A physical explanation of the temperature dependence of physiological processes mediated by cilia and flagella

Stuart Humphries[1]

School of Biological, Biomedical and Environmental Sciences, University of Hull, Kingston-upon-Hull HU6 7RX, United Kingdom

Edited by James H. Brown, University of New Mexico, Albuquerque, NM, and approved July 22, 2013 (received for review January 16, 2013)

The majority of biological rates are known to exhibit temperature dependence. Here I reveal a direct link between temperature and ecologically relevant rates such as swimming speeds in Archaea, Bacteria, and Eukaryotes as well as fluid-pumping and filtration rates in many metazoans, and show that this relationship is driven by movement rates of cilia and flagella. I develop models of the temperature dependence of cilial and flagellar movement rates and evaluate these with an extensive compilation of data from the literature. The model captures the temperature dependence of viscosity and provides a mechanistic and biologically interpretable explanation for the temperature dependence of a range of ecologically relevant processes; it also reveals a clear dependence on both reaction rate-like processes and the physics of the environment. The incorporation of viscosity allows further insight into the effects of environmental temperature variation and of processes, such as disease, that affect the viscosity of blood or other body fluids.

biological fluid dynamics | Arrhenius

Eukaryotic cilia and prokaryotic flagella are whip- or corkscrew-like cellular organelles responsible for a wide range of biological functions (Fig. 1*A*). The temperature dependence of processes driven by these organelles has been recognized for well over a century (1) and can be seen in unicells and both poikilo- and homeothermic metazoa. What has been less commonly noted is the dependence of cilial and flagellar movement on the viscosity of the medium in which they operate.

When an object such as a cilium moves in a fluid, it experiences an external load (drag) that depends on the fluid's viscosity. In systems operating at low Reynolds (Re) numbers ($\mathrm{Re} = \rho l v/\mu$, where ρ is fluid density, l a length scale, v velocity, and μ dynamic viscosity), this viscous drag dominates the dynamics of movement because the product $l \times v$ (inertia) is small compared with μ (viscosity). Temperature determines the kinetic energy of molecules, driving the well-known response of reaction rates, but the kinetic energy of molecules in liquids also determines their ability to move with respect to one another, thus leading to the negative relationship between temperature and viscosity seen in most liquids. Though several studies have shown the importance of temperature-dependent viscosity to individual systems (2–5), the effect has largely been ignored, and cannot be described directly by Arrhenius kinetics. Models based on Arrhenius kinetics attribute changes in fitness with temperature to an exponential increase in chemical reaction rates with temperature (6).

Explaining the mechanics of temperature dependence of biological processes is key to deciphering organismal performance and interactions in a changing climate. However, despite considerable interest in the mechanics of cilial and flagellar movement, previously developed models have not incorporated important physicochemical effects of temperature that are known to influence cilial and flagellar movement rates, such as the viscosity–temperature relationship of the fluids in which they operate. Thus, the use of Arrhenius kinetics as a mechanistic description of the relationship between biological rates and temperature may not always be appropriate. Arrhenius kinetics are useful, particularly when incorporated into larger predictive frameworks, such as the metabolic theory of ecology (7–9), but alternative models are capable of providing additional understanding of the processes specific to particular systems (10).

Here I report results from a model where cilial and flagellar responses to external load are determined by the dynamic stiffness of the molecular motors driving these organelles (e.g., dynein), and demonstrate that core functional relationships between temperature and biological rates linked to flagellar movement can be derived from purely physical effects. I develop a model of beat frequency for cilia and flagella to examine their temperature dependence in terms of both reaction rates and physics of the environment. For brevity, I describe the model mainly as it relates to a eukaryotic cilium, but the full derivation and those for rotating Archaeal and Bacterial flagella (where torque plays the role of stiffness) are provided in *SI Appendix*.

The starting point for the model is the force balance of a moving cilium. By assuming that the power (rate of energy transfer) available to a cilium is proportional to that required to produce a given cilial beat frequency, it is possible to derive a set of simple expressions for beat frequency given the physical relationships outlined in Fig. 1*B* (*SI Appendix*, Eqs. **S12** and **S13**). The key points of this model are that it includes the effect of temperature on the viscosity of both the cell contents, and the external medium against which the cilium acts, and includes a linear term relating external viscosity to the stiffness of the cilium. For a rotating prokaryotic flagellum, this stiffness term is equivalent to the torque in the system.

