

Beyond Arrhenius: Nonlinear and Negative Temperature Scaling of Biological Rates from Multi-Step Mechanisms

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Temperature dependence is a central constraint on biological timing, particularly in ectothermic development where reaction rates directly set developmental pace. A widely used framework for describing temperature dependence is the Arrhenius equation, which predicts an exponential increase in rates with temperature. However, biological rates often deviate from this prediction when measured across broader temperature ranges. While negative apparent activation energies are often attributed to protein denaturation, this cannot explain the similar behavior observed at temperatures where enzymes remain stable. These broader scaling patterns remain mechanistically unexplained. Here we present a general Markov chain framework for modeling biological timing as cascades of reversible, temperature-dependent steps. Across datasets, we identify a three-regime scaling structure: Arrhenius-like behavior at low and high temperatures, separated by a quadratic-exponential regime at intermediate temperatures. We show that this pattern arises naturally from differences in activation energies across steps in the network. The quadratic exponential regime is an emergent feature of averaging across many steps and is robust to variation across network realizations. In contrast, Arrhenius-like scaling at the extremes tends to be more variable and originates from smaller subnetworks. Apparent negative activation energies arise from network topology and reversibility in multi-step processes, even without protein denaturation. Our framework provides a general mechanistic framework for diverse temperature-scaling behaviors in biology and may help predict how developmental and physiological processes respond to environmental change. Although we focus on development, the model is broadly applicable to biological systems governed by multi-step reaction networks.

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I. INTRODUCTION

Temperature influences biological rates across all levels of organization and is particularly critical during early embryonic development, where reaction kinetics directly set developmental timing. Because ectothermic organisms do not regulate internal temperature, their developmental processes are especially sensitive to thermal variation [1–3], making it essential to understand how biological timing scales with temperature.

Biological processes exhibit complex temperature dependencies across scales, from enzymatic catalysis to organismal growth. These dependencies are often represented by thermal performance curves, which display optimal performance at specific temperatures and decline at extremes. Such curves provide insights into how temperature governs life processes,

ranging from enzymatic activity to embryonic development [4,5]. While thermal performance curves describe how biological rates depend on temperature under steady conditions, many ectotherms in nature experience substantial short-term fluctuations around these curves. The magnitude of such fluctuations is strongly habitat-dependent: large aquatic systems often buffer rapid temperature changes, whereas shallow pools, intertidal zones, and terrestrial microhabitats can undergo pronounced diurnal swings. Embryos and other immobile life stages may be particularly exposed, although some species mitigate extremes through careful selection of oviposition sites. Climate change is projected not only to raise mean temperatures but also to amplify the frequency and amplitude of extreme thermal events [3], making short-term variability an increasingly relevant component of the thermal environment. Separating the effects of mean temperature from short-term variability is essential for understanding temperature-dependent biological rates across ectotherms. At the mechanistic level, however, many of these temperature dependencies ultimately arise from the kinetics of underlying biochemical reactions, which are often approximated using Arrhenius-type models.

The Arrhenius equation [6–8] is widely used in biology to describe the temperature dependence of reaction rates. Initially derived for chemical reactions, it takes the

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form

$$\kappa(T) = \frac{1}{A} e^{-\frac{E}{RT}}, \quad (1)$$

where $\kappa(T)$ is the rate constant, A is a pre-exponential factor, E is the activation energy, R is the universal gas constant, and T is the temperature in kelvin. This response function is typically plotted as $\ln \kappa(T)$ versus $1/T$, or using the associated time $\tau(T) = 1/\kappa(T)$:

$$\ln \tau(T) = \ln A + \frac{E}{R} \frac{1}{T}, \quad (2)$$

which yields a simple linear relationship. The Arrhenius equation predicts a monotonic increase in rate, which holds reasonably well over moderate temperature ranges [9,10].

If the Arrhenius equation were universally valid and processes had similar activation energies, a small increase in temperature would speed up rates in a predictable way. For example, using the commonly invoked rule-of-thumb $Q_{10} = 2$ (meaning that reaction rates double for a 10 °C increase in temperature), a 3 °C warming would increase rates by a factor of $2^{3/10} \approx 1.23$, corresponding to an acceleration of about 23%. In reality, activation energies vary substantially across biological processes, so even under ideal Arrhenius kinetics, different steps accelerate by different amounts and their relative timings drift with temperature, potentially disrupting coordination.

Moreover, empirical observations show that even modest temperature increases often lead to pronounced deviations from Arrhenius behavior, especially at the upper end of the physiological temperature range. For instance, a temperature change of just a few degrees can trigger high fever in humans or completely arrest development in ectothermic embryos. In these non-Arrhenius regimes, biological rates frequently plateau, decline, or cease altogether. Such breakdowns arise from a range of factors, including protein denaturation, the activation of stress response pathways, and the nonlinear dynamics of complex biochemical networks [11–14]. These phenomena underscore the limitations of the Arrhenius framework in fully capturing biological temperature responses, particularly near physiological boundaries or across broad thermal ranges. They also highlight the need for mechanistic models that can explain not only species-level differences, but also variability across different realizations of biochemical networks.

To understand how such deviations appear throughout development, we examine temperature scaling across both early and later stages. Early embryonic cleavages are often driven by well-defined biochemical oscillators with minimal regulation, while later stages involve more complex and tightly regulated processes. Yet, as we show below, non-Arrhenius behavior occurs across this entire developmental spectrum, suggesting that it arises from general features of biological timing rather than stage-specific mechanisms.

Figures 1(a) and 1(b) illustrate this distinction. Figure 1(a) shows a schematic of the early, rapid cleavage divisions in the *X. laevis* frog embryo, regulated by a periodic cell cycle oscillator [11]. These divisions occur without gap phases, checkpoint controls, or transcription [20,21], allowing fast, regular cycles of proliferation. In contrast, Fig. 1(b) sketches

the slower and more variable timing of later developmental transitions, which involve diverse regulatory pathways, including gene expression, signaling, and morphogenesis.

Temperature-scaling behavior across these developmental stages is illustrated in Figs. 1(c)–1(e). In Fig. 1(c), the early cell cycle period of *Xenopus laevis* is plotted on a logarithmic scale against inverse absolute temperature. The data appear approximately linear at lower temperatures, consistent with Arrhenius scaling, but deviate significantly at higher temperatures. To describe these deviations more accurately, several extensions to the Arrhenius model have been proposed. These include the Quadratic Exponential (QE) equation [11,14,17,22], the double exponential (DE) equation [16], the polynomial exponential (PE) equation [23], and others [24,25] (Table I). These models introduce additional temperature-dependent terms to improve data fits (see Supplemental Material, Note 1 [19]). Figure 1(c) shows that the upward curvature in the *X. laevis* data is more accurately described by the QE model than by a single Arrhenius fit. Figure 1(d) extends this analysis to five species: *X. laevis*, *X. tropicalis*, *D. rerio*, *C. briggsae*, and *C. elegans*, showing the temperature dependence of early cleavage timings. Most species display Arrhenius-like scaling at low temperatures, but deviate at higher temperatures. These deviations are abrupt in *C. elegans*, *C. briggsae*, and *D. rerio*, and more gradually in *Xenopus*. In some cases, the slope even becomes negative at high temperatures, implying negative local activation energies. The best-fitting model varies by species: the DE function fits the more abrupt deviations better, while the simpler QE law fits gradual ones equally well (Table I). Figure 1(e) shows four later developmental intervals in *D. melanogaster*, including embryonic and pupal durations. These processes are more heterogeneous than early cleavages, yet they also deviate from Arrhenius scaling, especially over broader temperature ranges. Here, too, the QE equation provides an excellent fit.

These observations demonstrate that deviations from Arrhenius scaling occur across both oscillatory and nonoscillatory developmental processes, motivating the need for a general mechanistic explanation.

As shown in Figs. 1(c)–1(e), both the QE and DE equations do a better job at describing cleavage and developmental timing than the Arrhenius equation. In species such as *C. elegans*, *C. briggsae*, and *D. rerio*, the DE law fits better due to abrupt deviations and negative local activation energies. In contrast, for *X. laevis*, *X. tropicalis*, and later stages of *D. melanogaster*, the QE law fits well with fewer parameters. These results suggest that different empirical models are suited to different biological contexts, depending on species, developmental stage, and underlying regulatory complexity.

Many of the models described above are empirical, aiming to fit the experimental data without necessarily explaining the underlying mechanisms. The Arrhenius equation itself was originally proposed as an empirical description of the exponential increase in reaction rates with temperature. Only later was a theoretical basis developed by Kramers [26,27] and Eyring [28], who linked reaction rates to transitions over energy barriers in chemical reactions.

More recently, we provided a mechanistic explanation for non-Arrhenius scaling in the early embryonic cell cycle of

