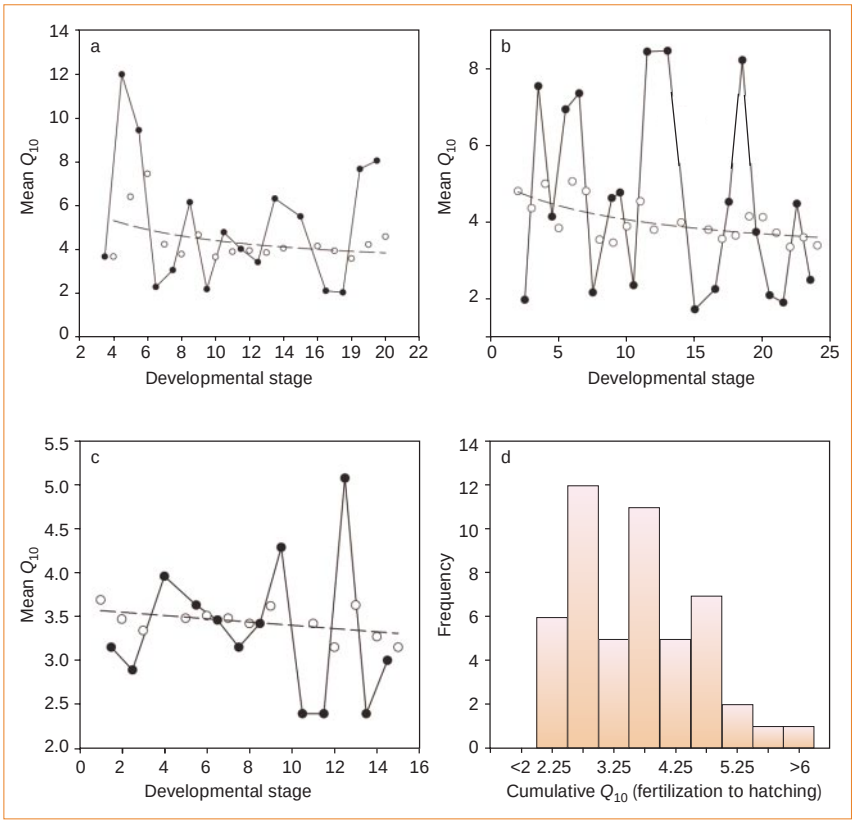

Figure 1 Stage- and species-specific variation in thermal sensitivity. Literature data are shown for **a,** tailed frog[2]; **b,** chinook salmon[3]; **c,** sea urchin[4]; and **d,** various teleost fish[5]. Development times (D) at various temperatures (T, in °C) were fitted to the exponential model $D = ae^{bT}$, and mean van't Hoff Q_{10} values were calculated as e^{-10b}, where b is the slope of the regression equation. Interval Q_{10} values (filled circles) are for the duration of defined embryonic stages; cumulative Q_{10} values (open circles) are for the period from fertilization to a particular stage.

$Q_{10} = \exp(-10\alpha)$; Arrhenius Q_{10} = van't Hoff Q_{10} at 0 °C).

This variation is unlikely to be the result of measurement errors. Stage durations are typically determined to an accuracy of within a few per cent of the reported time. Comparisons between species also indicate considerable variability. For example, van't Hoff Q_{10} values for the duration of the embryonic period (fertilization to hatching) of various species of teleost fishes ($n = 50$) range between 2.1 and 6.9 (Fig. 1d)[5]; the equivalent range for Arrhenius Q_{10} values is about 2.5 to about 7.4.

Diversity is the salient characteristic in terms of temperature sensitivity at the stage and species level. It is necessary to look across stages and species to see any indication of uniformity. There are two principal reasons for this apparent narrowing of thermal responsiveness. One is a simple averaging effect. The net effect of stage-specific differences in thermal responsiveness becomes progressively less, the longer development proceeds (cumulative Q_{10} values in Fig. 1a–c). Mean response similarly converges in cross-species comparisons as more species are included in the analysis. The other reason is that evolutionary adaptation tends to reduce cross-species thermal sensitivity[5,6]. For example, rate functions of individual fish species typically show greater thermal sensitivity than would be predicted from cross-species comparisons, both during development[5] and as adults[6].

Changes in development rate have important implications for physiology (growth efficiency, for example) and ecology (such as predator–prey match/mismatch). Knowing the extent to which temperature affects development rate is therefore important, especially when viewed in the context of global warming. Universal models based on cross-stage and cross-species comparisons are unfortunately of little use in predicting how a particular species will respond. It will be necessary to examine each stage and species separately to come up with realistic predictions.

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Allometry

How reliable is the biological time clock?

A theoretical framework that describes the effect of temperature and body size on biological processes has long been sought. Gillooly *et al.*[1] use an elegant combination of the first principles of biochemical kinetics and allometric growth to derive a general definition of biological time by which, simply from a knowledge of how warm and how large an animal is, it is possible to calculate how long it will take to develop. Here I argue that their application of chemical kinetics in formulating the theory is problematical, undermining their claim that the theory fits the evidence.

