



# Biomechanical Analysis Of Gait Adaptation In The Nematode *Caenorhabditis elegans*

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# **Locomotory adaptation of the nematode *C. elegans* to defined external loads**

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## ABSTRACT

To navigate different environments, an animal must be able to adapt its locomotory gait to its physical surroundings. The nematode *C. elegans*, between swimming in water and crawling on surfaces, adapts its locomotory gait to surroundings that impose ~10,000-fold differences in mechanical resistance. Here we investigate this feat by studying the undulatory movements of *C. elegans* in Newtonian fluids spanning nearly five orders of magnitude in viscosity. In these fluids, the worm undulatory gait varies continuously with changes in external load: as load increases, both wavelength and frequency of undulation decrease. We also quantify the internal viscoelastic properties of the worm's body and their role in locomotory dynamics. We incorporate muscle activity, internal load, and external load into a biomechanical model of locomotion and show that (1) muscle power is nearly constant across changes in locomotory gait, and (2) the onset of gait adaptation occurs as external load becomes comparable to internal load. During the swimming gait, which is evoked by small external loads, muscle power is primarily devoted to bending the worm's elastic body. During the crawling gait, evoked by large external loads, comparable muscle power is used to drive the external load and the elastic body. Our results suggest that *C. elegans* locomotory gaits are the product of one circuit that continuously adapts to external mechanical load in order to maintain propulsive thrust.

How do neural circuits produce and regulate the rhythmic patterns of muscle activity that drive animal locomotion? The nematode *C. elegans* – with its well-mapped nervous system(1), relatively simple anatomy(2), and rhythmic undulatory movements(3) – is an excellent model for exploring the neural basis of locomotion. As a first step toward a comprehensive understanding of motor behavior, we need to understand *C. elegans* locomotory biomechanics: how muscle activity produces movement within the mechanical constraints of the worm’s body and its physical environment.

*C. elegans* moves forward by propagating undulatory waves in a dorsal-ventral plane from head to tail(3). Bending is generated by alternating contraction and relaxation of two dorsal and two ventral muscle groups which run along the length of the worm’s body(2). Both the shape and speed of these undulations change in response to the physical environment(3, 4). When moving on moist surfaces such as agarose gels, *C. elegans* exhibits a crawling gait characterized by undulations with low frequency and short wavelength (5). By contrast, when moving through water, *C. elegans* exhibits a swimming gait characterized by undulations with higher frequency and longer wavelength (Table 1). The differences in the size and speed of undulations are modest in comparison with the difference in the scales of physical force during swimming and crawling. At the size and speed of *C. elegans*, forces due to surface tension (surface tension holds the crawling animal to the agar surface) are ~10,000-fold larger than forces due to viscosity when swimming in water (6).

Studies of nematode locomotion on or within gels of varying stiffness have shown a continuous change in locomotory patterns from gaits that resemble swimming in water to gaits that resemble crawling on surfaces (7, 8). Here, we sought the mechanical determinants of locomotory gait adaptation. Because it is difficult to quantify all the forces associated with locomotion on or through gels -- a complex set of elastic, tearing, viscous, and capillary forces -- we studied locomotion in Newtonian fluids, in which external forces on the worm body are proportional to speed and viscosity. To quantify the worm’s locomotory behavior we applied machine vision algorithms similar to those previously used to describe *C. elegans* behavior (4, 5, 9-11).

We also address the internal biomechanics of the worm's body. The worm's shape is maintained in part by internal hydrostatic pressure, which imparts rigidity that opposes bending. The mechanical properties of the *C. elegans* cuticle have been previously investigated by probing with a cantilever-based micromachine device(12). Here, we sought the elastic coefficients that are relevant to undulatory locomotion, those that characterize bending the entire worm modeled as a viscoelastic rod. We measured these elastic coefficients by quantifying the relaxation of the worm body after sudden bending deflections.