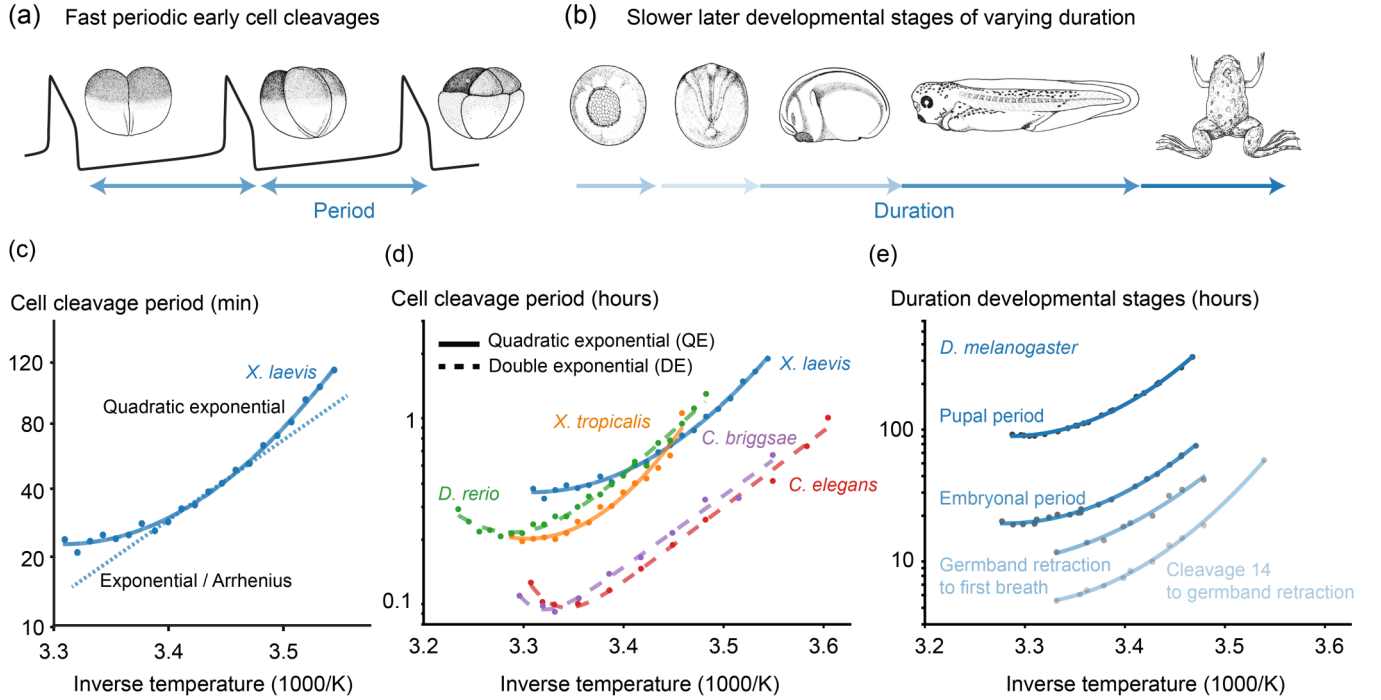


FIG. 1. Developmental timing scales non-Arrhenius. (a) Schematic of the first cleavages in the early *X. laevis* embryo (adapted from Ref. [15]), driven by periodic cell cycle oscillations [11]. (b) Schematic of selected later developmental stages during *X. laevis* development (adapted from Ref. [15]), showing varying and longer durations between stages. (c) Period of the early embryonic cell cycle in *X. laevis* [11], plotted on a logarithmic scale as a function of inverse absolute temperature. The best fits using the quadratic exponential (QE, solid line) and Arrhenius (dotted line) models are shown. (d) Periods of the early embryonic cell cycle for five species: *X. laevis*, *X. tropicalis*, *D. rerio* [11], and *C. briggsae*, *C. elegans* [16], with best fits using either the double exponential (DE, dashed line) or QE (solid line) models. (e) Timings of four developmental stages in *D. melanogaster*: from the 14th cell cleavage to germband retraction, germband retraction to first breath [17], total embryonic duration, and total pupal duration [18]. Since both DE and QE models fit the data equally well, only the simpler QE fit (solid line) is shown. Fits in panels (c) and (d) are reproduced from Rombouts *et al.* [11]. Fits in panel (e) were performed by the authors using the experimental datasets from Refs. [17,18] (see Supplemental Material, Note 1 [19]).

Xenopus laevis, using both embryos and egg extracts [11]. By modeling the cell cycle as a relaxation oscillator using coupled ordinary differential equations (ODEs), we showed that different activation energies among temperature-sensitive biochemical processes can account for the observed deviations from simple Arrhenius behavior, and this model also serves as a biochemical benchmark in our later analysis. However, that framework is limited to oscillatory systems and does not extend to the temperature scaling of more complex, nonperiodic biological processes. In complementary work, Voits and Schwarz [14] developed a statistical model that explains quadratic exponential scaling using a graph-based interpretation of mean first-passage times from a biochemical master equation.

In this study, we present a general mechanistic framework based on Markov chains to explain non-Arrhenius temper-

ature scaling in biological timing. We first map the ODE model for the cell-cycle oscillator to an equivalent Markov chain model, recovering the period as a mean first-passage time. We then show that temperature-dependent multi-step cascades produce a mean first-passage time with Arrhenius flanks and a central QE regime, the latter consistent with prior graph-theoretic work [14]. Applying this framework to 121 published datasets spanning diverse rates, durations, and species, we consistently observe the Arrhenius–QE–Arrhenius pattern, with dataset-specific boundaries. The QE regime is a robust consequence of averaging across many steps, whereas the flanking Arrhenius regimes are governed by smaller subnetworks and vary more across realizations. Simple motifs (cycles, competing paths) also account for apparent negative activation energies. Together, the ODE-to-Markov bridge and cascade theory provide a unified, testable

TABLE I. Proposed modifications of the Arrhenius law for timings. Note that E_2 can be negative.

Scaling function	Form	Parameters
Single exponential (Arrhenius)	$Ae^{\frac{E}{RT}}$	A, E
Double exponential (DE)	$A_1e^{\frac{E_1}{RT}} + A_2e^{\frac{E_2}{RT}}$	A_1, A_2, E_1, E_2
Quadratic exponential (QE)	$Ae^{\left(\frac{E}{RT} + \frac{B}{T^2}\right)}$	A, B, E

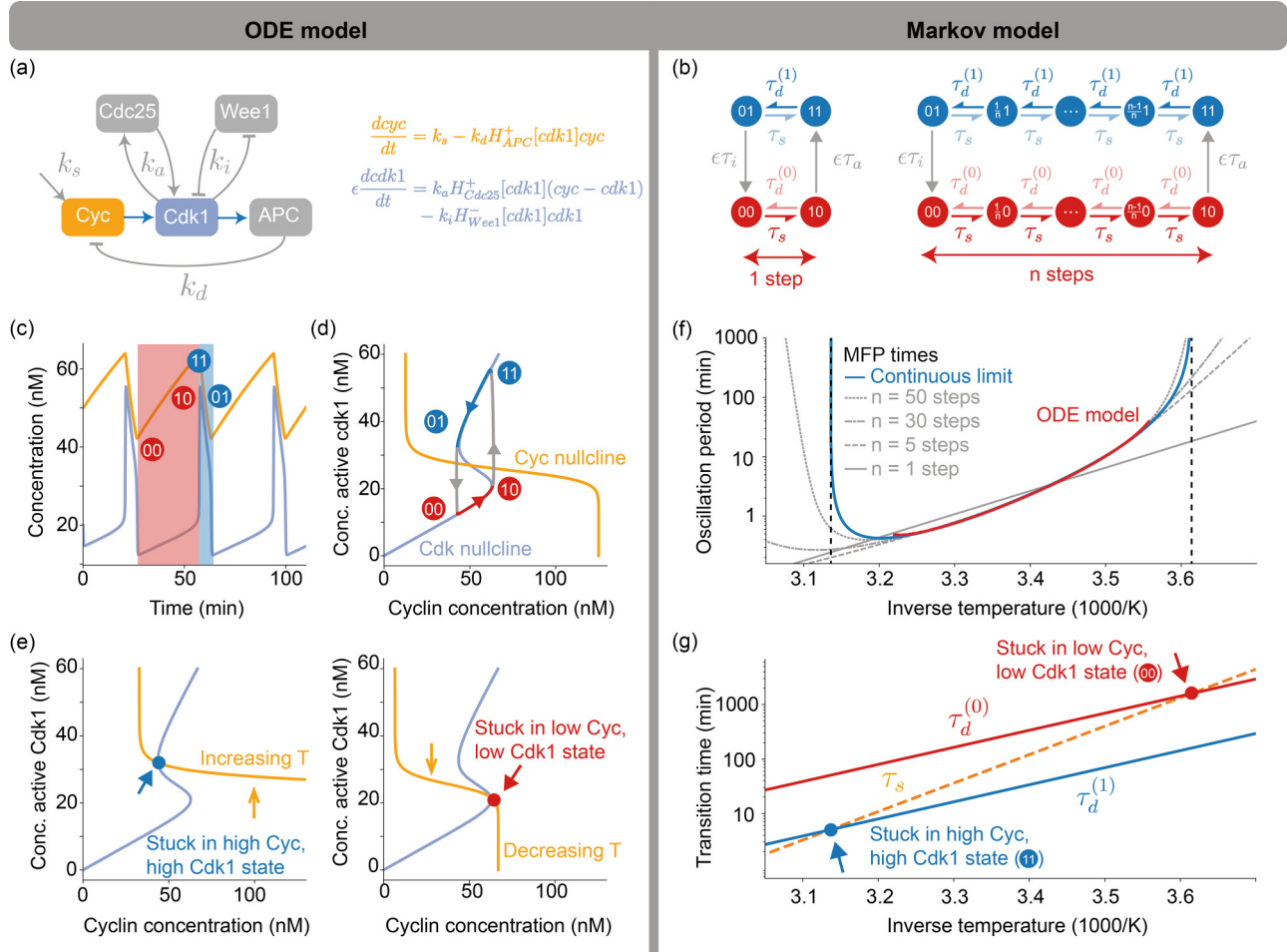


FIG. 2. The temperature scaling of the period of a simple embryonic cell cycle model can be explained using Markov chains. (a) A 2-ODE embryonic cell cycle model [11,29]. The terms $H_{\pm}^{\pm}[x]$ represent rising (+) and falling (−) Hill functions, which depend on the variable x . (b) Markov chain approximation of one cycle of the 2-ODE embryonic cell cycle model. The MFP time $\langle T \rangle$, calculated between the initial state (00) and the final state (00) (passing through all intermediate states in an anticlockwise direction), is identified with the period of the cycle (left, one-step approximation; right, n -step approximation). (c) A numerical simulation of the 2-ODE cell-cycle model, demonstrating a limit cycle as a time series, for the specific parameter set in Table S1 of the Supplemental Material [19]. (d) A numerical simulation of the 2-ODE cell-cycle model, demonstrating a limit cycle of the relaxation-oscillator type in phase space, for the specific parameter set in Table S1 of the Supplemental Material [19]. The nullclines are also drawn. (e) Illustration of how the shifting of the nullclines of the 2-ODE cell-cycle model with temperature can create new fixed points that cause the model to get stuck and destroy the limit cycle (Left, increasing T ; Right, decreasing T). (f) Comparison of the period of the Markov model, calculated as an MFP time, and the ODE model. Different step sizes and the continuous limit with “infinitely” small step sizes are shown. The ODE model uses the same specific parameter set from Table S1 of the Supplemental Material [19] as the other panels. The activation energies from the Markov model come from the same parameter set, while the pre-exponential factors of the Markov models have each been fitted to the period of the ODE model, following the procedure outlined in Supplemental Material, Note 4C [19]. The asymptotes (dashed black lines) of the continuous limit of the n -step model are also shown. (g) Transition times τ_s , $\tau_d^{(1)}$, and $\tau_d^{(0)}$ of the fitted continuous limit Markov model in function of temperature. The temperatures where the model gets stuck in a high Cyc and Cdk1 state, or in a low Cyc and Cdk1 state, correspond to asymptotes (dashed black lines) of the continuous limit in panel (f). (See also the Supplemental Material, Note 4C [19].)

account of how network structure shapes thermal responses in development and related processes.

II. RESULTS

A. The early embryonic cell cycle oscillator’s period is controlled by reaction-specific temperature sensitivities

1. A model based on coupled ordinary differential equations

The early embryonic cell cycle of the frog *Xenopus laevis* functions as an autonomous biochemical oscillator driven

by Cyclin-dependent kinase 1 (Cdk1). This core regulatory network, which consists of Cyclin synthesis, bistable Cdk1 activation, and APC/C-mediated Cyclin degradation [Fig. 2(a)], is sufficiently simple to be described by coupled ODEs parametrized from experiments [29,30] (Supplemental Material, Note 2 [19]). The resulting dynamics correspond to a stable limit cycle in the (Cyc, Cdk1) phase plane [Figs. 2(c) and 2(d)].

This ODE framework has proven useful for understanding how temperature shapes the period of the cell cycle

oscillator [11]. When all reaction rates scale with temperature according to identical Arrhenius activation energies, the oscillation period itself follows a simple Arrhenius law. However, when activation energies differ between reactions, temperature changes move the nullclines and can eliminate the limit cycle altogether. This network-level effect produces curved Arrhenius plots in this model, including regimes with negative effective activation energies, and generates thermal limits even though every individual reaction obeys Arrhenius kinetics.

