Swimming Behavior of the Nematode *Caenorhabditis elegans*: Bridging Small-Scale Locomotion with Biomechanics

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Abstract— **Undulatory locomotion, as seen in the nematode *Caenorhabditis elegans*, is a common swimming gait of organisms populating the world of low Reynolds number. While the nematode's motility is expected to be a strong function of its material properties, measurements remain scarce. Here, we first reveal the robustness of *C. elegans*' swimming gait with increasing mechanical load. By coupling kinematic data with a simple model, we measure non-invasively *C. elegans*' material properties including Young's modulus and tissue viscosity. By comparing wild-type nematodes with mutant strains carrying muscular dystrophy, we find that tissue properties are sensitive to changes in muscle functional properties. Altogether, our findings suggest that *C. elegans* is an attractive model to bridge small-scale motility research with biomechanical knowledge.**

Keywords— **swimming, locomotion, model organism, material properties, low Reynolds number.**

I. Introduction

Small-scale organisms move in the realm of low Reynolds (*Re*) number, where viscous forces dominate over inertial forces. Hence, locomotion must result from non-reciprocal motion to break time-reversal symmetry [1]. An attractive example of such a low *Re* number swimmer is found in the nematode *Caenorhabditis elegans*. This small organism, approximately 1 mm long, is a well established model system used extensively for biological research [2] and characterized by its undulatory locomotion [3].

C. elegans' motility results from the coordinated activity of body wall muscle cells and is a strong function of tissue properties such as body stiffness and muscle tonus. While it is generally accepted that *C. elegans*' body tissues are viscoelastic [3], quantitative data on its material properties including tissue stiffness and viscosity remain largely unexplored. Recently the nematode's cuticle has been experimentally probed [4]. Such measurements provided a Young's modulus on the order of 400 MPa; this value is closer to stiff rubber than soft tissues. Overall, there is still a dearth of knowledge on mechanical properties of *C. elegans*' body.

To gain insight on the interplay between locomotion and material properties, we study the motility behavior of swimming *C. elegans* in response to changes in mechanical loading. Using kinematic data coupled with a simple model, we measure non-invasively the material properties of *C. elegans* and their dynamic response to changes in external mechanical load. In a final step, we show that our methods are sensitive to changes in muscle functional properties using mutant strains carrying Muscular Dystrophy.

II. Experimental Methods

Experiments are conducted in shallow acrylic channels (1.5 mm wide and 500 µm deep) to minimize 3D nematode motion. Channels are filled with an aqueous solution of M9 buffer [2]. The motion of *C. elegans* is imaged using an inverted microscope and a high-speed camera (125 fps).

Figure 1 shows a wild-type *C. elegans* swimming in buffer solution. Image processing is used to extract the nematode's centroid and create 2D skeletons from segmented body shape. Skeletons represent the nematode's body centerline. *C. elegans* move in a highly periodic fashion, as indicated by the sweeping motion of the tail (Fig. 1). Snapshots of skeletons over a beating period *T* reveal an envelope of well-confined body postures (Inset, Fig. 1).

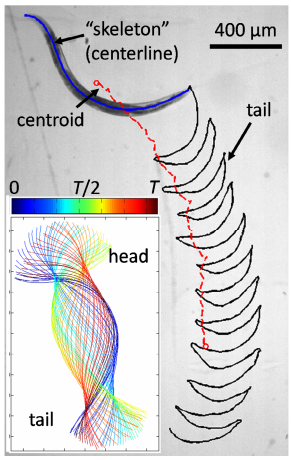

Fig. 1 Wild-type *C. elegans*' swimming in a pure buffer solution. Inset: color-coded temporal evolution of skeletons over one beating period *T*

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The nematode's swimming speed (U) is calculated by differentiating its centroid position over time. To characterize *C. elegans*' motility, we measure the curvature $\kappa(s,t)=d\varphi/ds$ along the nematode's body (Fig. 2a). Here, φ is the angle made by the tangent to the x-axis along the nematode's centerline, and s is the arc-length coordinate spanning the nematode's head (s=0) to tail (s=L). The beating frequency ($f=\omega/2\pi$) is obtained from Fast Fourier Transforms (FFT) of the κ field at multiple body positions s/L (Inset, Fig. 2a).