We incorporate our analyses of internal and external mechanics into a biomechanical model of locomotory gait. We find that this model can explain the load-dependent changes in gait exhibited by *C. elegans*, providing the necessary biomechanical framework for further analysis of the circuits for worm locomotion.

## RESULTS

### *C. elegans displays different gaits when swimming and crawling*

The kinematics of worm undulatory locomotion in the reference frame of the worm body can be represented by the time-varying curvature of the body centerline (Fig. 1). Here, we use a body coordinate,  $s$ , to describe position along the centerline from head ( $s = 0$ ) to tail ( $s = L$ ), where  $L$  is the length of the worm. The time-varying curvature is defined as the partial derivative of the tangent angle to the centerline with respect to the body coordinate:

$$\kappa(s, t) = \frac{\partial \theta}{\partial s} \quad [1]$$

Using this metric of time-varying curvature, we quantified the locomotory gait during swimming and crawling. We recorded dark field video sequences of worms during periods of regular forward movement (Fig. 2) and used image analysis software to measure the worm's curvature as a function of space and time (see Methods). From the curvature measurements, we calculated the wavelength and frequency of the undulatory gait (Fig. 2). Consistent with prior reports, worms swimming in buffer with the viscosity of water displayed undulations with long wavelength and high frequency, whereas worms crawling on agar surfaces displayed undulations with short wavelength and low frequency (Table 1).

Next, we quantified the locomotion of worms immersed in solutions containing high molecular weight dextran (MW 2,000,000), which display Newtonian flow characteristics(13) (see Methods). In our experiments the Reynolds number  $< 0.05$ , meaning that inertial forces are negligible in comparison to viscous forces. The advantage of using Newtonian fluids at low Reynolds numbers, as opposed to gels or non-Newtonian fluids, is that in our case the external force resisting transverse movement of the worm body is proportional to the speed of movement and to the coefficient of viscous drag to transverse movement. In describing the external viscous forces encountered by the moving worm, it is useful to describe its undulations in terms of its

transverse displacement  $y(t)$  in the reference frame of the moving worm (Fig. 1). Thus, the external force per unit length that is transverse to the worm's body during lateral movement is:

$$F_{ext} = C_N \times \frac{dy}{dt} \quad [2]$$

where  $C_N$  is the coefficient of viscous drag to transverse movement and  $dy/dt$  is the speed of transverse movement (Fig 1).

We placed worms in chambers containing Newtonian viscous fluids, and found that increasing viscosity from 1 to 28,000 mPa·s induces a continuous transition between undulations that resemble swimming in water and crawling on agarose, as measured by the wavelength, frequency, and amplitude of the undulations (Table 1, Fig. 2, Fig. 3a-c, Supplemental Movies). At intermediate values of the range of viscosities that we studied, worms exhibited steady undulations that were intermediate in wavelength and frequency to swimming and crawling (Fig. 3b). If locomotory behavior exhibited bistable switching between distinct crawling and swimming gaits, intermediate gaits might be construed from time-averages of two distinct gaits. In our experimental setup, we found no evidence for such switching. Abrupt switching between swimming and crawling gaits would be expected to increase the statistical variation in measured wavelength. To the contrary, we did not find the standard deviation of wavelength to be larger for intermediate viscosities, compared with low or high viscosities (Supplemental Fig. S2). Instead, the normalized standard deviation of wavelength was nearly constant over the range of viscosities studied.

### **Internal resistance to bending**

Next, we sought to quantify the internal elastic and viscous forces that resist bending during undulatory locomotion. Following Guo and Mahadevan (14), we characterize the internal elastic and viscous forces per unit length of the undulating worm in terms of a coefficient of internal elasticity ( $b$ ) and a coefficient of internal viscosity ( $b_v$ ). Net mechanical load owing to these internal forces, in terms of these coefficients and the transverse displacement of the undulating worm is:

$$F_{int} = by_{ssss} + b_v \frac{\partial y_{ssss}}{\partial t} \quad [3]$$

where  $(\cdot)_s$  denotes the partial derivative with respect to  $s$ .