The power available for cilial motion is dependent on a supply of energy characterized by the concentration of energy transfer molecules (for Eukaryotes, usually ATP) and their rate of delivery to the molecular motors (dynein) comprising the ciliary axoneme (a central mechanical organelle). The rate at which the molecular motors hydrolyze ATP and the number of these motors (proportional to ciliary length) (11) also determine available power. Under simplifying assumptions (*SI Appendix*), delivery and hydrolysis of ATP can be described by a temperature-dependent diffusion process. For bacteria, the transport of ions across the cell membrane drives rotation of the flagellum and is driven by diffusion and an electrochemical gradient, again described through a temperature-dependent process. In contrast, the power required to drive a cilium at a given rate depends essentially on its length, the local drag coefficients of the cilium as it moves, and the frequency at which it does so. Here, the drag experienced by the cilial rod will be viscosity (and so temperature)

ECOLOGY

Author contributions: S.H. designed research, performed research, analyzed data, and wrote the paper.

[1] E-mail: s.humphries@hull.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1300891110/-/DCSupplemental.

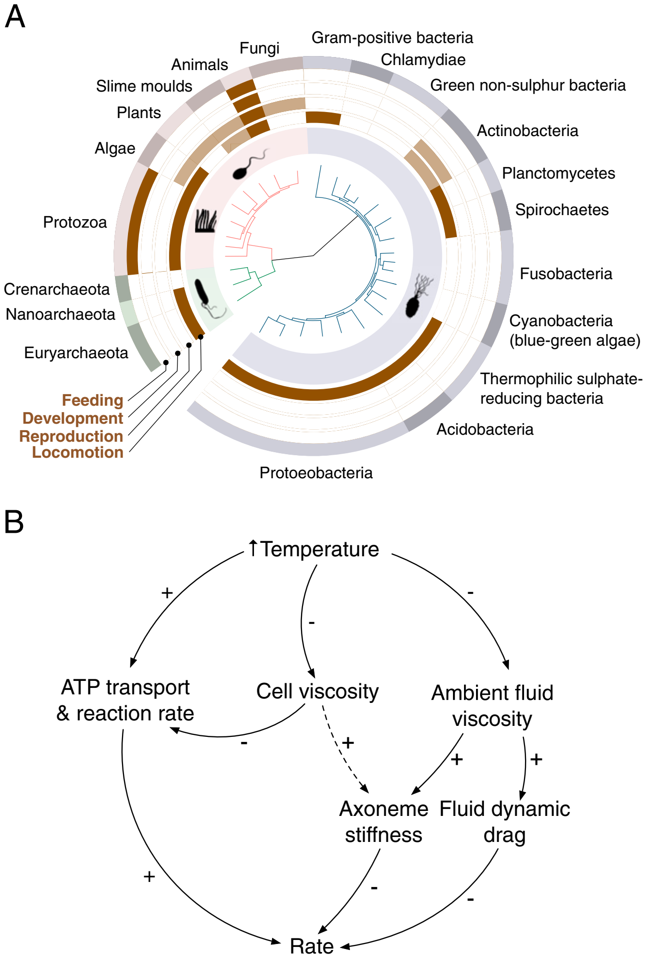

Fig. 1. Flagellar and cilial distribution and model outline. (*A*) The distribution of cilia and flagella and their function on the tree of life. The phylogenetic tree and inner ring illustrate the three major branches of Archaea, Eukaryotes, and Bacteria. Sequential rings indicate the presence (dark), restricted appearance (pale), or absence (white) of cilia or flagella used for locomotion (inner), reproduction, development, and feeding (outer). The outer ring indicates phylogenetic groupings. Data sources are cited in *SI Appendix*, Table S1. (*B*) Graphical representation of the physical relationships described by my model. Increasing temperature can be seen to lead to increased reaction rates, but also to a decrease in the viscosity of environmental and cellular fluids. The dashed line indicates a minor effect not considered by the model.

dependent. To these terms must be added the power required to overcome the stiffness of the cilial rod, which includes terms for the Young's modulus of the cilium and the wavelength of the wave passing along it (12). Evidence suggests that the stiffness of systems built from molecular motors such as dynein varies directly with external load (13–15), which in this case will be determined by the external fluid viscosity. Cytoplasmic dynein takes smaller, but more powerful, steps under load (16), and the stiffness of sperm increases with binding of dynein (17), suggesting that increased load results in lower translation speeds and greater numbers of bound molecules per unit time. Sznitman et al. (18) report a linear relationship between Young's modulus and external viscosity in *Caenorhabditis elegans*, suggesting a similar gearing in muscular systems. For rotational motors, the torque generated is proportional to the rotational drag of the cell body (19), which is linearly dependent on viscosity. In both these cases, raised viscosity increases the power required for a given movement through both increased external load and internal resistance to movement (e.g., stiffness or torque).