Fluids of different viscosities (μ) are prepared by adding small amounts of carboxy-methyl cellulose (CMC) to the buffer solution. Viscosity is varied from 1.0 to 12 mPa-s, by changing the polymer concentration from 0 to 2000 ppm. Since CMC is a flexible polymer, viscoelastic effects are expected to arise in the fluid. However, within the range of shear rates considered, viscoelastic and strain-rate dependent viscosity behaviors are minimized.

To characterize further low Re number flow properties, we measure the velocity fields obtained from *C. elegans* swimming using particle image velocimetry (PIV). The fluid is seeded with 2.2µm green fluorescent polymer microspheres; 2D velocity fields are calculated using a cross-correlation based code.

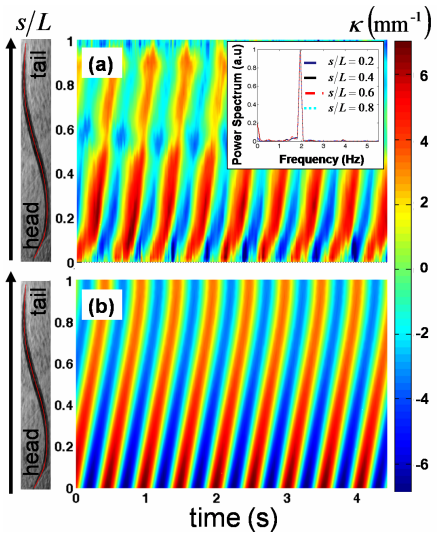

Fig. 2 (a) Contour plot of the measured curvature (κ) along the nematode's body in pure buffer solution. Inset: beating frequency (f) obtained from FFT. (b) Corresponding curvature field obtained from the model

III. Kinematics of Undulatory Swimming

The spatio-temporal evolution of κ is shown in Fig. 2(a), for a wild-type nematode swimming in pure buffer solution. The contour plot reveals the existence of periodic waves of bending motion, which propagate along the nematode body. Note that the magnitude of κ decays from head to tail.

The nematode's swimming behavior is characterized as a function of fluid viscosity or mechanical load. Results for the average swimming speed (U), the beating frequency (f), and the body displacement amplitudes (A) are shown in Fig. 3 ($n\geq20$ for each fluid viscosity). We find that the nematode's locomotion is robust; for all fluids, the swimming speed is sustained ($U\approx0.35$ mm/s). For the range of viscosities, $Re=\rho UL/\mu$ is below unity (Re=0.03-0.4), where ρ is the fluid density. Hence, *C. elegans*' motility can be considered to be viscous dominated even in the pure buffer solution.

The nematode's beating frequency (f) decreases slightly with increasing μ (Fig. 3b). However, this change remains small; a ten-fold increase in μ yields a 20% reduction from 2 Hz to an asymptotic value of ~1.7 Hz. The spatial form of the nematode's swimming gait is unaffected over the viscosity range; body displacement amplitudes remain near 0.25 mm (Fig. 3c). *C. elegans* maintains a consistent swimming gait as it senses its fluidic environment [5]. The data in Fig. 3 indicates that *C. elegans* may respond to the increased mechanical load by adapting its swimming kinematics to maintain a constant swimming speed. This adaptability suggests that *C. elegans* may not be power-limited [6].