For example, if Cyclin synthesis is more temperature-sensitive than Cyclin degradation, increasing temperature shifts the Cyclin nullcline upward until it no longer intersects the Cdk1 nullcline, arresting the system in a high-Cdk1, M-phase-like steady state [Fig. 2(e)]. Conversely, sufficiently low temperatures stabilize a low-Cdk1, interphase-like steady state. Thus, non-Arrhenius temperature scaling emerges naturally from imbalances in reaction-specific temperature sensitivities within the network.

A second route to non-Arrhenius scaling arises when individual reactions themselves exhibit intrinsically non-Arrhenius (e.g., biphasic) temperature dependence. In this case, the overall period becomes dominated by the reaction(s) with thermal optima rather than by differential Arrhenius scaling [11]. Experiments in cycling *Xenopus* egg extracts indicate that both mechanisms are relevant in vivo: Cyclin synthesis is both more temperature-sensitive than degradation and displays biphasic temperature dependence [11].

2. A model based on Markov jump processes

The ODE analysis shows that temperature scaling can be a network property. However, extending this analysis beyond relaxation oscillators remains a significant challenge. Boolean models motivate a discrete state space for regulatory networks [31], but their discrete time hinders analysis of temperature-dependent periods. We therefore map the two-ODE oscillator to a continuous-time Markov jump process on a discrete state graph with protein concentrations having binary states: active (1) or inactive (0). We introduce a continuous-time framework into the state graph by assigning transition rates κ_{ij} to transitions between states $(i) \rightarrow (j)$. For small time intervals dt , the probability of such a transition is given by

$$P[i \rightarrow j] \approx \kappa_{ij} dt. \quad (3)$$

This describes a Markov jump process, a stochastic, continuous-time process on a discrete state space with the property that future transitions depend only on the current state, making these processes both analytically and numerically tractable [32].

In a Markov process, the time required to transition from state i to state j is called the first passage time τ_{ij} . We are especially interested in the first passage time from the initial state 1 to the final state n , denoted as $\tau_{1n} = \mathcal{T}$. As $\mathcal{T}$ varies between realizations, it is treated as a random variable. However, the mean first passage (MFP) time $\langle \mathcal{T} \rangle$, a fixed value for the process, can be calculated and used to study biological transition timings (Supplemental Material, Note 3 [19]). For simplicity, we assume that, for large n , the higher-order moments of $\mathcal{T}$ are negligible compared with the mean

(the variance-to-mean ratio vanishes as $1/\sqrt{n}$), a reasonable approximation for large-scale biological systems evolved for specific purposes [33–35].

Temperature dependence is introduced into the Markov jump process by assigning Arrhenius scaling to the transition rates. Specifically, the inverse rate $\tau_{ij} = \kappa_{ij}^{-1}$ is modeled as

$$\tau_{ij} \equiv (\kappa_{ij})^{-1} = A_{ij} e^{E_{ij}/(RT)}. \quad (4)$$

where A_{ij} is the pre-exponential factor, E_{ij} is the activation energy, R is the universal gas constant, and T is the absolute temperature in kelvin.

To approximate the two-ODE model, we developed a simplified Markov chain in which Cyclin B and active Cdk1 are limited to binary states: ON (1) or OFF (0) (Supplemental Material, note 4 [19]). The ON state represents a high concentration of Cyclins and high Cdk1 activity, while the OFF state corresponds to a low concentration of Cyclins and low Cdk1 activity. This yields four possible system states: (0, 0), (1, 0), (1, 1), and (0, 1). Transition times τ_{ij} were assigned based on the corresponding rate constants in the two-ODE model. For example, τ_s represents the time for Cyclins to be synthesized [transitions (0, 0) $\rightarrow$ (1, 0) and (0, 1) $\rightarrow$ (1, 1)], while τ_d governs Cyclin degradation [transitions (1, 0) $\rightarrow$ (0, 0) and (1, 1) $\rightarrow$ (0, 1)]. Similarly, $\epsilon\tau_a$ and $\epsilon\tau_i$ were used for the faster activation and inhibition of Cdk1, where ϵ is a small dimensionless constant that serves to separate timescales, leading to transitions (1, 0) $\rightarrow$ (1, 1) and (0, 1) $\rightarrow$ (0, 0), respectively. For the temperature dependence we used (4) with activation energies that were the same as that of the corresponding rate constants in the ODE oscillator and pre-exponential factors that were allowed to differ. To replicate the two-rate dynamics of Cyclin degradation, we introduced two different pre-exponential factors, $A_d^{(0)}$ (low degradation when APC is OFF) and $A_d^{(1)}$ (high degradation when APC is ON). These factors were scaled to match the ratio of extremal values in the Hill function of the two-ODE model. (Supplemental Material, Note 4A [19]).

The resulting Markov process is represented as a weighted directed graph [Fig. 2(b), left]. While backward reactions are possible, they were omitted in the transitions for Cdk1 activation and inhibition for simplicity. At a given temperature, the MFP time for the system to traverse all states in a counter-clockwise direction, starting from (0, 0) and returning to (0, 0), was calculated and identified with the period of the cycle. The results were then compared with the temperature-dependent period of the two-ODE model, by determining a best fit of the remaining undetermined pre-exponential factors. However, this rudimentary four-state model does not reproduce the strong curvature of the ODE period in the Arrhenius diagram at thermal extremes.

To improve the match between the Markov chain and two-ODE models, intermediate states were added for Cyclin B. Instead of binary states, Cyclin B concentrations were divided into n states, allowing transitions in steps of $1/n$. For simplicity it was assumed that the transition times between each of the intermediate states were the same. The right-hand side of Fig. 2(b) shows the resulting state graph. As n increases, the MFP time of the Markov chain converges to the period of the ODE model [Fig. 2(f)]. In the continuous limit ($n \rightarrow \infty$), the

system exhibits vertical asymptotes at extreme temperatures (dashed lines), corresponding to thermal limits where oscillations cease (Supplemental Material, Notes 4B and 4C [19]).

The Markov model provides an intuitive explanation for the breakdown of Arrhenius scaling when $E_s \neq E_d$. Because transitions are stochastic in both directions, net forward drift is observed when the mean waiting times satisfy

$$\tau_d^{(1)} < \tau_s < \tau_d^{(0)}.$$

When E_d and E_s differ, their scaling with temperature diverges, causing one of these inequalities to fail at extreme temperatures [see Fig. 2(g)]. This results in the system becoming trapped in backward cycles during either Cyclin synthesis or degradation, halting oscillations (when $n \rightarrow \infty$). Physically, this corresponds to one process dominating the other, preventing the system from completing a full cell cycle. In short, the Markov formulation reproduces the ODE period and the loss of oscillations at thermal extremes, while making a forward-backward bias explicit. This, in turn, motivates the three-regime scaling we derive next for linear cascades.

B. Cascades of reversible transitions explain temperature scaling in developmental systems

Motivated by the ODE to Markov mechanism above, we now examine linear cascades of reversible steps as a simplified model of developmental progression. This shifts the focus from periodic cycles to one-way progressions, where a system transitions from an initial to a final state through a series of potentially reversible steps. Many later developmental events can be conceptualized this way, as sequential, partly reversible processes rather than a dedicated oscillatory mechanism.

To generalize this framework, we allow the transition times between states to differ. The resulting state graph, a linear cascade of cycles, is shown schematically in Fig. 3(a). It consists of n states, with $n - 1$ intermediate reversible transitions with a forward and backward component. Each i th forward transition connects states i and $i + 1$, while each i th backward transition connects states $i + 1$ and i .¹ The process begins in state 1 and terminates in state n .² In the Supplemental Material, Note 6A [19], it is shown that, for this linear cascade, the MFP time from 1 to n over all realizations of the Markov process can be calculated analytically:

$$\langle \mathcal{T} \rangle = \sum_{i=1}^{n-1} \mathcal{T}_i^*, \quad (5)$$

where the effective transition times $\mathcal{T}_i^*$ are defined by³

$$\mathcal{T}_i^* = \tau_{i,f} \left(1 + \sum_{k=1}^{i-1} \prod_{j=i-k}^{i-1} \frac{\tau_{j,f}}{\tau_{j,b}} \right), \quad (6)$$

¹Irreversible forward transitions can also be incorporated by setting the corresponding backward transition time to infinity.

²Note that we did not include a transition $\tau_{n-1,b}$ since this would not make a difference in the computation of the MFP time.

³Equation (6) does not account for the $(n - 1)$ st backward transition, so it is not shown in Fig. 3(a).

and the longest effective transition time $\mathcal{T}_{\max}^*$ at temperature T :

$$\mathcal{T}_{\max}^*(T) = \max\{\mathcal{T}_i^*(T), \quad i \in [1, n - 1]\}. \quad (7)$$

The scaling of the MFP time depends on the distribution of the ratios between forward and backward transitions $r_i = \tau_{i,f}/\tau_{i,b}$. When forward transitions are faster (i.e., $r_i \ll 1$), it simplifies to the sum of all forward transition times, $\langle \mathcal{T} \rangle = \sum \tau_{i,f}$.⁴ However, when backward transitions become significant $r_i = O(1)$ or $r_i > 1$, $\langle \mathcal{T} \rangle$ increases, as each effective transition time $\mathcal{T}_i^*$ is amplified by a factor that accounts for the ratios of forward and backward transitions in prior cycles. This factor is always greater than 1, meaning that faster backward transitions increase the MFP time considerably (see also Supplemental Material, Note 6B [19]).

To model temperature-dependent large-scale biological processes, we pick a reference temperature T_0 and generate random transition statistics in three steps:

(i) *Baseline times at T_0* . At the reference temperature, we assign random forward transition times $\tau_{i,f}(T_0)$ and backward transition times $\tau_{i,b}(T_0)$ to the cascade. A forward bias can be explicitly incorporated by tuning the distributions from which the transition times are drawn, such that $r_i \ll 1$ for most transitions. For example, this can be done by making the distribution of the r_i 's narrowly peaked around a mean $\mathbb{E}[r_i] \ll 1$.

(ii) *Activation energies*. To each of the transitions we assign a random forward or backward activation energy ($E_{i,f}$ or $E_{i,b}$), which is assumed to be independent from the transition time at the reference temperature.

(iii) *Arrhenius extrapolation from T_0* . for any temperature T , the forward or backward ($\bullet = f$ or b) transition times are given by

$$\ln \tau_{i,\bullet}(T) = \ln \tau_{i,\bullet}(T_0) + \frac{E_{i,\bullet}}{R} \left(\frac{1}{T} - \frac{1}{T_0} \right).$$

In Supplemental Material, Note 6C.1 [19], we derive in the limit of many steps ($n \rightarrow \infty$) that these statistics lead to the following QE scaling law for the MFP time $\langle \mathcal{T} \rangle \approx \mathbb{E}[\langle \mathcal{T} \rangle]$ in the neighborhood of T_0 [where we assume a forward bias; see Eq. (63)]:

$$\langle \mathcal{T} \rangle \approx (n - 1) \mathbb{E}[\tau_{i,f}(T_0)] \exp \left(\mathbb{E}[E_{i,f}] \beta + \frac{\text{Var}[E_{i,f}]}{2} \beta^2 \right), \quad (8)$$

where $\beta = 1/(RT) - 1/(RT_0)$ is a rescaled inverse temperature and $\mathbb{E}[\dots]$ and $\text{Var}[\dots]$ denote the mean and variance, respectively, with respect to the distribution of the parameters. This prediction holds for a wide class of activation-energy distributions with finite mean and variance, without requiring fine-tuning of individual steps.