Explaining Temperature–Frequency Scaling

An initial test of the model is that it accurately describes the response of cilia and flagellar beat frequencies (and rotational frequencies in archaeal and bacterial flagella) to experimental manipulation of viscosity (30 datasets; Fig. 2 *A* and *C*). Akaike information criterion differences (ΔAIC) indicate that previous predictions (20) of a simple scaling of beat frequency to fluid viscosity (μ_f) for sperm tails as $\mu_f^{-0.5}$ are not supported (aggregate $\Delta AIC = 16.5$, where positive values support the current model). Rather, scaling of frequency with viscosity takes the form $f \propto \sqrt{\mu_f + c}/\sqrt{\mu_f}$ ($\propto c_i/\sqrt{\mu_f + c_j}$ for prokaryotes), where f is frequency and $c_{i,j}$ are constants.

I next compare statistical fits of my model to data from a range of bacterial and eukaryotic taxa against those for an Arrhenius model. Using 17 datasets data from the literature, ΔAIC indicate that there is support (aggregate $\Delta AIC = 5.9$) for selecting my model over an Arrhenius model to explain the scaling of frequency with temperature (Fig. 2 *B*, *D*, and *E*). Here, Arrhenius kinetics suggest a scaling where $f \propto e^{-1/T}$, whereas that of the current model is $f \propto \sqrt{T} e^{-T}$ ($\propto \sqrt{T + c}\, e^{-T}$ for bacteria). However, the point here is not that my model is better, but that it provides a mechanistic explanation of the temperature dependency with parameters that have a real biological interpretation. In this light, a more appropriate criterion is that my model is able to explain the data equally as well as the Arrhenius model; this is supported by the aggregate mean ΔAIC falling within a ±10 range around zero, indicating support for both models.

In generalizing the temperature response and quantifying the effect of load on frequency, the model provides a simple formulation based on biologically meaningful variables. By linking temperature-dependent molecular kinetics directly to cilial and flagellar movement rates, it is now possible to provide a mechanistic link between temperature change and a key biological rate. By incorporating load dependence of dynein force output and stiffness (and rotational torque in prokaryotes), the response of the molecular engines involved both to changes in temperature and in their external media can be better understood. The implications of this load dependence are that we see that viscosity plays a role in the temperature responses commonly recorded across taxa. With this insight we can posit a central scaling relationship between temperature and frequency driven by physical processes, about which further regulation of frequency by neural or chemical stimuli can occur, but which may limit the range of their control.

Understanding the relationships among temperature, fluid viscosity, and cilial beat frequencies has direct application to the experimental methods used to probe cilial mechanics (many of which studies provided the data used here). The core of eukaryotic flagella and cilia is the axoneme, a central rod-like structural and mechanical organelle that bends in response to dynein-generated shear. Separating and attributing the effects of temperature to viscosity and reaction rate-driven components will enable a better understanding of dynein and axoneme mechanics. A clearer understanding of the interactions between biochemical and mechanical effects of temperature change also has bearing on the rheology of mucus secretions. For example, given the impairment of ciliary motility in mammalian respiratory tracts when breathing cold air, and the implications for seasonal respiratory infections (21), we might expect some tuning of mucosal secretion toward shear thinning (22) to reduce energy costs. In a similar vein, despite considerable study (23), exploration of the consequences of fever responses in mammals seem never to have included the impact of increased body temperature on mucociliary transport.