We estimated the coefficients of internal elasticity and viscosity by measuring the time scale of relaxation of the worm body following deformation in Newtonian fluids varied between 1 and 25 mPa·s. To do this, we held a live worm with a glass micropipette immersed in each viscous solution. We used another micropipette with a hooked end to bend the worm to one side and release it (Fig 4a and Supplemental Movie S5). Using high speed video microscopy, we measured the angle between the holding pipette and the vector connecting the end of the pipette and the worm's head as a function of time (Fig 4b).

We found that worm movement following release had a fast exponential component, owing to passive relaxation of the worm body, and a slow linear component, owing to active movement of the live worm. We quantified the exponential time constant as a function of the viscosity of the surrounding fluid. As expected from linear viscoelastic theory, this exponential time constant was linearly related to external viscosity (see Methods). From the slope of the linear relationship between the time constant and external viscosity (Fig 4c), we estimated the coefficient of elasticity to be  $b \approx 4 \times 10^{-12} \text{ N m}^3$ . From the  $y$ -intercept of this linear relationship, we found that mechanical load due to internal viscosity is negligible in comparison to the load due to internal elasticity or external viscosity. We found the upper limit of the coefficient of internal viscosity  $b_v < 1.5 \times 10^{-14} \text{ Nm}^3\text{s}$ .

### **Propulsive thrust generated by undulation depends on its angle of attack**

Slender animals like worms generate propulsive thrust by undulating in the direction that is perpendicular to net movement. Each body segment of an undulating worm contributes propulsive thrust depending on its angle of attack,  $\theta_a$ , the angle between the vector of net movement and the tangent vector to the body segment (see Figure 1). In viscous fluids and in simple descriptions of solid friction, total propulsive thrust is proportional to  $\langle \sin^2(\theta_a) \rangle$ , the



average taken over all body segments along the undulating worm. In Newtonian fluids at low Reynolds numbers, one finds that

$$\frac{V}{V_{und}} = \left( \frac{C_N}{C_T} - 1 \right) \langle \sin^2(\theta_a) \rangle \quad [4]$$

where  $V$  is forward swimming speed,  $V_{und}$  is the propagation speed of undulations in the reference frame of the worm body, and  $C_N$  and  $C_T$  are the coefficients of viscous drag to transverse movement and longitudinal movement, respectively (see Fig 1).

According to Eq. 4, propulsive thrust decreases rapidly with a drop in  $\theta_a$ . We quantified  $\theta_a$  of worms swimming in viscous fluids ranging from 1 to 28,000 mPa·s. Despite changes in undulatory wavelength, frequency, and amplitude, the peak angle of attack remains roughly constant, modestly increasing from 45 degrees to 55 degrees with 28,000-fold increase in viscosity (Fig. 3).

### **How the angle of attack depends on the parameters of undulatory locomotion**

How might the worm maintain its angle of attack despite dramatic changes in external load? To answer this question, we sought a relationship between the angle of attack and the biomechanical parameters of undulatory movement. To do this, we incorporated muscle activity into our biomechanical model. Undulatory waves are caused by alternating contraction and relaxation of muscle groups along the dorsal and ventral sides of the worm. Thus, muscle activity generates time-varying torque along the centerline,  $M(s,t)$  (Fig. 1).

In an actively undulating worm, the muscles work against both external load (given by Eq. 2) and internal elastic and viscous loads (given by Eq. 3). Balancing external and internal loads in the direction transverse to the body with the transverse force due to muscle activity gives:

$$M_{ss}(s,t) = C_N \frac{\partial y}{\partial t} + b y_{ssss} + b_\nu \frac{\partial y_{ssss}}{\partial t} \quad [5]$$