Intuitively, when all forward activation energies have the same activation energy, the overall MFP time will also scale

⁴This MFP time is equivalent to that of a cascade of irreversible transitions, which is discussed in Supplemental Material, Note 5 [19].

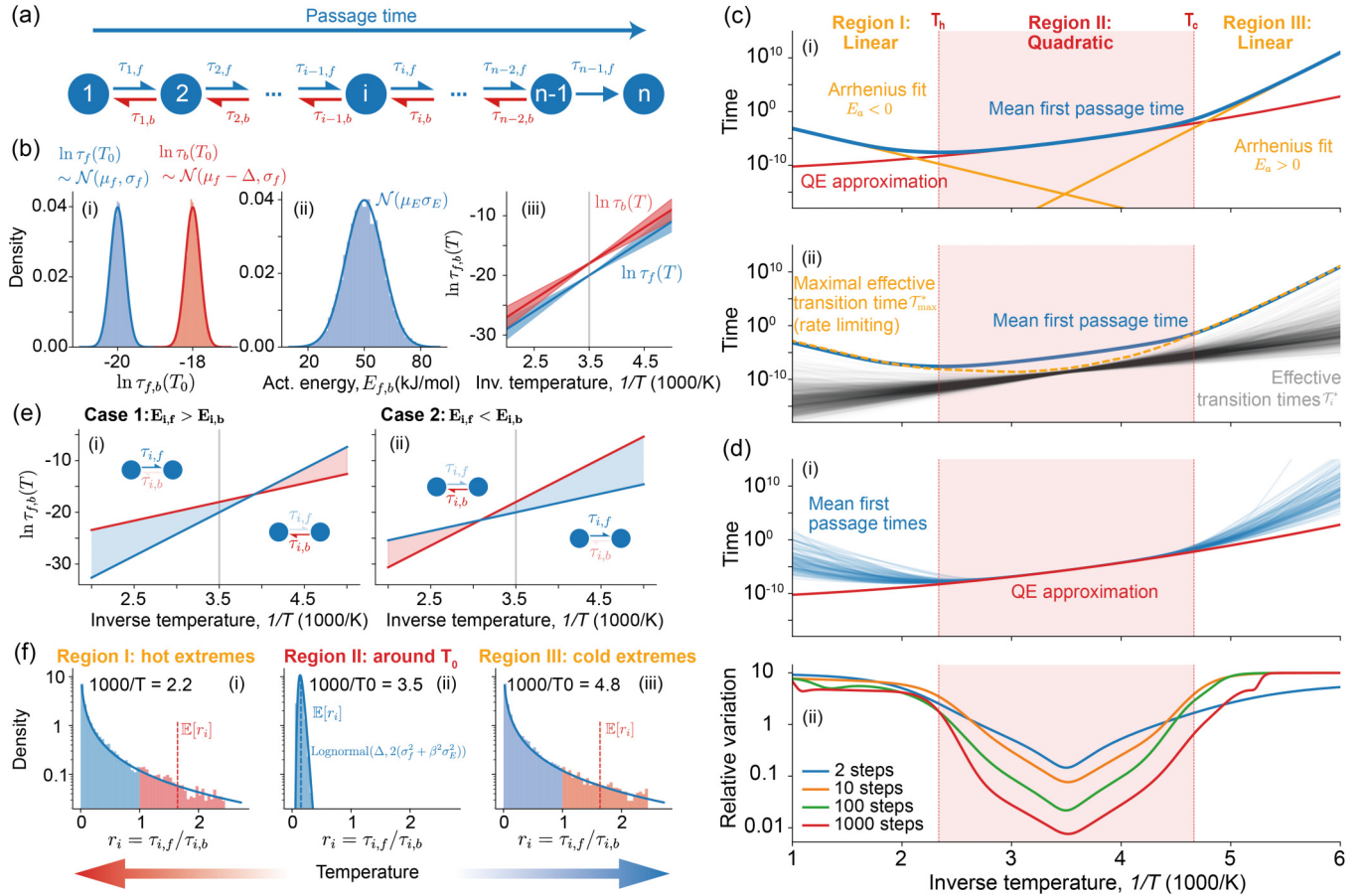


FIG. 3. The mean first passage time of a linear cascade of reversible processes exhibits three distinct temperature-scaling regimes. (a) Schematic of a linear cascade of reversible Markov jump processes. (b) Ingredients of the transition times' temperature dependence: (i) natural logarithms of forward (blue) and backward (red) transition times are distributed normally at the reference temperature T_0 , with the backward distribution shifted by Δ with respect to the forward one; (ii) activation energies of forward and backward transitions are drawn from normal distribution with the same mean and variance; (iii) resulting Arrhenius scaling of forward (blue) and backward (red) transition times (mean $\pm$ s.d.). Gray vertical line indicates inversed reference temperature ($1/T_0$). (c) Simulation of the temperature dependence of the MFP time for system (a) with $n = 1000$ steps and other parameters from Table S2 of the Supplemental Material [19]: (i) Arrhenius scaling is fitted at high and low temperatures (Regions I and III, yellow lines). In Region II, the analytical QE approximation from (8) provides a good fit. Boundaries of the QE region are analytically determined from (10) and (11). (ii) MFP time plotted alongside with effective transition times (family of thin black curves) and the longest effective transition time (dashed yellow curve). (d) Variation of MFP time temperature scaling across 100 random realizations of system (a) with $n = 1000$ and other parameters from Table S2 of the Supplemental Material [19]: (i) MFP time from 100 realizations are plotted alongside with the QE approximation; (ii) Relative variation of MFP time (s.d./mean) across realizations versus temperature for different sizes of a linear cascade (a). (e) Examples of individual transition time scaling when $E_{i,f} > E_{i,b}$ (i) and $E_{i,f} < E_{i,b}$ (ii). In both panels, blue and red lines show scaling of individual forward and backward transition line, respectively; blue and red shadings show the areas where either forward or backward transition from state i is more preferable, as shown by the respective pictograms. (f) Forward-to-backward transition time distribution illustrating the fraction of forward-dominated ($r < 1$, blue bars) and backward-dominated ($r > 1$, red bars) transitions, at different temperatures symmetric to the reference one.

perfectly Arrhenius-like with this activation energy if backward transitions can be neglected. This is indeed reflected in (8), which reduces to an Arrhenius law when $\text{Var}[E_{i,f}] = 0$. The additional quadratic curvature in the Arrhenius diagram that arises in the generic case corresponds to the second cumulant in the expansion of $\mathbb{E}[\exp(E_i\beta)]$ [see Eq. (41) in Supplemental Material, Note 5 [19]], and can then be understood as emerging from a variability in slopes of individual transition times.

Under the additional assumption that the higher-order cumulants of $E_{i,f}$ and $E_{i,b}$ are sufficiently small [see Eqs. (43) and (44) in Supplemental Material, Note 5 [19]], one finds

that the QE scaling law is valid within a specific temperature range:

$$\frac{1}{T_h} < \frac{1}{T} < \frac{1}{T_c}, \quad (9)$$

where forward transitions dominate. Here T_c and T_h are the temperature limits of the QE scaling at cold and warm temperatures, and $\Delta(1/T) = 1/T_c - 1/T_h$ defines the width of the QE region. They have simple symmetric analytical expressions when the distribution of forward and backward activation energies is the same and when the variance of r_i

is small at the reference temperature [see Eq. (61) in Supplemental Material, Note 6B [19]]

$$\frac{1}{T_c} = \frac{1}{T_0} + R \sqrt{\frac{-\ln(\mathbb{E}[r_i(T_0)])}{\text{Var}[E_i]}}, \quad (10)$$

$$\frac{1}{T_h} = \frac{1}{T_0} - R \sqrt{\frac{-\ln(\mathbb{E}[r_i(T_0)])}{\text{Var}[E_i]}}, \quad (11)$$

$$\Delta\left(\frac{1}{T}\right) = 2R \sqrt{\frac{-\ln(\mathbb{E}[r_i(T_0)])}{\text{Var}[E_i]}}. \quad (12)$$

These expressions are only valid when there is a forward bias at the reference temperature, i.e., $\mathbb{E}[r_i(T_0)] < 1$. Otherwise, there will be no QE temperature-scaling regime.

In Supplemental Material, Note 6C.2 [19], we show that, outside of this range, the law of large numbers cannot be applied to (5). The reason is that here, the forward bias is destroyed and backward transitions dominate the effective transition times in (6). As multiple fast backward transitions can link together to create a strong backward bias, the variation of $\mathcal{T}_i^*$ will increase with n , in such a way that the convergence to the mean ensured by the law of large numbers will no longer be possible. As a result, the individual transition time $\mathcal{T}_{\max}^*$ will dominate. In the low-temperature (high-temperature) limit, it will usually have a positive (negative) activation energy.

To illustrate these generic theoretical predictions, we set up a numerical implementation of this system. Figure 3(b)(i) shows the chosen lognormal distribution of the forward and backward transition times at the reference temperature: $\ln \tau_{i,f}(T_0) \sim \mathcal{N}(\mu_0, \sigma_0^2)$ and $\ln \tau_{i,b}(T_0) \sim \mathcal{N}(\mu_0 - \Delta, \sigma_0^2)$. In this, we defined a parameter $\Delta < 0$ to set a forward bias at T_0 (forward reactions faster than backward reactions). Next, as shown in Fig. 3(b)(ii), we picked for the forward and backward activation energies a normal distribution $\mathcal{N}(\mu_E, \sigma_E^2)$ with biologically plausible values ($\mu_E \simeq 50 \text{ kJ mol}^{-1}$, $\sigma_E \simeq 10 \text{ kJ mol}^{-1}$; Ref. [36]). This leads to the temperature-dependent distribution of transition times, plotted in Fig. 3(b)(iii).

Within the range in (9), the MFP time indeed follows a QE scaling law as described in (8) [Fig. 3(c)(i)]. This behavior arises from the collective contribution of many forward-dominated transitions with comparable timescales in the sum given by (5) [Fig. 3(c)(ii)]. As a result, the system displays strongly reduced variability across realizations, particularly as the number of steps increases [Fig. 3(d), Supplemental Material, Fig. S4 [19]]. This regime is therefore both more predictable and more robust, reflecting the emergent regularity of large ensembles of similarly activated steps. Here, “robust” refers to statistical robustness of timing, i.e., reduced variability across realizations and parameter perturbations, rather than evolutionary or ecological robustness. From the analytical expressions in (10) and (11), we can predict the boundaries of the QE regime and understand that its validity largely depends on the relative width of the activation energy distribution (σ_E) and the directional bias (Δ).