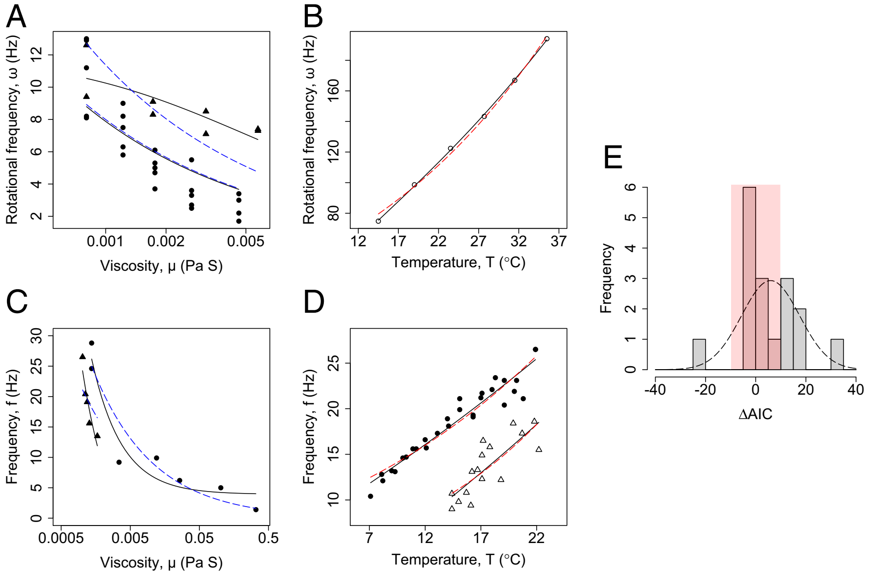

Fig. 2. Examples of data and model fits for flagellar rotational frequency of the bacterium *Escherichia coli* (*Upper*) and the cilial beat frequency of the bivalve *Mytilus edulis* (*Lower*). (*A* and *C*) Response to viscosity, log spaced. (*B* and *D*) Response to temperature. Solid lines are fits for my model, dashed for a function based on scaling as $\mu_f^{-0.5}$ (blue) or on the Arrhenius equation (red). Within each panel, symbol shapes indicate different datasets. Open symbols indicate data from individual cells, filled symbols data from experimental means. (*E*) Frequency of ΔAIC for model fits to data from the literature relating temperature to frequency response (*SI Appendix*). Positive ΔAIC indicates support for my model, negative for the Arrhenius equation. The shaded area indicates the ±10 ΔAIC region where both models might be supported. The dashed line is a normal distribution based on the mean and SD of the ΔAIC values, with an aggregate mean ΔAIC of 5.9. Citations for datasets are given in *SI Appendix*, Tables S4–S7.

Linking the Microscale to the Macroscale

Given the roles of cilia and flagella in motility and fluid transport, the microscale processes described here are pivotal to macroscale processes. Resistive force theory (24) for flagellar motion predicts a linear relation between motile cell velocity and beat frequency (and rotational frequency in prokaryotes) (25), indicating that the locomotion of the majority of low Reynolds-number swimmers should scale with temperature in the same way as does beat frequency. To investigate this, I conducted a meta-analysis of 15 datasets, which shows that beat (Eukaryotes, $n = 11$ datasets and nine species) and rotational (Bacteria, $n = 4$ datasets and three species) frequencies correlate strongly and linearly with swimming speeds (fixed-effect estimate of $r = 0.80$; Fig. 3). Thus, swimming speeds of bacteria, protozoan parasites, and metazoan gametes depend directly on the frequency (rotational or beat) of their cilia or flagella.

On the basis of the linear relationship between swimming speed and beat/rotational frequencies, it seems highly likely that the same dependency will exist in other processes driven by cilia: fluid flow rates are linearly related to beat frequency in frog esophageal cilia (26) and in magnetic biomimetic cilia (27), and several lines of theory suggest such a relationship (28). In the metazoa mucus transport (29), fluid pumping in groups such as sponges, molluscs, and other marine invertebrates (30), and the nodal flows involved in left/right asymmetries in vertebrates (31), are all driven by ciliary movement.

To test if macroscale processes could be driven by biophysical responses to temperature, I compared model fits for data on filtration rates of metazoan animals and swimming speeds of prokaryotes (Bacteria and Archaea) and metazoan gametes. For 38 datasets from the literature, ΔAIC indicate that there is little difference in support (aggregate ΔAIC = 1.3; Fig. 4 *B*, *D*, and *E*) between an Arrhenius model and the current model to explain the scaling of rates with temperature. Previous models (32) have linked wave parameters to swimming speed in bacteria to predict $(\mu_f + \text{constant})^{-1}$ scaling with viscosity, whereas Roberts (28) derived a $\mu_f^{-0.5}$ scaling for ciliate microorganisms. The theoretical prediction of Roberts was used here for comparison with the similar scaling predicted for individual flagella, but is similarly not supported ($n = 31$, aggregate ΔAIC = 7.2). Though the predicted $\mu_f^{-0.5}$ scaling is not supported, the relationships predicted by the current model presented here can be described very approximately by other power laws, providing an explanation for the range of exponents recently reported for scaling of rates with viscosity in a range of aquatic organisms (33).