The amount of force generated by the muscles of an undulating worm resembles a traveling sinusoidal wave,  $M(s, t) = M_0 \sin(\omega t + 2\pi s/\lambda)$ , allowing us to use Eq. 5 to estimate the angle of attack as a function of undulatory wavelength and frequency:

$$\theta(s, t) = \frac{M_0(\lambda/2\pi)}{\sqrt{b^2 + (\omega C_N)^2(\lambda/2\pi)^8}} \cos(\omega t + 2\pi s/\lambda + \phi) \quad [6]$$

where

$$\phi = \arctan \left( b(2\pi)^4 / C_N \omega \lambda^4 \right) \quad [7]$$

Thus, the angle of attack is explicitly dependent on  $C_N$ , which is proportional to external viscosity. How, then, is the angle of attack conserved during a 28,000-fold increase in viscosity? One possibility is that the term in Eq. 6 that involves internal elasticity might be sufficiently larger than the term for viscous drag ( $b > \omega C_N (\lambda/2\pi)^4$ ), such that changes in viscosity have little effect on the angle of attack. Another possibility is that changes in muscle torque, undulatory wavelength, and/or undulatory frequency might compensate for changes in external viscosity. As shown below, the key to evaluating these possibilities lies in accounting for how muscle power is used during locomotion at different viscosities.

### **Muscle power is used differently at low and high viscosity**

The total muscle power produced per unit length along the worm body is

$$P(s, t) = M(s, t) \frac{\partial \kappa(s, t)}{\partial t} \quad [8]$$

where  $\kappa(s, t)$  describes time-varying curvature along the centerline and  $M(s, t)$  describes time-varying muscle torque along the centerline (Figure 1). This leads to:

$$P(s, t) = \omega \kappa_{max}^2 \left[ b \sin(\omega t + 2\pi s/\lambda) \cos(\omega t + 2\pi s/\lambda) + \omega C_N (\lambda/2\pi)^4 \cos^2(\omega t + 2\pi s/\lambda) \right] \quad [9]$$

where the maximum curvature of the worm is given by

$$\kappa_{max} = \frac{M_0}{\sqrt{b^2 + (\omega C_N)^2(\lambda/2\pi)^8}}$$

Muscle power consists of two terms. The first term is the muscle power used to deform the elastic body of the worm, and the second term is the muscle power used to shear the surrounding viscous fluid. During each undulation cycle, the peak power delivered to the elastic body is

$P_e = \omega \kappa_{max}^2 b/2$  and the peak power delivered to the viscous fluid is

$$P_\eta = \omega^2 \kappa_{max}^2 C_N (\lambda/2\pi)^4 .$$

In Fig. 5a, we quantify the relative amounts of muscle power that the worm uses to drive its own elastic body and to drive the surrounding fluid, each as a function of external viscosity. At the lower viscosities that we studied,  $P_\eta < P_e$ , which also defines the regime where  $b > \omega C_N (\lambda/2\pi)^4$ . In this regime, the angle of attack can be constant despite changes in viscosity, not requiring changes in undulatory wavelength or frequency (Fig. 5a). This observation is consistent with our experimental observation that both undulatory frequency and wavelength exhibit asymptotic behavior in the limit of low viscosities.

At the higher viscosities that we studied,  $P_\eta > P_e$ . In this regime, significant muscle power is used both to shear the surrounding viscous fluid as well as bend the elastic body. To preserve constant angle of attack as viscosity increases, the worm gradually decreases both frequency and wavelength and gradually increases muscle force.

Finally, our biomechanical can be used to estimate any phase differences between the traveling wave of muscle activity and the traveling wave of the undulation itself. In the regime of the swimming gait, peak muscle torque coincides with peak curvature of the worm body. However, as viscosity increases, a phase difference develops. For the crawling gait, we predict  $\sim 60^\circ$  phase difference between peak muscle activity and peak curvature. This phase difference between muscle torque and body curvature might be testable by directly imaging the activity of muscle cells (e.g., using calcium imaging) in freely moving animals.

