I.K. performed the experiments, I.K. and analysed the data with input from T.K., I.K. wrote the manuscript with input from T.K. Both authors gave their final approval for publication.

Competing interests

We have no competing interests.

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References

[1] DeWitt TJ, Scheiner SM, 2004 Phenotypic variation from single genotypes: a primer. In TJ DeWitt, SM Scheiner, eds., *Phenotypic plasticity: Functional and conceptual approaches*. Oxford University Press, Inc.

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

[3] Chevin LM, Lande R, Mace GM, 2010 Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLoS Biol* **8**, e1000357

[4] Scheiner SM, 2002 Selection experiments and the study of phenotypic plasticity. *Journal of Evolutionary Biology* **15**, 889–898

[5] Nussey DH, Postma E, Gienapp P, Visser ME, 2005 Selection on heritable phenotypic plasticity in a wild bird population. *Science* **310**, 304–306. doi:10.1126/science.1117004

[6] Day T, Rowe L, 2002 Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. *The American Naturalist* **159**, 338–350

[7] Ingleby FC, Hunt J, Hosken DJ, 2010 The role of genotype-by-environment interactions in sexual selection. *Journal of Evolutionary Biology* **23**, 2031–2045. doi:10.1111/j.1420-9101.2010.02080.x

[8] Falconer DS, 1952 The problem of environment and selection. *The American Naturalist* **86**, 293–298

[9] Kolmodin R, Strandberg E, Madsen P, Jensen J, Jorjani H, 2002 Genotype by environment interaction in nordic dairy cattle studied using reaction norms. *Acta Agriculturae Scandinavica, Section A — Animal Science* **52**, 11–24. doi:10.1080/09064700252806380

[10] Knap PW, Su G, 2008 Genotype by environment interaction for litter size in pigs as quantified by reaction norms analysis. *Animal* **2**, 1742–1747. doi:10.1017/S1751731108003145

[11] Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR, 2008 Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences* **105**, 6668–6672. doi:10.1073/pnas.0709472105

[12] Kingsolver JG, Diamond SE, Buckley LB, 2013 Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology* **27**, 1415–1423. doi:10.1111/1365-2435.12145

[13] Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, McCann KS, Savage V, Tunney TD, O'Connor MI, 2014 Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society of London B: Biological Sciences* **281**. doi:10.1098/rspb.2013.2612

[14] Levy O, Buckley LB, Keitt TH, Smith CD, Boateng KO, Kumar DS, Angilletta MJ, 2015 Resolving the life cycle alters expected impacts of climate change. *Proceedings of the Royal Society of London B: Biological Sciences* **282**. doi:10.1098/rspb.2015.0837

[15] Kingsolver JG, Ragland GJ, Diamond SE, 2009 Evolution in a constant environment: Thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. *Evolution* **63**, 537–541. doi: 10.1111/j.1558-5646.2008.00568.x

[16] Niehaus AC, Angilletta MJ, Sears MW, Franklin CE, Wilson RS, 2012 Predicting the physiological performance of ectotherms in fluctuating thermal environments. *Journal of Experimental Biology* **215**, 694–701. doi: 10.1242/jeb.058032

[17] Rezende EL, Castañeda LE, Santos M, 2014 Tolerance landscapes in thermal ecology. *Functional Ecology* **28**, 799–809. doi:10.1111/1365-2435.12268

[18] 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

[19] Kingsolver JG, Higgins JK, Augustine KE, 2015 Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time-dependent effects. *Journal of Experimental Biology* **218**, 2218–2225. doi:10.1242/jeb.120733

[20] Ketola T, Kristensen TN, 2017 Experimental approaches for testing if tolerance curves are useful for predicting fitness in fluctuating environments. *Frontiers in Ecology and Evolution* **5**, 129. doi:10.3389/fevo.2017.00129

[21] 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

[22] 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. doi:10.1093/icb/icr097

[23] Johnston IA, Dunn J, 1987 Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. *Symposia of the Society for Experimental Biology*

[24] Padilla DK, Adolph SC, 1996 Plastic inducible morphologies are not always adaptive: the impotance of time delays in a stochastic environment. *Evolutionary Ecology* **10**, 105–117

[25] Feder ME, Hofmann GE, 1999 Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology* **61**, 243–282. doi:10.1146/annurev.physiol.61.1.243. PMID: 10099689

[26] Bird A, 2002 DNA methylation patterns and epigenetic memory. *Genes & Development* **16**, 6–21. doi: 10.1101/gad.947102

[27] Bannister AJ, Kouzarides T, 2011 Regulation of chromatin by histone modifications. *Cell Research* **21**, 381–395