Linking microscale temperature dependency to larger processes puts theories built on Arrhenius-like relationships on a firmer methodological and predictive footing. Measurable biological rates such as swimming, pumping, and filtration provide a suite of ecologically relevant phenomena that are driven by comprehensible sets of temperature-dependent subprocesses. Understanding the temperature dependence of molecular motors will pave the way to understanding the effects of circadial and inflammatory temperature variation on the motility of mammalian cells and of motile pathogens such as trypanosomatids (e.g., *Leishmania* and *Trypanosoma*) or bacteria (e.g., *Salmonella* and *Helicobacter*). In an ecological context, differential swimming responses of predator and prey can lead to measurable benefits to motile bacteria (34, 35), suggesting substantial adaptive and ecological significance (36).

Biological Interpretations

The model presented here provides a mechanistic link between the scaling of flagellar frequencies with viscosity and the recorded scaling of these parameters with temperature. The model suggests that the intrinsic properties of molecular motors such as dynein determine the response to external load, which itself translates into temperature–rate relationships when load (viscosity) varies with temperature. Such an interpretation suggests that temperature effects are dominated by impacts on the supply of energy (e.g., diffusion of ATP, reaction rates, enzyme efficiencies), but that there is also a contribution from the physical properties of the medium in the form of the viscosity of the external fluid and of the cell. A simple conceptualization is that

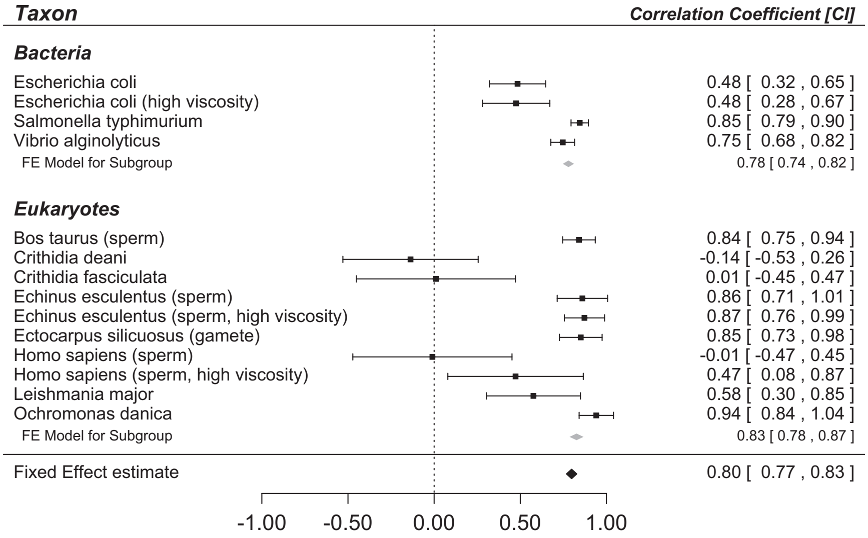

Fig. 3. Forest plot of correlation coefficient *r* (squares, proportional to weights used in meta-analysis) for studies examining relationships between beat/rotational frequencies of cilia and flagella and swimming speed of the cell. Summary measures (diamonds, fixed-effect estimates) are shown for two subgroups (Bacteria and Eukaryotes) and for the whole dataset. The overall fixed-effect estimate ($r = 0.80$) indicates a strong linear correlation between frequency and speed. Associated confidence intervals are shown as horizontal bars (*Right*). Dotted vertical line indicates no effect. Citations for datasets and individual scatterplots are given in *SI Appendix*, Table S2 and Fig. S6.

the main impact of temperature on ciliary-derived biological rates is to set the balance between available power (through changes to cytoplasmic viscosity, diffusion, and reaction rates) and power required to overcome external loads (external fluid viscosity). The presence of viscous loads on molecular transport has rarely been addressed, but given the similarities of dynein with other eukaryotic molecular motors, such as actin and myosin, the model should also be applicable to both cellular motility and intracellular transport mechanisms. The slowing of movement and metabolism at high pressure in organisms living in the ocean depths (37) should also be amenable to study using this system. Hydrostatic pressure is a force acting on cellular components and thus can be incorporated simply as an additional component to viscous drag.

Few data on the contribution of viscosity effects to temperature-dependent changes in flagellar-beat frequency exist, but one estimate for the mussel data (2) used in Fig. 2 is ~80%. For cilia-driven swimming speeds, estimates range from 40% to 100% of the effect of temperature as attributable to viscosity. However, viscosity change is expected to be less easily acclimated to than, for instance, temperature-dependent changes in enzyme efficiency (38), and so the true value may lie close to the lower end of these estimates.