## DISCUSSION

Gradually increasing external mechanical resistance on a swimming worm -- which we did by increasing the viscosity of the external Newtonian fluid -- induces a continuous transition of locomotory gait, gradually decreasing the wavelength and frequency of undulations until the worm gait resembled that of crawling on agarose surfaces. Thus, the different gaits exhibited by *C. elegans* represent a continuous adaptability of an underlying locomotory circuit to external mechanical load. Nevertheless, swimming and crawling are qualitatively different from a mechanical perspective. During swimming, external load is insignificant in comparison to internal elasticity. During crawling, external load and internal elasticity are comparable.

The worm, like other organisms that are smaller than the capillary length of water, must be able to move through fluids and fluid interfaces that impose external loads spanning several orders of magnitude. Our analysis has uncovered the strategic value of the worm's changes in locomotory gait over this range. Over 28,000-fold changes in viscosity, total muscle power varies by less than a factor of 2, but muscle power that is dissipated in external viscous shear varies by ~1000-fold. The purpose of gait change in *C. elegans* is to maintain propulsive thrust, allowing the worm to maintain the angle of attack of its undulation with the constraint of limited muscle power expenditure.

The neuromuscular mechanisms that underlie load-dependent adaptation of locomotory gait are not yet known. Our results provide well-defined constraints for any such mechanistic model of the underlying locomotory circuit, and a biomechanical framework within which to explore such models. Our analysis shows how patterns of muscle activity are transduced into locomotory undulation in different physical surroundings. Further work will aim to show how the locomotory circuit drives these patterns of muscle activity.

## METHODS

### *Worm strains and cultivation*

Wild-type worms (N2 Bristol) were cultivated on *E. coli* OP50 NGM plates at 20C according to standard methods. Development was synchronized by hypochlorite bleaching, and all experiments were performed with adult worms 12-18 hrs after the final molt.

### *Viscous fluids*

Viscous solutions were composed of 0-45% (w/w) dextran (2,000,000 MW) dissolved in either NGM buffer (for 0-30%) or 10 mM HEPES (pH 6.0) (for 35-45%) . The viscoelastic properties of each dextran solution was measured using a AR-G2 rheometer with cone-plate geometry (TA Instruments, New Castle NJ). For each solution that we used, we measured the viscosity using a shear rate of  $1 \text{ s}^{-1}$ , and further verified that viscosity varied by less than a factor of 1.5 over a range of shear rates from  $10^{-1}$  to  $10^3 \text{ s}^{-1}$ . Over the range of dextran solutions that we used (1-45% by mass), the viscosity increased nearly 5 orders of magnitude, providing a large range of experimental viscosities with Newtonian flow characteristics (i.e., negligible dependence of viscosity on shear-rate).

### *Measuring locomotory gait*

To quantify the crawling gait, worms were placed on 2 mm thick layers of 2% agarose in NGM buffer. To quantify the swimming gait in NGM buffer or in viscous fluids, worms were washed in NGM buffer and transferred to 100-200  $\mu\text{l}$  fluid droplets in chambers composed of two glass slides separated by approximately 150  $\mu\text{m}$  using coverslips. To prevent worms from adhering to glass surfaces when using pure NGM buffer, 0.1% (w/w) bovine serum albumen (BSA) was added to the solution.

We recorded image sequences of worms using either an inverted Nikon microscope under 2X-4X magnification with dark field illumination or a custom-built microscope using a zoom lens

and dark field illumination provided by a ring of red LEDs. Image sequences were recorded on a computer at 30 Hz with a CCD camera (Imaging Source, Charlotte, NC) using IC Capture software (Imaging Source, Charlotte, NC).

Image analysis was performed using MATLAB (Mathworks, Natick, MA) (Supplementary Figure S1). Briefly, image sequences were identified in which the worm performed consistent forward movement without reversals or turns. In these sequences, each image was background-subtracted, filtered by a disk-shaped smoothing filter with diameter equal to one fifth the worm diameter, then thresholded to give a binary image. The anterior and posterior ends of the worm were identified as the points of maximum convex curvature on the anterior and posterior halves of the boundary of the thresholded image. A centerline extending from the head to the tail of the worm was calculated such that the centerline was equidistant to nearest boundary points along the two sides of the worm boundary. A least-squares cubic smoothing spline fit to the centerline was then calculated. Curvature was calculated as the derivative with respect to the body coordinate of the unit vector tangent to the centerline.