In contrast, outside this range, the distribution of effective transition times $\mathcal{T}_i^*$ includes large tails, outliers dominate the total MFP time, and the law of large numbers can no longer be applied [Fig. 3(c)]. In these extreme temperature regions,

the MFP timescales in an Arrhenius-like manner with either a positive or negative constant activation energy, depending on the temperature limit: positive activation energy in the cold regime and a negative activation energy in the warm regime. In both cases, the dynamics are effectively dominated by the longest rate-limiting transition time $\mathcal{T}_{\max}^*$ [panel (ii) in Fig. 3(c)]. This leads to strong sensitivity to stochastic variation: despite identical distributions of activation energies across realizations, substantial variability emerges in the observed slopes, transition sharpness, and crossover temperatures. This variability persists even for large systems (large n), indicating that the statistical averaging in long cascades does not apply in these regimes [Fig. 3(d), Supplemental Material, Fig. S4 [19]].

To better understand where the deviations from the QE scaling law originate, we focus on the scaling of individual reactions. Since the activation energies of forward and backward transitions of individual reaction are generally unequal, two cases are possible: (1) $E_{i,f} > E_{i,b}$ and (2) $E_{i,f} < E_{i,b}$. In both cases, forward transitions are preferable around the reference temperature T_0 , favoring the QE approximation. However, backward transitions in case (1) dominate at lower temperatures, while the opposite holds for the case (2) [Fig. 3(c) and Supplemental Material, Fig. S3 and Note 6D [19]]. Thus, moving away from T_0 in either direction facilitates the backward-dominated transitions with $r_i = \tau_{i,f}/\tau_{i,b} > 1$ through either case-(1) reactions on the colder side or case-(2) reactions on the warmer side [Fig. 3(f)]. Such backward-dominated transitions trap the cascade in the local cycle motifs, thereby extending overall MFP time beyond the QE prediction at the temperature extremes.

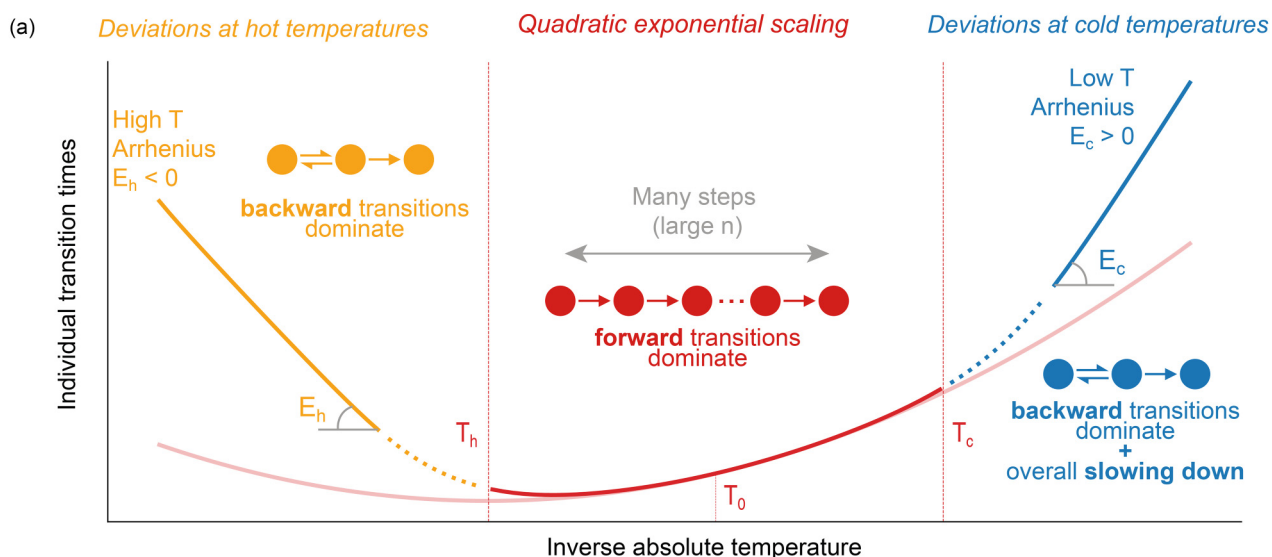
Together, these results show that complex biological processes can transition between distinct scaling regimes [Fig. 4(a)]. Quadratic exponential scaling arises in intermediate thermal regimes due to the cumulative contribution of many steps in large cascades, while single-exponential (Arrhenius) behavior dominates at thermal extremes due to the disproportionate influence of a small number of transitions. This framework provides a mechanistic basis for understanding both the robustness and variability observed in temperature-dependent biological timing.

C. Simple network motifs explain the mechanisms behind non-Arrhenius scaling and negative activation energies

Figure 4(a) illustrates how single-exponential Arrhenius behavior, whether with positive or negative activation energies, dominates at thermal extremes, due to the disproportionate influence of a small number of rate-limiting transitions. We focus here on three minimal network motifs that play a critical role in generating deviations from simple Arrhenius responses at these temperature extremes.

The simplest case is two sequential reactions with different activation energies [Fig. 4(b)]. At low temperatures, the system’s overall rate scales with the reaction having the largest activation energy, while at high temperatures, it scales with the smallest. This produces the widely used double-exponential response curve. However, at both extremes, the system still exhibits scaling with a positive activation energy. In fact, it is shown in Supplemental Material, Note 7A [19] that any

The general temperature scaling of large-scale biological cascades



Small network motifs that lead to non-Arrhenius scaling

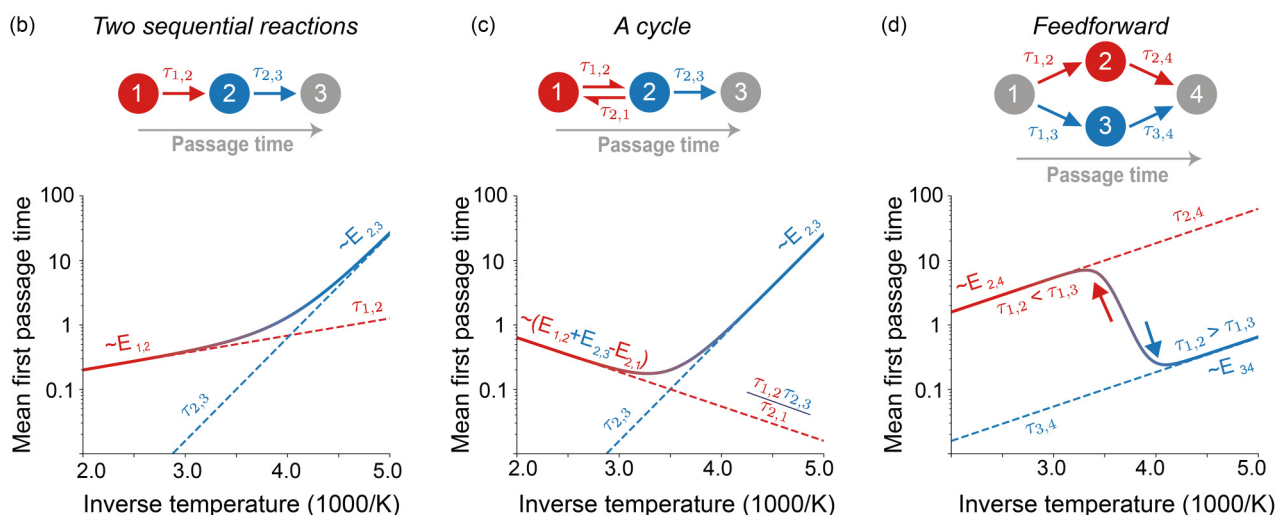


FIG. 4. General scaling motifs underlying temperature-dependent biological timing. (a) Schematic overview showing how biological systems combine quadratic exponential scaling at intermediate temperatures—driven by the collective averaging of many similar transitions—with Arrhenius-like single-exponential scaling at low- and high-temperature extremes, where one or few rate-limiting steps dominate. (b) Sequential two-step motif: at low temperatures, the slower high-barrier reaction (blue) sets the overall rate; at high temperatures, the faster low-barrier reaction (red) dominates, but both regimes maintain positive effective activation energies. (c) Cycle motif: while at low temperatures the forward transition (blue) determines the MFP time, at high temperatures, reverse transitions (red) become dominant, trapping the system and producing an emergent negative macroscopic activation energy despite all microscopic steps following Arrhenius scaling. Here, $E_{23} > E_{12}$ and $E_{12} + E_{23} < E_{21}$. (d) Feedforward motif: two parallel forward pathways (red: $1 \rightarrow 2 \rightarrow 4$, blue: $1 \rightarrow 3 \rightarrow 4$) contribute, with temperature shifting dominance between them (increasing T : along red pathway following the red arrow, decreasing T : along blue pathway following the blue arrow), producing a negative activation energy within a narrow intermediate temperature range. The parameters to reproduce this figure are given in Table S3 of the Supplemental Material [19].

cascade of irreversible forward Arrhenius-like reactions can never generate negative activation energies in $\langle T \rangle$. So how can minimal network motifs, where each microscopic transition obeys the Arrhenius law with a fixed positive activation energy, give rise to a system where the MFP timescales with a negative activation energy?

We identify two minimal state-graph motifs that illustrate how negative activation energies can emerge from systems

with only positive microscopic activation energies. The first is a simple cycle [Fig. 4(c), Supplemental Material, Note 7B [19]], where the system can transiently loop between states 1 and 2 before reaching the endpoint 3. At high temperatures, when reverse transitions become significantly faster than forward ones, the MFP timescales with an effective negative activation energy. The second motif involves two parallel forward-directed paths [Fig. 4(d), Supplemental Material,

Note 7C [19]), where temperature determines the likelihood of taking one path over the other. Within a narrow intermediate temperature range, the shift in path dominance produces a negative activation energy.

These motifs show that macroscopic negative activation energies can arise even when all microscopic transitions obey Arrhenius scaling, emphasizing the role of network topology rather than individual reaction chemistry. Both motifs feature multiple possible routes to the endpoint, with the most probable path shifting with temperature. In contrast, systems with only a single possible path, such as the two-step sequential motif, cannot generate negative macroscopic activation energies in their MFP scaling.

The temperature-dependent scaling in the cycle and feedforward motifs is qualitatively distinct. For the cycle, the macroscopic activation energy can become negative in the high-temperature limit, as the system becomes increasingly trapped in reverse loops. For the feedforward motif, negative activation energies emerge only in an intermediate transition region, where the dominant contribution to the MFP time shifts between two competing forward paths that control behavior at opposite thermal extremes.

D. The Markov framework captures temperature scaling across diverse species, biological timescales, and temperature ranges

To evaluate the applicability of the Markov cascade model, we assembled a diverse set of experimental datasets densely sampled in temperature, i.e., many measurements per curve across a broad thermal span, covering ecosystem biodiversity traits such as richness and abundance of species, growth and developmental processes, including early embryonic cell cycles and stage transitions across multiple species [11,16–18,37–80]. In total, we analyzed 121 datasets from 48 sources, comprising 2174 measurements with on average 18 temperature points per curve (and minimum 5), with a typical temperature span of 30 ± 17 K (minimum 10 K).