[28] Gaydos LJ, Wang W, Strome S, 2014 H3K27me and PRC2 transmit a memory of repression across generations and during development. *Science* **345**, 1515–1518. doi:10.1126/science.1255023

[29] de la Paz Sanchez M, Aceves-García P, Petrone E, Steckenborn S, Vega-León R, Álvarez-Buylla ER, Garay-Arroyo A, García-Ponce B, 2015 The impact of Polycomb group (PcG) and Trithorax group (TrxG) epigenetic factors in plant plasticity. *New Phytologist* **208**, 684–694. doi:10.1111/nph.13486

[30] Kooke R, Johannes F, Wardenaar R, Becker F, Etcheverry M, Colot V, Vreugdenhil D, Keurentjes JJ, 2015 Epigenetic basis of morphological variation and phenotypic plasticity in *Arabidopsis thaliana*. *Plant Cell* **27**, 337–348. doi:10.1105/tpc.114.133025

[31] Kronholm I, Johannesson H, Ketola T, 2016 Epigenetic control of phenotypic plasticity in the filamentous fungus *Neurospora crassa*. *G3: Genes|Genomes|Genetics* **6**, 4009–4022. doi:10.1534/g3.116.033860

[32] Wibowo A, *et al.*, 2016 Hyperosmotic stress memory in *Arabidopsis* is mediated by distinct epigenetically labile sites in the genome and is restricted in the male germline by DNA glycosylase activity. *eLife* **5**, e13546. doi: 10.7554/eLife.13546

[33] Luna E, Ton J, 2012 The epigenetic machinery controlling transgenerational systemic acquired resistance. *Plant Signaling & Behavior* **7**, 615–618

[34] Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, Sun JY, Agrawal AA, Felton GW, Jander G, 2012 Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiology* **158**, 854–863. doi:10.1104/pp.111.187831

[35] Colot HV, Park G, Turner GE, Ringelberg C, Crew CM, Litvinkova L, Weiss RL, Borkovich KA, Dunlap JC, 2006 A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proceedings of the National Academy of Sciences* **103**, 10352–10357

[36] McCluskey K, Wiest A, Plamann M, 2010 The fungal genetics stock center: a repository for 50 years of fungal genetics research. *J Biosci* **35**, 119–126

[37] Metzenberg RL, 2003 Vogel's medium N salts: Avoiding the need for ammonium nitrate. *Fungal Genetics Newsletter* **50**, 14

[38] White B, Woodward D, 1995 A simple method for making disposable race tubes. *Fungal Genetics Newsletter* **42**, 79

[39] R Core Team, 2013 *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria

[40] Kuznetsova A, Brockhoff PB, Christensen RHB, 2015 *lmerTest: Tests in Linear Mixed Effects Models*. R package version 2.0-29

[41] Holm S, 1979 A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6**, 65–70

[42] Venables WN, Ripley BD, 2002 *Modern Applied Statistics with S*. New York: Springer, 4th edn.

[43] Kingsolver JG, Woods HA, 2016 Beyond thermal performance curves: Modeling time-dependent effects of thermal stress on ectotherm growth rates. *The American Naturalist* **187**, 283–294. doi:10.1086/684786. PMID: 26913942

[44] Ketola T, Mikonranta L, Zhang J, Saarinen K, Örmälä AM, Friman VP, Mappes J, Laakso J, 2013 Fluctuating temperature leads to evolution of thermal generalism and preadaptation to novel environments. *Evolution* **67**, 2936–2944

[45] Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, Young RA, 2001 Remodeling of yeast genome expression in response to environmental changes. *Molecular Biology of the Cell* **12**, 323–337. doi:10.1091/mbc.12.2.323

[46] Chechik G, Koller D, 2009 Timing of gene expression responses to environmental changes. *Journal of Computational Biology* **16**, 279–290. doi:10.1089/cmb.2008.13TT

[47] Christiano R, Nagaraj N, Fröhlich F, Walther TC, 2014 Global proteome turnover analyses of the yeasts *S. cerevisiae* and *S. pombe*. *Cell reports* **9**, 1959–1965

[48] Sahar S, Sassone-Corsi P, 2013 The epigenetic language of circadian clocks. In A Kramer, M Merrow, eds., *Circadian clocks. Handbook of experimental pharmacology*, vol. 217. Berlin, Heidelberg: Springer

[49] Raduwan H, Isola AL, Belden WJ, 2013 Methylation of histone H3 on lysine 4 by the lysine methyltransferase SET1 protein is needed for normal clock gene expression. *Journal of Biological Chemistry* **288**, 8380–8390