The speed of the undulatory wave, in the reference frame of the worm body, was calculated using least-square linear fits to the zero crossings of curvature over the central 80% of the body length. Because the slope of positive-derivative zero crossings could slightly differ from the slope of negative-derivative zero crossings, an equal number of the two were used to calculate wave speed in each image sequence (Supplementary Figure S1). Undulatory frequency was calculated by dividing the number of cycles by the elapsed time during the image sequence. Undulatory wavelength was calculated as the ratio between speed and frequency.

To quantify the angle of attack that defines propulsive thrust, we computed the tangent angles along the worm centerline as a function of body coordinate and time. To eliminate the effect of slow changes in worm orientation that could occur over several cycles, we filtered the tangent angles using a temporal low-pass filter with time scale equal to the worm undulatory period, producing a slow-offset-subtracted angle. The average angle of attack, as well as  $\langle \sin^2(\theta_a) \rangle$ , were calculated using the slow-offset-subtracted angles, averaged over body coordinate over an integral number of undulations.

### *Measuring the internal viscoelasticity of the worm body*

To estimate the internal elasticity and viscosity of the worm body, we used a method similar to that of Sauvage (6). A glass capillary pipette was drawn over a flame, broken, and flame polished to narrow the opening to a diameter of about 20 microns. An adult N2 worm was washed in NGM buffer and its tail was partly drawn into the pipette by vacuum. The pipette holding the worm was attached to a Petri plate containing viscous fluid (1- 25 mPa·s), such that the live worm was held about 2-3 mm above the bottom of the plate with its undulations in the plane of observation . The worm was monitored using darkfield illumination with a 4X objective on an inverted microscope. We used a glass pipette with a finely drawn hooked tip to bend the body to the ventral or dorsal side and then release it (Suppl. Movie S5). The rapid relaxation of the worm body to its original position was recorded at 5000 frames per second using a high speed video camera (Vision Research Phantom V9).

We tracked the angle of the vector between the tip of the pipette and the tip of the worm's head after release from the hooked pipette. The exponential component of the time course of relaxation can be related to the coefficients of internal elasticity and viscosity of the worm body. With no active muscle torque, the passive relaxation of the body is described by:

$$C_N \frac{\partial y}{\partial t} + b y_{ssss} + b_\nu \frac{\partial y_{ssss}}{\partial t} = 0 \quad [7]$$

A stationary bend is described by:

$$y(s, t) = a(t) \cos(2\pi s/\lambda) \quad [8]$$

Solving for  $a(t)$ , we arrive at

$$a(t) = a(0)e^{-t/\tau} \quad \text{with } \tau = \frac{C_N(\lambda/2\pi)^4 + b_\nu}{b} \quad [9]$$

When the body is allowed to relax, the response is dominated by the mode with lowest spatial frequency. In the case of a viscoelastic rod with one free end and one fixed end, the wavelength of this mode is  $\lambda = 4L_f$ , where  $L_f$  is the length of the free portion of the worm. Thus, the exponential time constant for relaxation is:

$$\tau = \frac{C_N(2L_f/\pi)^4 + b_\nu}{b}$$

The coefficient of viscous drag for transverse movement of a slender body with length  $L_f$  and diameter  $d$  in a solution with viscosity  $\eta$  is:

$$C_N = \frac{4\pi\eta}{\ln(2L_f/d) + 0.5}$$

Thus,  $C_N \approx 3.2\eta$ . Taken together, these equations suggest an affine dependence of the exponential time constant of relaxation with external viscosity:

$$b = \left(\frac{2L_f}{\pi}\right)^4 \frac{\partial C_N}{\partial \eta} \frac{\partial \eta}{\partial \tau}$$

$$b_\nu = \tau(\eta \rightarrow 0)b$$

Using a linear fit to the data for the exponential time constant versus external viscosity, we obtained the values  $b = 4 \times 10^{-12} \text{ Nm}^3$  and  $b_\nu < 1.5 \times 10^{-14} \text{ Nm}^3\text{s}$ . The latter represents an upper bound because, in the limit of low viscosity, we leave the limit of low Reynolds numbers, causing us to underestimate external frictional drag, thus overestimating  $b_\nu$ . In any case, forces due to internal elasticity are about 100 times larger than forces due to internal viscosity.