Each temperature response curve was fit with a composite model: a central QE regime [Eq. (8)], flanked by single-exponential regimes at low and high temperatures (See Supplemental Material, note 1 [19]). This approach enables a unified description of thermal scaling across biologically relevant ranges, with transitions between regimes governed by the underlying distribution of activation energies and the balance of forward and backward transitions in the Markov chain. Figure 5(a) shows representative examples, highlighting the diversity of observed scaling behaviors, from processes following QE across the full range to cases with deviations at either thermal extreme. Across all datasets, the model provided excellent fits (median $R^2 = 0.98$, 90% CI [0.89, 0.99]), accurately capturing both gradual and sharp transitions in scaling. The majority of experimental temperature responses, approximately 59%, were fully described by the QE law, while the rest displayed deviations at temperature extremes: 32% only at warm temperatures, 6% only at cold temperatures, and 3% at both ends [Fig. 5(b), Figs. S5–S10 of the Supplemental Material [19]]. In addition, 18% of analyzed datasets, such as metabolic rate measurements in *A. phoenixis* in Fig. 5(a), displayed what we call a “negative” scaling—a type of scaling characterized by a negative local slope in the

QE region. Although one should be careful in interpreting these numbers, since they can be biased by the availability of measured data (See Fig. S12 of the Supplemental Material [19]), this pattern suggests that while many biological systems operate within a robust QE regime near their thermal midpoint, transitions to Arrhenius-like scaling are common at extreme temperatures. At the same time, datasets exhibiting deviations at colder temperatures were less represented among the analyzed sources. It may reflect the fact that the processes slow down faster at the colder side, making their experimental measurement more challenging. More detailed grouping datasets by the type of process or system revealed an important trend: the likelihood that the temperature response curve is well-described by the QE law alone increases with the spatial scale of the underlying process or system, ranging from the level of molecules and cells to multicellular organisms and ecological communities [Fig. 5(c)]. This finding is consistent with our model’s prediction: in larger systems, one expects more regulatory steps and greater diversity, leading to better convergence to QE scaling across the measured temperature range.

Moving forward, we aimed to address the following question: Can the variation in thermal operating ranges across experimental datasets be explained by the mean and variance of the activation energy, following our model? Below, we test two predictions:

Prediction (i). From (8) and (12), we expect that a lower variance of activation energy results in a reduced scaling curvature and a wider QE window, i.e., less temperature-sensitive response [Fig. 5(d)].

Prediction (ii). The mean activation energy controls the local slope around the reference temperature T_0 ; if the linear term in the QE law remains unchanged, it is equivalent to shifting the reference temperature T_0 along the scaling curve, thereby tuning the thermal operation range [Fig. 5(e)].

First, we validated the relationship between the QE range width $\Delta(1/T)$ and activation energy variance $\sigma_E^2 = \text{Var}[E_i]$ [Fig. 5(f)]. On the log-log plane, the datapoints were scattered around the diagonal with the slope of $-1/2$, as predicted from (12) of our model. Points corresponding to enzyme activity clustered tightly around low energy variance, yielding the widest QE region, whereas the developmental rates, grouped around moderate σ_E^2 , displayed a considerably narrower QE window. Remarkably, the temperature responses of developmental rates measured *in vitro* differ substantially from those assessed *in vivo*, and thus cluster separately [Fig. 5(f), Fig. S5 of the Supplemental Material [19]].

Second, we looked at the reference temperature T_0 against mean activation energy $\mu_E = \mathbb{E}[E_i]$ [Fig. 5(g)]. Most of the analyzed temperature response curves exhibit positive μ_E , typically ranging from 40–80 kJ/mol (See Fig. S11A of the Supplemental Material), consistent with systems composed of many elementary steps, each drawing from a moderate activation energy distribution (0–200 kJ/mol) [36]. These observed mean activation energy values also overlap those reported by Dell *et al.* [10]. However, as mentioned above, approximately 1/5 of the datasets show negative scaling, with $\mu_E < 0$ [Fig. 5(b)]. These outliers are enriched for bacteria, viruses, archaea, thermophilic fungi, and viability in heat-tolerant plants, taxa that

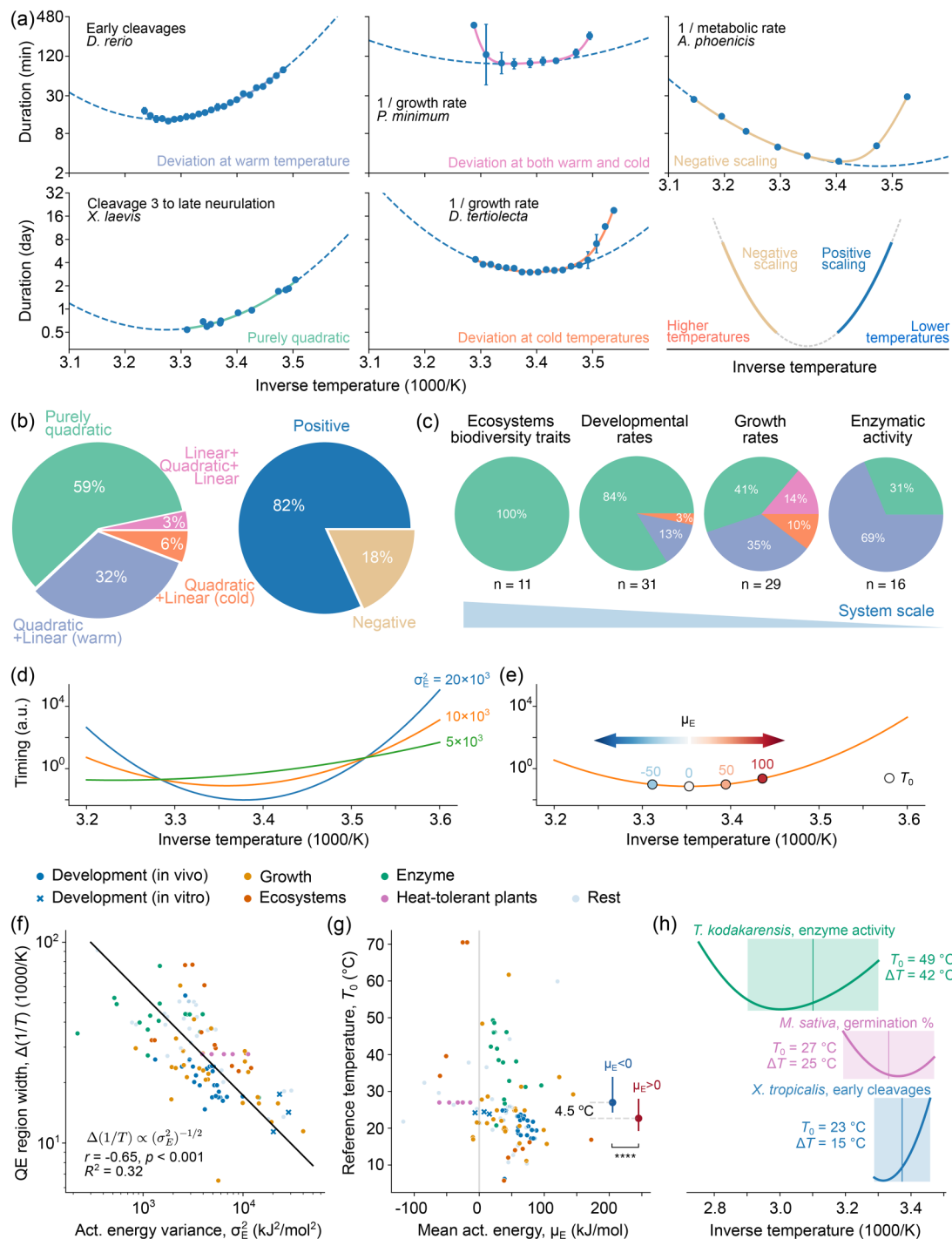


FIG. 5. The Markov cascade model explains temperature scaling across diverse biological systems. (a) Example fits of experimental data using a composite model with a central quadratic exponential (QE) regime flanked by exponential regimes at low and high temperatures. (b) Proportions of $N = 121$ datasets classified as fully QE or showing deviations at warm, cold, or both extremes (left chart) and as positive or negative scaling (right chart). (c) Fractions of datasets classified as fully QE or exhibiting deviations grouped by the process type in a descending order of spatial scale (from left to right). (d), (e) Predicted effect of variation in model parameters σ_E^2 and μ_E on temperature scaling. While panel (d) shows how change in energy variance affects scaling curvature, panel (e) displays how change in mean energy shifts the reference temperature (circles on the parabola). (f) Log-log plot of the QE region width $\Delta(1/T)$ versus σ_E^2 . Diagonal line shows the best fit of $\Delta(1/T) = \alpha(\sigma_E^2)^{-1/2}$. (g) Log-log scatterplot similar as in (f) but in (μ_E, T_0) space. The reference temperature T_0 is compared across datasets with positive and negative scaling, shown using median and interquartile ranges in red and blue, respectively ($p < 0.01$ via the Mann-Whitney test). (h) Examples of representative temperature performance curves to demonstrate the predicted effects of σ_E^2 and μ_E : (bottom) *X. tropicalis* early cleavages as a reference, (middle) negative scaling in *M. sativa* germination percentage, and (top) reduced energy variance in *T. kodakarensis* enzyme activity scaling. In all curves, shading and a vertical line indicate the QE region and the reference temperature, respectively.

routinely experience severe thermal conditions (Fig. S11B of the Supplemental Material [19]). Comparison of the reference temperature T_0 in these two groups shows that the $\mu_E < 0$ group has a higher median T_0 by approximately 4.5 °C relative to the population with $\mu_E > 0$, consistent with a predicted change of the operating range toward warmer conditions [Fig. 5(e)]. Nonetheless, a group of datasets in the $\mu_E > 0$ population exhibits a high reference temperature T_0 comparable to that of the negatively scaled curves. This group is largely occupied by the enzymatic activity data, characterized by an extended QE region and the lowest fitted σ_E^2 [Figs. 5(f) and 5(g)]. In fact, the apparent activation energy $\mu_E - \sigma_E^2/T_0$ links these three parameters; thus, given a similar level of μ_E , a variation in σ_E^2 can explain the variation in T_0 , observed in the $\mu_E > 0$ group (lower σ_E^2 leads to higher T_0 and vice versa). We hypothesize that the lower σ_E^2 can elevate the reference temperature T_0 by stretching the entire QE region.

Thus, both parameters— μ_E and σ_E^2 —set the thermal operation ranges of experimental data in accordance with the model predictions. In Fig. 5(h), we illustrate and summarize these effects by plotting three characteristic temperature response curves alongside each other—scaling of *X. tropicalis* early cleavages period used as a reference (bottom), contrasted by *M. sativa* germination percentage scaling with $\mu_E < 0$ (middle) and by the scaling of enzyme activity in *T. kodakarensis* with reduced σ_E^2 (top).