[50] Pokholok DK, *et al.*, 2005 Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* **122**, 517–527. doi:10.1016/j.cell.2005.06.026

[51] Ardehali MB, Mei A, Zobeck KL, Caron M, Lis JT, Kusch T, 2011 *Drosophila* Set1 is the major histone H3 lysine 4 trimethyltransferase with role in transcription. *EMBO J* **30**, 2817–2828. doi:10.1038/emboj.2011.194

[52] Crosthwaite SK, Heintzen C, 2010 Detection and response of the *Neurospora crassa* circadian clock to light and temperature. *Fungal Biology Reviews* **24**, 114 – 122. doi:http://dx.doi.org/10.1016/j.fbr.2010.10.001

[53] Gardner GF, Feldman JF, 1981 Temperature compensation of circadian period length in clock mutants of *Neurospora crassa*. *Plant Physiology* **68**, 1244–1248. doi:10.1104/pp.68.6.1244

[54] Adhvaryu KK, Morris SA, Strahl BD, Selker EU, 2005 Methylation of histone H3 lysine 36 is required for normal development in *Neurospora crassa*. *Eukaryotic Cell* **4**, 1455–1464. doi:10.1128/EC.4.8.1455-1464.2005

[55] Venkatesh S, Smolle M, Li H, Gogol MM, Saint M, Kumar S, Natarajan K, Workman JL, 2012 Set2 methylation of histone H3 lysine 36 suppresses histone exchange on transcribed genes. *Nature* **489**, 452–455

[56] Hampsey M, Reinberg D, 2003 Tails of intrigue: Phosphorylation of RNA polymerase II mediates histone methylation. *Cell* **113**, 429 – 432. doi:http://dx.doi.org/10.1016/S0092-8674(03)00360-X

[57] Morris SA, Shibata Y, Noma Ki, Tsukamoto Y, Warren E, Temple B, Grewal SIS, Strahl BD, 2005 Histone H3 K36 methylation is associated with transcription elongation in *Schizosaccharomyces pombe*. *Eukaryotic Cell* **4**, 1446–1454. doi:10.1128/EC.4.8.1446-1454.2005

[58] Maiti M, Lee HC, Liu Y, 2007 QIP, a putative exonuclease, interacts with the *Neurospora* Argonaute protein and facilitates conversion of duplex siRNA into single strands. *Genes Dev* **21**, 590–600. doi:10.1101/gad.1497607

[59] Lee HC, *et al.*, 2010 Diverse pathways generate microRNA-like RNAs and Dicer-independent small interfering RNAs in fungi. *Molecular Cell* **38**, 803 – 814. doi:http://dx.doi.org/10.1016/j.molcel.2010.04.005

[60] Chen J, Nolte V, Schlötterer C, 2015 Temperature-related reaction norms of gene expression: Regulatory architecture and functional implications. *Molecular Biology and Evolution* **32**, 2393–2402. doi:10.1093/molbev/msv120

[61] Fast I, Hewel C, Wester L, Schumacher J, Gebert D, Zischler H, Berger C, Rosenkranz D, 2017 Temperature-responsive miRNAs in *Drosophila* orchestrate adaptation to different ambient temperatures. *RNA* doi: 10.1261/rna.061119.117

[62] Sengupta P, Garrity P, 2013 Sensing temperature. *Current Biology* **23**, R304 – R307. doi: http://dx.doi.org/10.1016/j.cub.2013.03.009

Tables

Table 1: Gene ID is based on genome assembly NC12. The function column describes the biochemical activity of the protein. milRNA = microRNA-like RNA.