### *Measuring the coefficient of external viscous drag*

In our behavioral assays, each worm exhibited undulatory locomotion between two horizontal glass plates separated by 150  $\mu\text{m}$ . Viscous coupling between the undulating worm and the nearby



glass surfaces could alter the coefficients of viscous drag ( $C_N$ ). To quantify the correction to this coefficients in our imaging chambers, we measured the sedimentation speeds of anesthetized worms in a buffer containing NGM + 0.1% BSA + 25 mM sodium azide. We placed worms in two types of chambers: (i) vertically oriented thin chambers, identical to those used in our experiments and (ii) a bulk liquid chamber comprised of a 2.5 cm x 2.5cm x 4cm transparent plastic container. Using a CCD camera, zoom lens, and tracking software, we measured the average sedimentation speeds of worms falling in the transverse direction (oriented within  $10^\circ$  of the horizontal). We found that worms sedimented with an average speed of  $0.66 \pm 0.04$  mm/s (mean  $\pm$  SD, N=10) in bulk fluid and  $0.068 \pm 0.007$  mm/s (mean  $\pm$  SD, N=10) in the thin chamber. Thus, the coefficient of viscous drag to transverse movement is  $\sim 9.7$  times larger in the thin chamber compared with bulk fluid of the same viscosity. Note that such a correction was unnecessary when we measured the internal viscoelasticity of the worm, because, in those experiments, the worm was held at least 2 mm from any surfaces of the chamber.

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## FIGURE CAPTIONS

**Figure 1. The kinematics of an undulating worm.** (a) Diagram of a worm moving in a viscous fluid. Body coordinate  $s$  describes path length along worm body, starting from the head. Posture  $y(s,t)$  describes lateral displacement of worm body centerline.  $\theta$  describes angle of each body component with respect to direction of movement. (b) Worm body is modeled as a rod with elasticity (represented by spring), internal damping (represented by dashpot), and active muscular torque  $M(s,t)$ .

**Fig. 2. Modulation of *C. elegans* locomotion.** Dark field images and time-dependent curvature patterns of adult worms (a) swimming in NGM buffer with viscosity 1 mPas, (b) in dextran solutions with viscosity 980 mPas, (c) in dextran solution with viscosity 28000 mPas., (d) crawling on 2% agarose surface. The worm head is to the left in all images. Body curvature as a function of time (in seconds) and normalized body coordinate (varying from 0 at the head to 1 at the tail). Body curvature is represented using the non-dimensional product of curvature (the inverse of radius of curvature) and body length.

**Fig. 3. Locomotory parameters** (a) Mean wavelength of undulation scaled by worm body length  $L$  in different viscous solutions. (b) mean undulatory frequency; (c) mean curvature amplitude of undulation scaled by reciprocal of body length; (d) peak angle of attack, in degrees.

**Figure 4. Measurements of internal elasticity and viscosity.** (a) images from a video sequence in which worm position decays from deformed posture in NGM medium (viscosity 1 mPas). (b) Normalized worm bending angle for three viscosities. Lines show least-squares exponential fit for each viscosity. (c) Decay time scaled with fourth power of length of worm outside pipette, as function of viscosity. Data represents 15 decays from a total of 5 worms. Line: least-squares linear fit.

**Figure 5.** (a) Estimated external viscous power, peak internal elastic power, and peak total power as a function of viscosity. (b) Maximum torque, from Eqn. 1, and phase difference between torque and curvature as a function of viscosity, from Eqn. 7.