E. Negative quadratic exponential temperature scaling can be recapitulated in the Markov cascades

Data reveal two distinct settings in which the apparent activation energy can be negative: (i) at the *hot extreme*, where the macroscopic slope becomes negative; and (ii) within the intermediate QE range, where a local negative slope is observed over a finite window. In our theory, the hot-edge case has a simple mechanistic account via reverse-loop trapping in cycles [cf. Fig. 4(c)]. In contrast, the mechanism in the QE window case is unclear.

Although assigning negative microscopic activation energies [Figs. 6(a)–6(c)] can reproduce the observed temperature-scaling phenomenologically [Fig. 5(a) and 5(f)], this interpretation is unlikely for enzymatic reactions and obscures the network-level mechanisms responsible for negative apparent slopes. While negative activation energies are not forbidden for elementary chemical reactions [81], the typical transitions in biological systems we have in mind are enzymatic reactions. These are usually well described by transition-state theory as proceeding via an unstable activated complex atop a positive potential-energy barrier [8,36]. We therefore sought thermodynamically consistent network mechanisms in which negative apparent activation energies emerge within the QE window from combinations of microscopic steps that each obey Arrhenius scaling with positive activation energies.

To illustrate this, we turn to minimal network motifs composed solely of positive-energy transitions, for which we have previously demonstrated the possibility of negative scaling [Figs. 4(b)–4(d)]. For simplicity, we focus on a cycle motif and consider a Markov cascade of such cycles [Fig. 6(d), Supplemental Material, Note 8 [19]]. In this setup, forward and backward one-step transitions, each positively

scaled but differing in mean activation energies [Fig. 6(e)], together produce negative scaling of individual cycles at higher temperatures and positive scaling at lower temperatures [Fig. 6(f)]. As expected, the MFP time of a cascade of such cycles then displays negative QE scaling over a defined temperature range under physically realistic conditions. The simple cycle examined here serves merely as one example, as many other multi-step motifs could plausibly underlie negatively scaled reactions (see, for example, Supplemental Material, Note 8C [19]). Motifs of the modified chain, shown in Fig. 6(d), contain irreversible steps that mimic such biological mechanisms as a checkpoint or a bistable switch. Since both of them emerge, e.g., through positive or double-negative feedback loops in the underlying regulatory networks, we hypothesize that biological processes characterized by negative activation energy can be distinguished by more complex biochemical regulation.

III. CONCLUSION

Temperature fundamentally shapes biological timing. While the Arrhenius equation captures how individual activated steps accelerate with temperature, its limits become clear across broader thermal ranges and complex multi-step processes. Here, we introduced a Markov cascade framework that provides a mechanistic account of these departures.

For reversible multi-step cascades, we show that the mean first-passage time generically exhibits three temperature-scaling regimes: a quadratic-exponential regime near a reference temperature, with Arrhenius-like behavior at low and high temperatures. The extent of the QE regime and the crossover temperatures are set by the mean and variance of activation energies and by the balance of forward and backward transitions.

Apparent negative activation energies need not imply negative microscopic barriers or enzyme denaturation. In our framework, they emerge naturally from network topology. Simple motifs, such as cycles or feedforward parallel pathways, are sufficient to generate such effects, emphasizing how global thermal responses can emerge from local, reversible steps.

The theory agrees with data: applied to more than 100 densely sampled datasets spanning taxa, rates, and developmental intervals, the composite QE–Arrhenius picture provides excellent fits, explains when coordination is preserved, and clarifies why breakdowns can occur near physiological limits. These results offer practical guidance for measurement (need for dense temperature sampling) and interpretation (treat warm-edge slowdowns and negative slopes as network effects unless denaturation is independently demonstrated), and they suggest how thermal robustness may be organized by network architecture in natural systems.

Our QE prediction is consistent with graph-theoretic first-passage analyses of large networks [14]. We specify when QE holds for finite linear reversible cascades, explain the re-emergence of Arrhenius scaling at the extremes and motif-driven negative apparent activation energies, and validate these predictions across a broad empirical dataset.

More broadly, the Markov cascade model provides a unified, testable framework for forecasting how biological timing

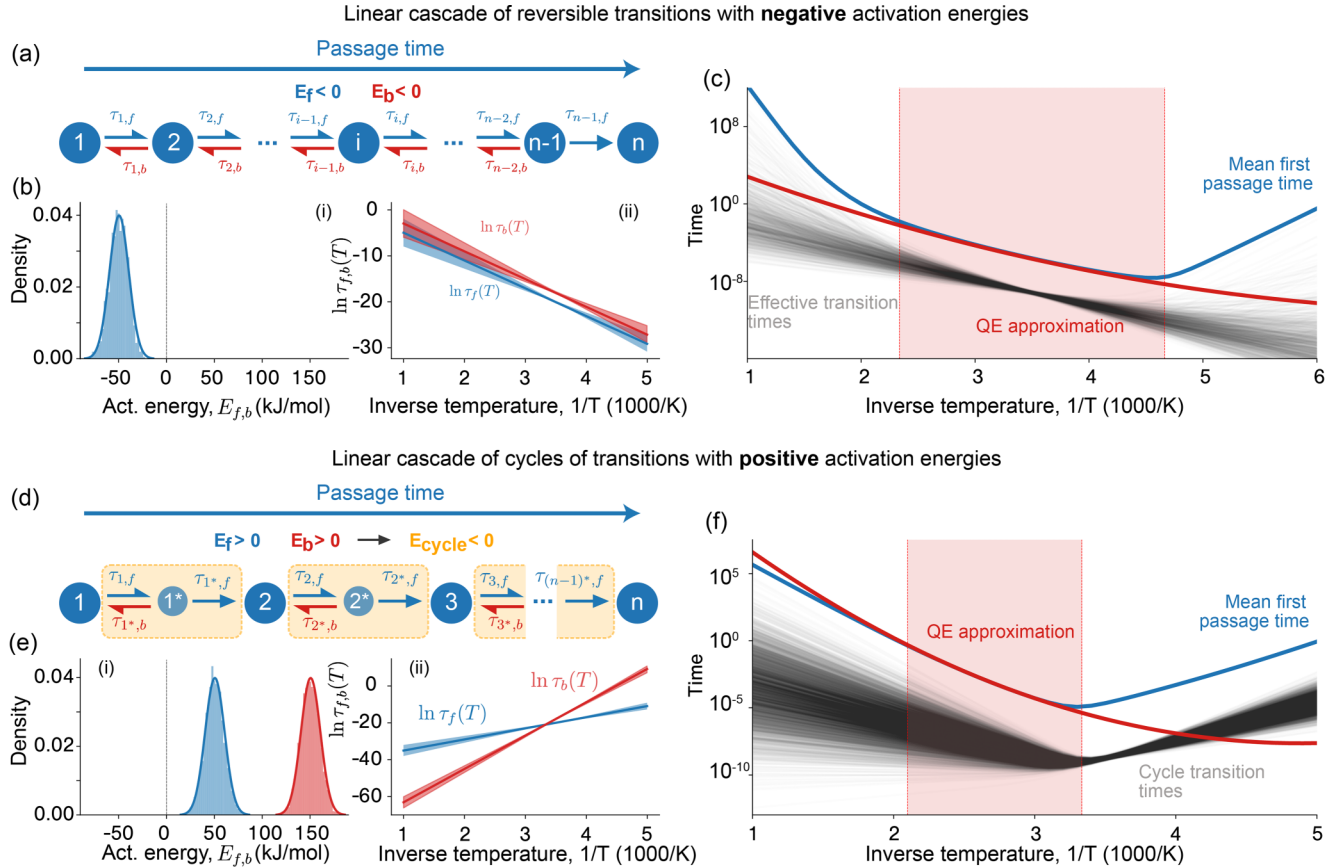


FIG. 6. Negative quadratic-exponential temperature scaling can be recapitulated in the Markov cascade of cycles. (a)–(c) Negative MFP time temperature scaling results from the negative scaling of individual reactions in a linear Markov cascade. (a) Schematic of a linear cascade, where each reaction has negative activation energy ($E_{f,b} < 0$). (b) Temperature scaling of individual reactions: (i) normal distribution from which activation energies $E_{f,b}$ are drawn; (ii) “negative” scaling of individual forward and backward reactions in blue and red, respectively (mean $\pm$ s.d.). These distributions are generated using the parameters in Table S4 (left) of the Supplemental Material [19]. (c) Resulting temperature scaling of MFP time in the linear cascade (blue). The bold red curve shows QE approximation, the thin black curves display effective transition times, and the shading indicates the range where MFP time scaling follows the QE approximation. The procedure for the simulations and the calculation of the analytical approximations is outlined in Supplemental Material, Note 8D [19]. (d)–(f) Cascade of cycles with an apparent negative scaling branch composed of positively scaled reactions can also exhibit negative MFP time scaling. (d) Schematic of a linear cascade of cycles, where each reaction has positive activation energy ($E_{f,b} > 0$), yielding negative apparent activation energy of cycles ($E_{cycle} < 0$). (e) Temperature scaling of individual reactions: (i) normal distributions from which activation energies $E_{f,b}$ are drawn; (ii) “positive” scaling of individual forward and backward reactions in blue and red, respectively (mean $\pm$ s.d.). These distributions are generated using the parameters in Table S4 (right) of the Supplemental Material [19]. (f) Resulting temperature scaling of MFP time in the linear cascade of cycles (blue). The bold red curve shows QE approximation, the thin black curves display individual cycle transition times, and the shading indicates the range where MFP time scaling follows the QE approximation. The procedure for the simulations and the calculation of the analytical approximations is outlined in Supplemental Material, Note 8D [19].

shifts with temperature. By linking microscopic activation statistics to macroscopic timescales, it identifies thresholds of developmental failure, guides targeted perturbations (e.g., altering network topology or biases) and can be extended beyond development to other multi-step physiological and ecological processes in a warming world.

IV. DISCUSSION

A. Robustness of biological timing to temperature changes

1. Development

Embryonic development consists of a cascade of coordinated biochemical and morphological events. Although absolute stage durations can vary severalfold with temper-

ature, the final anatomical outcome is often remarkably consistent. In *X. laevis* embryos, for example, the relative durations of developmental stages remain nearly invariant across a broad thermal range [11,17] [Fig. 7(a)]. Similar proportional scaling occurs in *Drosophila*, where the durations of embryonic and pupal development scale together across temperature [17,62] [Fig. 7(b)], in zebrafish somitogenesis, where somite spacing remains constant despite more than threefold variation in period [82], and in early *Drosophila* embryos, where transcriptional output scales with cell-cycle timing to maintain gene expression levels across temperatures [83].