Mutant	Gene ID	Genotype	Category	Function
dim-2	NCU02247	Δ*dim-2::hph* BC_5 2489	DNA methylation	DNA methyltransferase
dmm-2	NCU08289	Δ*dmm-2::hph* BC_5 2489	DNA methylation	Regulates spreading of DNA methylation
set-1	NCU01206	Δ*set-1::hph* BC_5 2489	Histone methylation	Methylates histone 3 lysine 4 (H3K4)
set-2	NCU00269	Δ*set-2::hph* BC_2 2489	Histone methylation	Methylates histone 3 lysine 36 (H3K36)
set-7	NCU07496	Δ*set-7::hph* BC_6 2489	Histone methylation	Methylates histone 3 lysine 27 (H3K27)
hda-1	NCU01525	Δ*hda-1::hph* BC_5 2489	Histone deacetylation	Histone deacetylase (Class I); partial loss of DNA methylation and increased acetylation
hda-4	NCU07018	Δ*hda-4::hph* BC_3 2489	Histone deacetylation	Inferred histone deacetylase; increased acetylation
nst-1	NCU04737	Δ*nst-1::hph* BC_5 2489	Histone deacetylation	Histone deacetylase (Class III); deacetylates H4K16
nst-2	NCU00523	Δ*nst-2::hph* BC_5 2489	Histone deacetylation	Inferred histone deacetylase
nst-4	NCU04859	Δ*nst-4::hph* BC_5 2489	Histone deacetylation	Inferred histone deacetylase
nst-7	NCU07624	Δ*nst-7::hph* BC_5 2489	Histone deacetylation	Inferred histone deacetylase
qde-1	NCU07534	Δ*qde-1::hph* BC_5 2489	RNA interference	RNA- and DNA-dependent RNA polymerase; initiation of the RNAi pathway
qde-2	NCU04730	Δ*qde-2::hph* BC_5 2489	RNA interference	Argonaute; mutations abolish milRNA processing ability
dcl-1	NCU08270	Δ*dcl-1::hph* BC_5 2489	RNA interference	Dicer ribonuclease; maturation of milRNAs affected
dcl-2	NCU06766	Δ*dcl-2::hph* BC_5 2489	RNA interference	Dicer ribonuclease; maturation of milRNAs affected
dcl-1 dcl-2	NCU08270, NCU06766	Δ*dcl-1::hph;* Δ*dcl-2::hph* BC_5 2489	RNA interference	Dicer ribonuclease double mutant; milRNA maturation defective
qip	NCU00076	Δ*qip::hph* BC_5 2489	RNA interference	Exonuclease; milRNA maturation defective
aof2	NCU09120	Δ*aof2::hph* BC_5 2489	Other	Inferred histone demethylase
lid2	NCU03505	Δ*lid2::hph* BC_5 2489	Other	Inferred histone H3K4 demethylase
elp3	NCU01229	Δ*elp3::hph* BC_5 2489	Other	Inferred histone acetyl transferase

Table 2: Empirical estimates for lag function parameters used in the models. Obtained by fitting equation 2 to the temperature shift data.

Temperature change	Notation	β_0	β_1	β_2
40 to 30 °C	l_1	4.45	2.03	2.06
30 to 40 °C	l_2	2.74	2.25	1.38

Table 3: Comparing all mutants to the control in a pairwise ANOVA. Differences in reaction norm elevation from the control are tested with the genotype effect, differences in reaction norm shape are tested with the genotype step duration interaction effect (G $\times$ E). For genotype effect df = 1, for step duration df = 7, for G $\times$ E df = 7, and for residuals df = 59. Significant G $\times$ E term indicates change in reaction norm shape, p-adj. indicates a p-value adjusted for multiple testing using the Bonferroni-Holm method.

Genotype	F-value Genotype	p-value Genotype	p-adj. Genotype	F-value G $\times$ E	p-value G $\times$ E	p-adj. G $\times$ E
aof2	61.073	1.13E-10	1.46E-09	0.654	0.709	1
dcl-1	0.000	0.997	1	0.163	0.992	1
dcl-2	0.582	0.448	1	0.273	0.962	1
dcl-1 dcl-2	0.482	0.49	1	0.081	0.999	1
dim-2	0.958	0.331	1	0.108	0.998	1
dmm-2	155.624	8.58E-19	1.37E-17	0.803	0.588	1
elp3	56.694	3.43E-10	4.11E-09	0.805	0.587	1
hda-1	641.911	4.52E-35	7.68E-34	2.025	0.0654	1
hda-4	10.298	0.00208	0.0208	0.303	0.95	1
lid2	128.872	5.63E-17	7.88E-16	1.021	0.426	1
nst-1	0.020	0.887	1	0.389	0.906	1
nst-2	1.181	0.281	1	0.223	0.978	1
nst-4	19.003	4.83E-05	0.000532	0.722	0.653	1
nst-7	1960.351	5.85E-43	1.17E-41	2.094	0.0948	1
qde-1	0.242	0.624	1	0.252	0.97	1
qde-2	144.391	4.65E-18	6.98E-17	1.019	0.427	1
qip	3.320	0.0731	0.658	0.289	0.956	1
set-1	1163.202	9.14E-43	1.74E-41	3.728	0.00191	0.0364
set-2	783.529	1.29E-37	2.32E-36	4.396	0.000491	0.00981
set-7	0.053	0.818	1	0.393	0.903	1