Though the data collated here was collected under controlled conditions, there are implications for changes in viscosity under natural conditions. Seawater viscosity more than doubles from tropical to arctic temperatures (at a salinity of 30‰, $\mu = 0.86$ mPa·s at 30 °C and 1.88 mPa·s at 0 °C). The nonlinear relationship

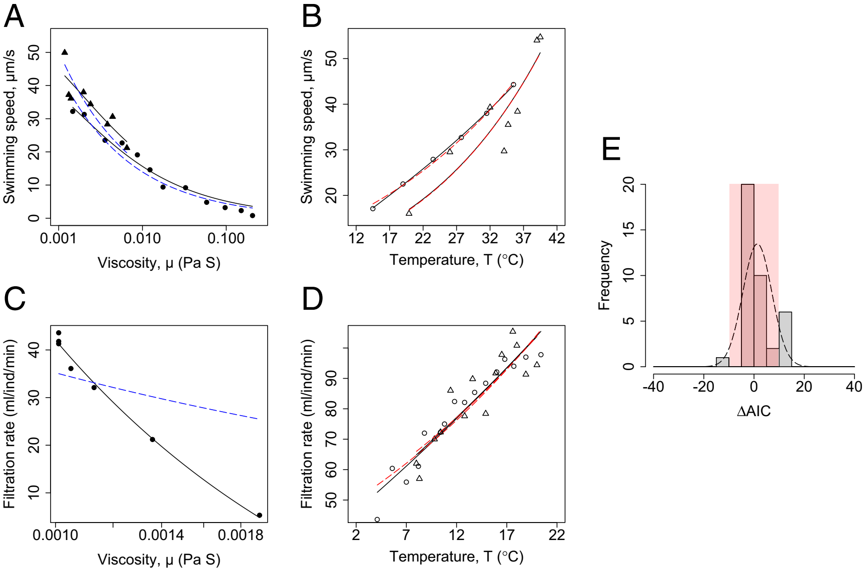

Fig. 4. Examples of data and model fits for swimming speed of the bacterium *E. coli* (*A* and *B*) and the filtration rate of the bivalve mollusk *Mytilus edulis* (*C* and *D*). Panels and symbols are as for Fig. 2, with an aggregate mean ΔAIC of 1.4 in *E*. Citations for datasets are given in *SI Appendix*, Tables S8–S11.

between temperature and viscosity (*SI Appendix*, Fig. S1*A*) means that a given temperature change at 20 °C will not have the same effect on viscosity as the same change at 10°, but as an example for a 12 °C annual variation in temperate seas, the change from 6 °C to 18 °C reduces both dynamic and kinematic viscosity by 27%. Though within complex multicellular organisms, temperature can be homeostatically controlled, there is still variation. For instance, mammalian ependymal cilia in the ventricular system are exposed to cerebrospinal fluid of increased viscosity in certain pathological conditions such as bacterial meningitis (39) and, although comparisons are complicated by the viscoelasticity of biological fluids, the effective viscosity of mammalian cervical mucus through which sperm must swim has been estimated at 0.2 Pa·s, 200× that of water (40).

We can interpret the temperature–rate relationships revealed here as near-Arrhenius scaling, with the corollary that activation energy (E) has a physical analog in the viscosity–temperature relationships of the cell and external medium and temperature dependence of diffusion rates or chemiosmotic potentials. In particular, my model can provide an additional explanation for some of the recently documented (41) widespread variation in activation energy for biological rates modeled with the Arrhenius equation. My model accounts for variation in biologically interpretable parameters such as wavelength, ATP concentration, or flagellum length, which would be attributed to variation in E using the Arrhenius equation. These results provide another indication that the Arrhenius formulation can sometimes be an oversimplification of the complexity underlying the temperature dependence of biological rates and ecological processes.

These results show that consideration of both biochemical and mechanical effects of temperature change can provide a universal scaling mechanism for functionally convergent organelles found in all three domains of life. Such a mechanistic understanding should greatly improve our ability to predict changes in physiological performance in the face of temperature variation.

Methods

I searched the literature for studies that measured the intraspecific response of flagellar or cilial beat (or rotational) frequency to temperature and/or viscosity. I also searched for studies on the swimming or filtration rate response of ciliated or flagellated organisms. Only datasets where four or more temperature or viscosity measurements were available after any trimming (see below) were used. I found 273 data sources. Where tabulated data were unavailable, data points were extracted directly from published figures using GraphClick. In its current form, the model cannot account for temperature-related decreases in rates such as those due to enzyme denaturation or high-temperature stresses. Where responses to temperature were unimodal, the data were restricted to those measurements up to and including the maximum value to give a monotonic response. This process yielded 17 intraspecific cilial or flagellar temperature responses, 39 swimming or filtration temperature responses, and 30 and 31 viscosity responses, for a total of 1,518 data points. The data reported in this paper are tabulated in *SI Appendix*, Tables S1–S11.