These examples illustrate striking developmental robustness, but this resilience is not universal. Recent single-cell profiling in zebrafish has revealed cell type-specific

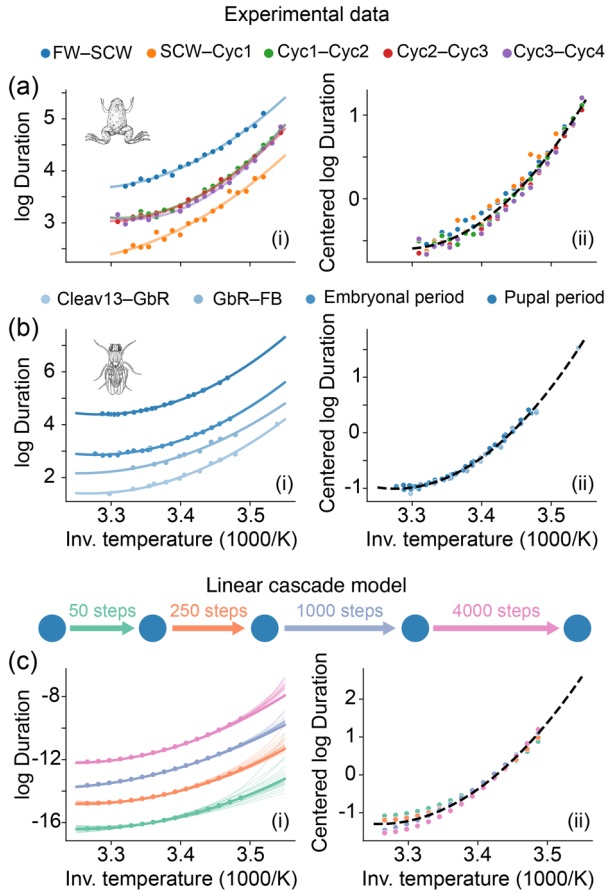


FIG. 7. Robustness of biological timing to temperature change. (a) Mean durations of five stages of early embryogenesis in *X. laevis* (Data from Ref. [11]). (b) Mean durations of four developmental stages in *D. melanogaster* [Similar to Fig. 5(e), data from Refs. [17,18]]. (c) Simulated mean durations of successive developmental stages using a linear Markov cascade of increasing size (from 50 to 4000 steps) overlaid with individual realizations. In panels (A)–(C), the left panels display the logarithm of biological timing per stage, while the right panels show the mean-centered logarithm of duration, i.e., duration with the extracted mean over the temperature range per stage, overlapped with the universal scaling curve.

vulnerabilities to thermal stress, leading to structural defects [84]. Such findings highlight that whole-embryo robustness can coexist with local sensitivities, and that thermal perturbations at the molecular level can propagate to higher-level coordination [85].

Robust preservation of relative timing is often attributed to active temperature compensation, such as opposing enzymatic reactions with tuned activation energies [86]. Our results show that robustness can also arise passively from network structure. In the QE regime, timing reflects an average over many similarly scaled reversible steps, naturally suppressing variability and preserving stage-to-stage ratios even when absolute rates change. Simulations [Fig. 7(c)] demonstrate that this effect persists even when different stages contain different numbers of steps or distinct reaction-rate distributions, and remains robust to stochastic variability in individual rates.

At thermal extremes, this passive robustness breaks down as averaging gives way to a small number of rate-limiting

steps. Variability increases and relative stage durations can shift, a pattern observed experimentally in several systems at high or low temperatures [86]. This is consistent with the idea that coordination is maintained only within the Arrhenius-like regime of physiological rates [16]. In our framework, such a breakdown corresponds to transitions from the central QE regime to Arrhenius-dominated flanks at the edges of the viable temperature range.

2. Temperature compensation

In chronobiology, the maintenance of relative timing across temperature is sometimes called temperature compensation of phase relationships. For example, the neuronal oscillator in the crab *Cancer borealis* preserves phase relationships across temperatures even though its period changes [87,88]. A stricter definition is used for systems such as the circadian clock, where the period itself is temperature-independent ($Q_{10} \approx 1$) [89–92].

Mechanistic explanations for strict compensation include antagonistic balance—opposing processes with matched activation energies [93,94], and enzyme-limited schemes, where several reactions share a common catalytic bottleneck [95,96]. Network-based models have also shown that temperature robustness can emerge from architecture and feedback alone [97–99].

Our framework extends these ideas: compensation can emerge not only from fine-tuned opposing processes but also from distributed averaging across many steps with constrained activation energy distributions. In some cases, this averaging naturally produces similar scaling for opposing reactions, leading to antagonistic balance without explicit tuning. In others, large control networks, whether realized as long sequential cascades (as analyzed here) or as more complex topologies [14], in the QE regime can exhibit very low apparent activation energies, $\mu_E - \sigma_E^2/RT_0$, balancing mean and variance in individual reaction activation energies to achieve robustness.

B. Negative activation energies

Negative apparent activation energies have long been reported in experimental studies of thermal scaling, particularly at warm temperatures, but their mechanistic basis has remained unclear.

At temperature extremes, they may arise from processes that deviate from simple Arrhenius kinetics, for example, biphasic behavior such as protein denaturation [100,101], or nonlinear system dynamics approaching bifurcations [11]. In Markov networks, cycles and feedforward motifs [Figs. 4(c) and 4(d)] can likewise produce negative slopes when transitions become dominated by reverse steps or competing paths.

Within the QE regime, negative activation energies can emerge more subtly as apparent network-level properties. Although each microscopic step obeys Arrhenius scaling with a positive energy, motifs such as asymmetric cycles generate local negative slopes, and cascades of such motifs can display an overall negative μ_E (Fig. 6). These values therefore do not reflect physically negative microscopic energies, but rather emergent signatures of distributed, multi-step regulation.

Negative μ_E values are enriched in organisms adapted to extreme or fluctuating environments [Fig. 5(g)]; however,

the biological or evolutionary significance of this association remains unclear.

C. Evolutionary and ecological implications

Across approximately 100 datasets, we find that many biological systems predominantly operate within the QE regime, yet deviations, particularly at elevated temperatures, are frequently observed. This pattern suggests that biological systems may have evolved to function optimally within a buffered thermal window, outside of which developmental timing becomes increasingly erratic or prone to failure. Supporting this idea, recent work [11] showed that, across multiple species, the native environmental temperature closely aligns with the minimum of the QE scaling curve. In other words, these organisms appear to have evolved to maximize developmental speed while remaining within the more robust, low-variability region of the QE regime. These findings carry important implications for understanding how organisms adapt to thermal environments and for predicting the biological consequences of climate change. Consistent with this view, Begasse *et al.* [16] observed that the fertile temperature window in *C. elegans* and *C. briggsae* overlaps with the range in which cell division adheres to Arrhenius kinetics, highlighting a correlation between developmental robustness and fertility limits. Our results emphasize the importance of characterizing thermal response curves at high resolution, not just at a few “representative” temperatures, to uncover the underlying structure of thermal sensitivity and to identify critical tipping points in developmental systems. Although we focused on steady temperatures, many ectothermic embryos experience substantial temporal variability [3]. Extending the Markov cascade framework to time-dependent temperatures, for example, by allowing transition rates to follow imposed thermal trajectories, may help quantify how fluctuation amplitude and timescale interact with network structure to shape developmental timing. Such extensions represent an important direction for future work.

D. Markov vs deterministic descriptions

Previous studies of temperature dependence in biological systems have largely relied on deterministic dynamical models [11,90,93,94,97–99]. Such models provide detailed descriptions of concentration dynamics and have been invaluable for dissecting specific regulatory circuits, but they become difficult to extend to heterogeneous, nonoscillatory, or large-scale developmental processes. In this work, we adopted a stochastic master-equation approach, in which Markov theory enables analytical treatment of first-passage times, reversibility, and network topology without requiring the integration of high-dimensional nonlinear dynamics. Although stochastic chemical master equations reduce to deterministic laws in the large-system limit [102], constructing faithful microscopic equivalents of reduced biological ODE models is generally nontrivial. Our ODE-to-Markov mapping of the embryonic cell-cycle oscillator provides a concrete example in which the macroscopic limit of a Markov jump process accurately reproduces deterministic periods and thermal limits. This demonstrates that Markov frameworks can

serve as effective coarse-grained descriptions of biological timing, particularly for multi-step, partially reversible processes where network structure plays a central role. Finally, in systems where intrinsic stochasticity is biologically relevant, Markov formulations also provide a natural framework for studying variability in timing, beyond mean first-passage times. Previous work has shown that higher-order moments of passage-time distributions can encode important functional constraints, such as precision and robustness, in biological networks [33,34]. Extending the present framework to account for such fluctuations represents a natural direction for future work.

E. Connections to empirical biophysical models

Our results also relate to the Sharpe–Schoolfield model, a biophysically inspired framework that combines temperature-dependent reaction kinetics with reversible enzyme inactivation at temperature extremes [24,25,103–105]. Like our model, it captures three distinct thermal regimes: intermediate speeding up with quadratic-like curvature, a linear scaling at cold extremes, and a slowdown with negative activation energy at hot extremes. However, it attributes the deviations at both ends to enzyme inactivation. In contrast, our Markov framework shows that similar triphasic scaling can emerge from statistical averaging and network effects, even without assuming enzyme denaturation. This provides a mechanistic foundation for empirical patterns observed across taxa. Other commonly used models, such as the QE and DE, typically capture only one or two scaling regimes; while useful for fitting smooth or abrupt deviations, they often fail to describe the full thermal range seen in biological data.

F. Toward predictive models of temperature scaling

Lastly, we circle back to Fig. 1(e), which shows the temperature scaling of developmental times in *D. Melanogaster* as collected by Crapse *et al.* [17]. In their work, they tried to get a mechanistic insight into these results by simulating development as a cascade of irreversible transitions with different activation energies. However, they found that this irreversible cascade could not account for the experimentally observed sharp deviations from the Arrhenius law at thermal extremes. Our work explains this discrepancy by showing the necessity of backward transitions to generate strong curvature and negative activation energies in the Arrhenius diagram. Recently, the idea of computing MFP times in Markov jump processes to study the temperature dependence of biological timings has also been exploited by Voits and Schwarz to derive a QE law for a generic class of large-scale random biochemical networks with forward and backward transitions, providing strong mechanistic support to the validity of the QE [14]. Comparing their results to our work, it should be noted that their initial assumptions do not hold for the specific network topology considered in our model: a linear cascade of reversible transitions.⁵ Still, we arrive at the same

⁵The key difference is that the cascade of reversible transitions has only one spanning tree that ends with its root on the final state, that is only one possible forward path toward the final state.

conclusion of QE scaling at intermediate temperatures. Additionally, the simplified setting of our model also opens up the possibility to investigate analytically how a single dominating network motif can lead to strong deviations from the QE at temperature extremes, described by a combination of QE and DE temperature scaling. Extending such an analysis to generic large-scale networks is an interesting direction for future research. Together, these insights highlight the need for mechanistic models that explain not only what scaling behavior occurs, but why and how it might shift under environmental change [106,107]. By combining statistical physics with biological realism, the Markov cascade model provides a versatile tool for studying temperature-dependent timing. It unifies empirical observations across diverse taxa and stages, and offers mechanistic explanations for scaling deviations that previously lacked theoretical grounding. As global temper-

atures continue to rise, such generalizable models will be essential for identifying thresholds of developmental failure and for predicting species-specific vulnerabilities.

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DATA AVAILABILITY

The data that support the findings of this article are openly available [108].

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