Gillooly *et al.*[1] build on their equations for metabolism (B; ref. 2) to predict that plots of $\ln(t/m^{1/4})$ against $T_c/(1+(T_c/273))$, where t is the development time, T_c is the animal's temperature in degrees Celsius, and m is its mass, yield a universal straight line with a common slope $\alpha = -\bar{E}/kT_0^2$, where E is the average activation energy for metabolic reactions, k is Boltzmann's factor and $T_0 = 273$ K. However, this relation is neither linear nor universal.

The temperature-dependence term in the equations used by Gillooly *et al.*[1,2] is the van't Hoff–Arrhenius law: $B(T) = B(T_0)e^{\bar{E}/k(T-T_0/T_0T)}$; Arrhenius showed that the Q_{10} is a simplification for conditions when T and T_0 are close together, a conclusion also reached by Gillooly *et al.*[1]. When the van't Hoff–Arrhenius law was first applied to development time[3], it became evident that the activation energy decreases as temperature increases. This is still apparent from Gillooly *et al.*[1]: $\bar{E}$ decreases with temperature by a factor of no more than 49% (Fig. 1a).

Therefore, as k and T_0 are constants, the slope $\alpha = -\bar{E}/kT_0^2$ increases with temperature and the relationship is not linear. There is further temperature dependence beyond the 'exact' expression $e^{(-\bar{E}/kT_0^2)(T_c/(1+(T_c/T_0)))}$ of Gillooly *et al.*[1]. The overall pattern in some of their figures might seem to be linear owing to the combination of many curvilinear relationships (one for each species) in a single plot and to the previous adjustment of many of the original data to intervals of 5 °C (Fig. 1b).

The main objection to the application of chemical kinetics to biology[4] is that vital action is arrested in the vicinity of 0 °C and not at −273 °C. Therefore, as the constants $B(T_0)$ and $a(T_0)$ should be zero for $T_0 = 273$ K, the equations of Gillooly *et al.*[1] have zero $a(T_0)$ in the denominator and cannot be solved. Ultimately, this is why the relationship is not linear: when body temperature approaches the biological zero, development ceases and the line becomes asymptotic to the y-axis. Gillooly *et al.*[1] do not solve the long-standing debate over whether reaction kinetics[5] or diffusion of reactants[4,6–8] is the limiting factor controlling temperature dependence in vital processes.

Combining temperature and body-mass dependence[1] is an interesting attempt to find out how the biological time clock works, but the clock seems to be imprecise. Data are presented on the development time for marine fish eggs in Fig. 2 of Gillooly *et al.*[1], who infer that the intercept is similar to that in their Fig. 1, but when these are plotted on the same *y*-axis scale they are strikingly different. Also, egg-development times for univoltine aquatic insects[9] were excluded from their compilation. Taking an arbitrary temperature of 20 °C, the biological clock runs fast by 20 days for univoltine aquatic insects and slow by 5 days for marine fish (Fig. 1c). The intercepts are therefore not similar, cautioning against the conclusion that the model of Gillooly *et al.* is common and invariant to all organisms.

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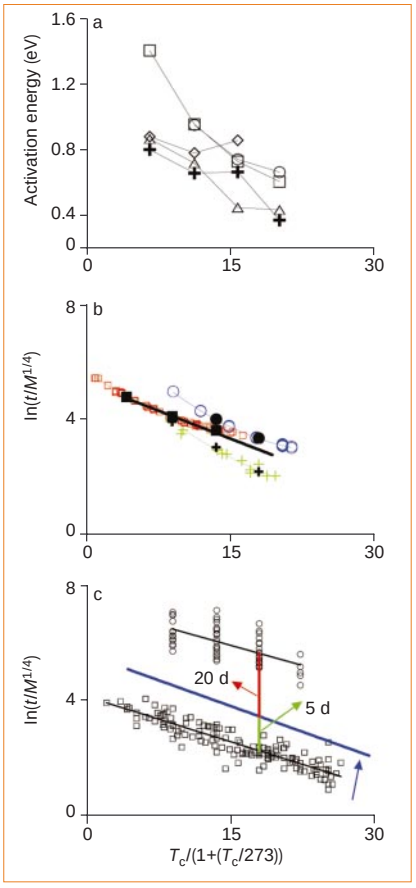

Figure 1 Effect of temperature on mass-corrected development time. **a,** Activation energy, $\bar{E}$, calculated following Gillooly *et al.*[1], for different temperature intervals plotted against the interval's mean temperature. Lines link the average activation energy for species of amphibians (squares), fish (rhomboids), multivoltine aquatic insects (circles) and zooplankton (crosses, embryonic development; triangles, post-embryonic development). **b,** Colour symbols show the complete data sets, not adjusted to 5 °C intervals, for chinook salmon (squares), garfish (circles) and bream (crosses). Data sources from references in Gillooly *et al.*[1]. Black symbols are the adjusted data for each of these species as used by Gillooly *et al.*[1]; black line is their linear fit for fish (their Fig. 1b). **c,** 'Biological time clock' line (blue arrow) obtained by Gillooly *et al.*[1] for aquatic ectotherms and birds (their Fig. 3). Red and green lines show prediction error of the 'biological time clock' at 20 °C for non-diapause eggs of univoltine aquatic insects (circles) and marine fish (squares) incubated at different constant temperatures. Errors in days were calculated from egg masses of 0.01 and 0.9 mg, the average for each group from Gillooly *et al.*[1].