Supplementary Information

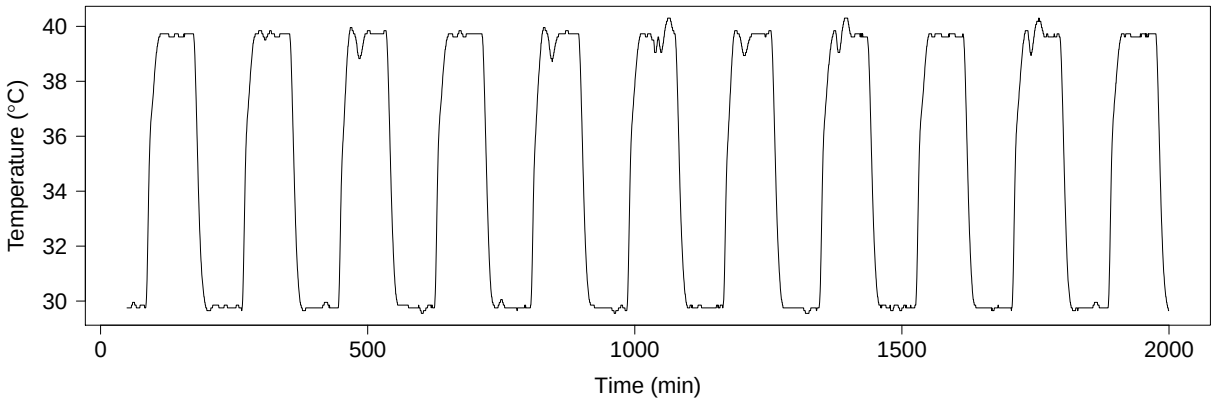

Figure S1: Realized temperatures from the experiment measuring growth rate in fluctuating environments. Temperature was recorded every 30 s with a datalogger. Step duration is 90 min in this example.

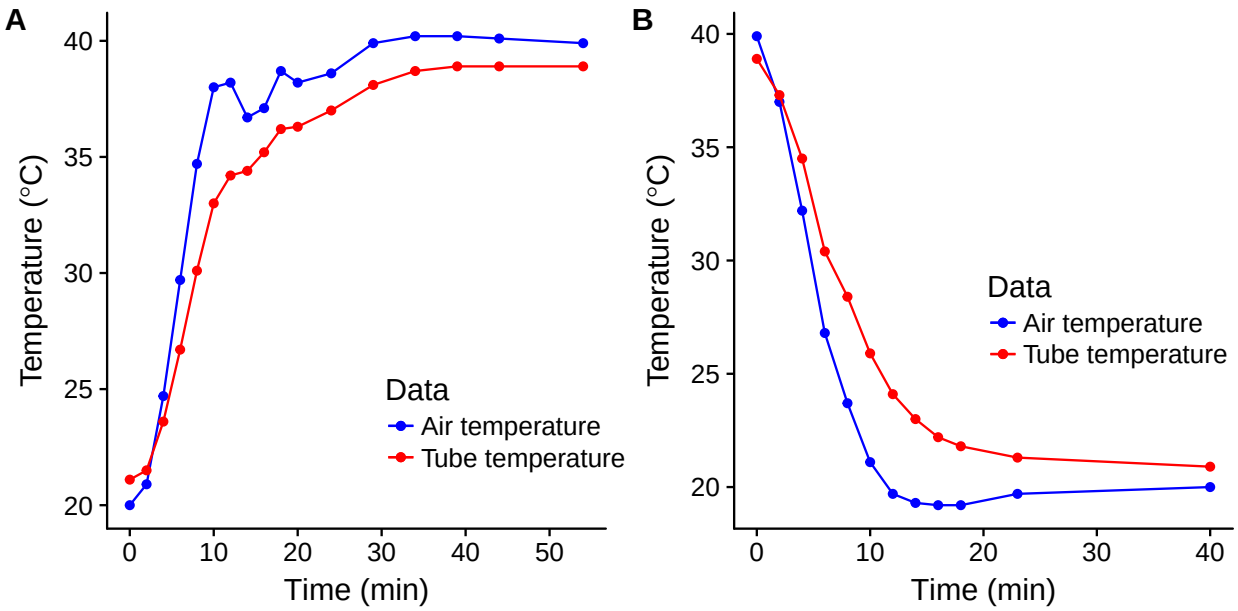

Figure S2: Changes in air and growth tube temperatures during growth chamber heating and cooling. In this preliminary experiment we measured changes from 20 to 40 °C, to explore how fast temperature equilibrates between air and medium in maximal fluctuation. In the experiment temperatures altered between 30 to 40 °C. A) Growth chamber heating. B) Growth chamber cooling.