All statistical analysis and model fitting was carried out in R (42). Models were fitted with nonlinear least-squares regression (package minpack.lm) using a modification of the Levenberg–Marquardt algorithm (function nlsLM). For the temperature version of my model (*SI Appendix*, Eqs. **S12** and **S22**), the parameter $(x + y)$ was constrained between −0.023 and −0.120, the maximum being the estimate of x for pure water, whereas the minimum is the maximum plus the highest 95% confidence interval of the y estimates (−0.0961; *SI Appendix*, Table S1) for cell contents from nine datasets on the response of cell viscosity to temperature. Apart from the prokaryote temperature model where constant c_x was constrained as $0 \leq c_x \leq$ (minimum temperature for data) to prevent square roots of negative numbers, all other fitting constants were constrained to be real and positive.

Model fit was assessed using the AIC estimated for the fitted nonlinear least-squares model and the fitted Arrhenius model (frequency vs. temperature; *SI Appendix*, Eqs. **S12**, **S21**, and **S30**) or power function (frequency vs. viscosity; *SI Appendix*, Eqs. **S13** and **S22**).

ACKNOWLEDGMENTS. P. S. Larsen, L. J. Morrell, G. D. Ruxton, B. Okamura, and C. D. Venditti provided helpful comments and discussion, and the paper was greatly improved by comments from the editor, J. Okie, and an anonymous reviewer. Support for this work was provided in part by Natural Environment Research Council Advanced Fellowship NE/B500690/1 (to S.H.).