Gillooly et al. *reply* — Rombough asserts that our model[1] is of limited use in predicting the temperature dependence of developmental time, but he has used a model with the same two primary variables, size and temperature, for the same purpose[2]. From his Fig. 1d above, he says that diversity among species is the salient feature for temperature dependence.

We replotted these data as the natural logarithm of mass-corrected incubation time against average incubation temperature in °C for fish with sizes and temperatures reported ($y = -0.18x + 4.34$; $r^2 = 0.72$; $n = 36$; Q_{10} ranges from 2 to 7; see supplementary information). The slope (−0.18) is close to the value predicted by our model (−0.14). Our model explains 72% of the variation, leaving only 28% to be explained by species differences, measurement errors and all other factors.

Rombough uses data on time intervals between stages to argue that the effect of temperature varies significantly during development. Most of this variation is measurement error because stages are defined arbitrarily, and times between them are short. Plotting cumulative times for three different stages from these data (Fig. 1) gives excellent fits and very similar slopes, showing that the temperature dependence remains nearly constant throughout development. Rombough actually presents additional support for our model.

López-Urrutia's main theoretical objection is that "vital action is arrested in the vicinity of 0 °C", so our model is undefined. Biological activity ceases at around 0 °C because of a phase transition, the freezing of water. We consider this to be a separate process from molecular kinetics (which ceases at absolute zero). Therefore, by extrapolating the $a(T)$ curve for $T > 0$ to $T = 0$, we obtain a *y*-intercept, $a(0)$, that is always non-zero. López-Urrutia argues that activation energy (E) decreases systematically with temperature, and our figures only seem to be linear because we have averaged across temperatures for species. Plotting E for narrow temperature intervals, as in his Fig. 1a, is subject to measurement errors; the confidence intervals are infinite. Systematic changes in E with T should give curvilinear relationships in plots of $\ln(t/m^{1/4})$, as in his Fig. 1b, c or our Fig. 1. Although one species seems slightly curvilinear in his Fig. 1b, most species showed linear relationships across all our data.

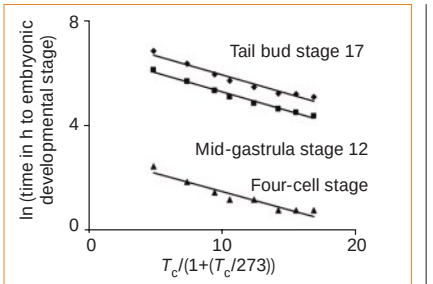

Figure 1 Plot of the natural logarithm of time to reach a specific embryonic development stage against incubation temperature for the tailed frog in Rombough's Fig. 1b (ref. 2). The slope of each line is close to the value predicted by our model (−0.13). For the tail-bud stage 17, $y = -0.15x + 7.4$ and $r^2 = 0.96$; for mid-gastrula stage 12, $y = -0.15x + 6.7$ and $r^2 = 0.98$; and for the four-cell stage, $y = -0.14x + 2.8$ and $r^2 = 0.92$.

Statistics provide a definitive answer for linearity. Curvilinear regression models (polynomials) did not give significantly better fits for any of our plots. Linearity is evident in López-Urrutia's Fig. 1c and in our Fig. 1, where linear fits account for 92–98% of the variation. Note that there is no averaging across temperatures for the fish in his Fig. 1c because each point represents a different species; all points in our Fig. 1 are for the same species.

In his Fig. 1c, López-Urrutia uses differences in intercepts, a, between fish in the wild, univoltine insects and the average for several taxonomic groups to question the reliability of the biological time clock. In our model, the coefficient a allows for variation in intercepts with metabolic rate, B_0, and hence for differences in development times depending on which taxa, environmental conditions and developmental stage are measured. It is the $M^{-1/4}$ dependence on body size and $e^{-E/kT}$ temperature dependence in our equation (6), not the coefficient a, that defines the biological time clock. Note, however, that in López-Urrutia's Fig. 1c, the slopes and therefore the E values are nearly identical.

Both authors overlook our central message by focusing on temperature. We never claimed to have derived the Boltzmann factor or Q_{10}. Our model does provide a theoretical framework that combines the effects of size, temperature and stoichiometry to explain most of the variation in developmental rates across diverse environments and taxonomic groups.

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