1. Calliburcès P (1858) Experimental research on the influence of heat on the events of the contractility of organs. *Comptes Rendu* 47:638–644. French.
2. Riisgård HU, Larsen PS (2007) Viscosity of seawater controls beat frequency of water-pumping cilia and filtration rate of mussels *Mytilus edulis*. *Mar Ecol Prog Ser* 343:141–150.
3. Kupriyanova EK, Havenhand JN (2005) Effects of temperature on sperm swimming behaviour, respiration and fertilization success in the serpulid polychaete. *Galeolaria caespitosa* (Annelida: Serpulidae). *Invertebr Reprod Dev* 48(1-3):7–17.
4. Podolsky RD (1994) Temperature and water viscosity: Physiological versus mechanical effects on suspension feeding. *Science* 265(5168):100–103.
5. Beveridge OS, Petchey OL, Humphries S (2010) Mechanisms of temperature-dependent swimming: The importance of physics, physiology and body size in determining protist swimming speed. *J Exp Biol* 213(Pt 24):4223–4231.
6. Arrhenius S (1889) About the reaction rate in the inversion of cane sugar by acids. *Z Physikal Chemie* 4:226–248. German.
7. Savage VM, Gilloly JF, Brown JH, Charnov EL (2004) Effects of body size and temperature on population growth. *Am Nat* 163(3):429–441.
8. Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. *Science* 293(5538):2248–2251.
9. Price CA, et al. (2012) Testing the metabolic theory of ecology. *Ecol Lett* 15(12):1465–1474.
10. Patterson MR (1992) A mass transfer explanation of metabolic scaling relations in some aquatic invertebrates and algae. *Science* 255(5050):1421–1423.
11. Alexander RM (1971) *Size and Shape* (Arnold, London).
12. Machin KE (1958) Wave propogation along flagella. *J Exp Biol* 35:796–806.
13. Gao YQ (2006) A simple theoretical model explains dynein's response to load. *Biophys J* 90(3):811–821.
14. Cross RA (2004) Molecular motors: Dynein's gearbox. *Curr Biol* 14(9):R355–R356.
15. Sznitman J, Purohit PK, Krajacic P, Lamitina T, Arratia PE (2010) Material properties of *Caenorhabditis elegans* swimming at low Reynolds number. *Biophys J* 98(4):617–626.
16. Mallik R, Carter BC, Lex SA, King SJ, Gross SP (2004) Cytoplasmic dynein functions as a gear in response to load. *Nature* 427(6975):649–652.
17. Okuno M, Asai DJ, Ogawa K, Brokaw CJ (1981) Effects of antibodies against dynein and tubulin on the stiffness of flagellar axonemes. *J Cell Biol* 91(3 Pt 1):689–694.
18. Sznitman J, Shen X, Purohit PK, Arratia PE (2010) The effects of fluid viscosity on the kinematics and material properties of *C. elegans* swimming at low Reynolds number. *Exp Mech* 50(9):1303–1311.
19. Xing J, Bai F, Berry RM, Oster G (2006) Torque-speed relationship of the bacterial flagellar motor. *Proc Natl Acad Sci USA* 103(5):1260–1265.
20. Brokaw CJ (1966) Effects of increased viscosity on the movements of some invertebrate spermatozoa. *J Exp Biol* 45(1):113–139.
21. Clary-Meinesz CF, Cosson J, Huitorel P, Blaive B (1992) Temperature effect on the ciliary beat frequency of human nasal and tracheal ciliated cells. *Biol Cell* 76(3):335–338.
22. Quraishi MS, Jones NS, Mason J (1998) The rheology of nasal mucus: A review. *Clin Otolaryngol Allied Sci* 23(5):403–413.
23. Blatteis CM (2003) Fever: Pathological or physiological, injurious or beneficial? *J Therm Biol* 28(1):1–13.
24. Gray J, Hancock G (1955) The propulsion of sea-urchin spermatozoa. *J Exp Biol* 32:802–814.
25. Lauga E, Powers TR (2009) The hydrodynamics of swimming microorganisms. *Rep Prog Phys* 72(9):096601.
26. Teff Z, Priel Z, Gheber LA (2008) The forces applied by cilia depend linearly on their frequency due to constant geometry of the effective stroke. *Biophys J* 94(1):298–305.
27. Shields AR, et al. (2010) Biomimetic cilia arrays generate simultaneous pumping and mixing regimes. *Proc Natl Acad Sci USA* 107(36):15670–15675.
28. Roberts AM (1981) *Biochemistry and Physiology of Protozoa*, eds Levandowsky M, Hunter SH (Academic, New York), 2nd Ed, pp 5–66.
29. Silberberg A (1982) Rheology of mucus, mucociliary interaction, and ciliary activity. *Prog Clin Biol Res* 80(Suppl):25–28.
30. Riisgård HU, Larsen PS (2000) Comparative ecophysiology of active zoobenthic filter feeding, essence of current knowledge. *J Sea Res* 44:169–193.
31. Cartwright JH, Piro O, Tuval I (2004) Fluid-dynamical basis of the embryonic development of left-right asymmetry in vertebrates. *Proc Natl Acad Sci USA* 101(19):7234–7239.
32. Keller JB (1974) Effect of viscosity on swimming velocity of bacteria. *Proc Natl Acad Sci USA* 71(8):3253–3254.
33. Larsen PS, Riisgård HU (2009) Viscosity and not biological mechanisms often controls the effects of temperature on ciliary activity and swimming velocity of small aquatic organisms. *J Exp Mar Biol Ecol* 381(2):67–73.
34. Matz C, Jürgens K (2005) High motility reduces grazing mortality of planktonic bacteria. *Appl Environ Microbiol* 71(2):921–929.

35. Pernthaler J (2005) Predation on prokaryotes in the water column and its ecological implications. *Nat Rev Microbiol* 3(7):537–546.
36. Lenski RE, Rose MR, Simpson SC, Tadler SC (1991) Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am Nat* 138(6):1315–1341.
37. Bartlett DH (2002) Pressure effects on in vivo microbial processes. *Biochim Biophys Acta* 1595(1-2):367–381.
38. Bolton TF, Havenhand JN (2005) Physiological acclimation to decreased water temperature and the relative importance of water viscosity in determining the feeding performance of larvae of a serpulid polychaete. *J Plankton Res* 27(9):875–879.
39. Yetkin F, et al. (2010) Cerebrospinal fluid viscosity: A novel diagnostic measure for acute meningitis. *South Med J* 103(9):892–895.
40. Smith DJ, Gaffney EA, Gadêlha H, Kapur N, Kirkman-Brown JC (2009) Bend propagation in the flagella of migrating human sperm, and its modulation by viscosity. *Cell Motil Cytoskeleton* 66(4):220–236.
41. Dell AI, Pawar S, Savage VM (2011) Systematic variation in the temperature dependence of physiological and ecological traits. *Proc Natl Acad Sci USA* 108(26): 10591–10596.
42. R Development Core Team (2006) R: A language and environment for statistical computing, version 2.2.1 (R Foundation for Statistical Computing, Vienna).