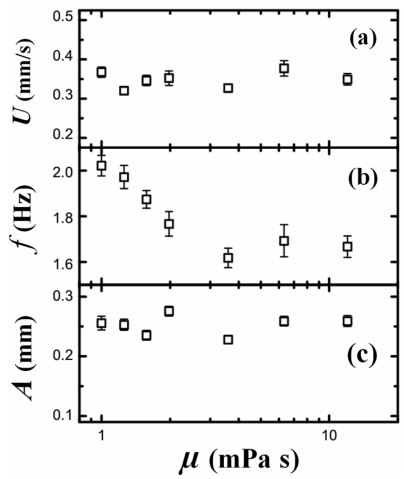

Fig. 3 *C. elegans*' (a) swimming speed, (b) beating frequency, and (c) body displacement amplitude, as a function of fluid viscosity

Our kinematic results are supported by quantitative flow data obtained with PIV. An example of an instantaneous 2D velocity field is shown in Fig. 4(a) for a swimming *C. elegans* ($Re\approx0.4$). Magnitudes of the flow are on the order of U. Regions of fluid circulation are observed along the nematode's body. For low Re number flows, vortices remain attached within the vicinity of the nematode's body

and are not shed into the fluid. Temporal signals for the mean fluid velocity magnitude (<|*V*|>) are extracted from PIV data (Fig. 4b). Signals are marked by a characteristic double period. One period is associated with the nematode beating frequency (f~2 Hz), while the other corresponds by definition to the velocity of its body displacements ($2f$). Note that results of Fig. 4(b) pertain to fluid properties only as nematodes remain transparent for PIV measurements.

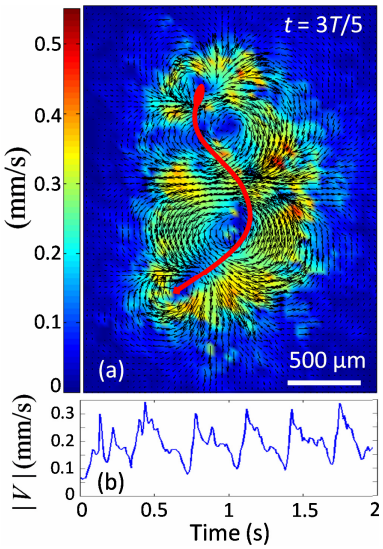

Fig. 4 (a) Velocity field of a *C. elegans* swimming in pure buffer solution at t=3T/5 (Re≈0.4). The nematode position is schematically depicted. (b) Mean velocity magnitude signal of fluid flow

IV. Swimming Model

We model the motion of *C. elegans* as a slender filament swimming at low Re number. The model is used along with kinematic data to estimate the nematode's material properties such as its Young's modulus (E) and effective tissue viscosity (η). The nematode's motion is described in terms of its centerline $y(s,t)$. The swimming *C. elegans* experiences no net total force or torque (moments) such that, in the limit of low Re, the equations of motion are

$$\frac{\partial \vec{F}}{\partial s} = C_t \vec{u}_t + C_n \vec{u}_n , \qquad (1)$$

$$\frac{\partial M}{\partial s} = -\left[F_y \cos(\phi) - F_x \sin(\phi)\right]. \qquad (2)$$

In Eq. (1), $\vec{F}(s,t)$ is the internal force in the nematode, C_i is the drag coefficient experienced by the nematode, $\vec{u}_i$ is the nematode velocity along the arc-length, and the subscripts t and n correspond to the tangential and normal directions, respectively. Estimates of drag coefficients are obtained from slender body theory [1].

In Eq. (2), M=M_p+M_a, where M_p is a passive moment and M_a is an active moment generated by the nematode muscles; the active and passive moments are parts of a total internal moment [3] The passive moment is given by the Voigt model such that $M_p = EI\kappa + \eta_p I(\partial\kappa/\partial t)$ [3]. Here, we assume two homogeneous effective material properties, namely a constant Young's modulus (E) and a constant tissue viscosity (η_p). The active moment generated by the muscle is given by $M_a = -\eta_a I(\partial\kappa/\partial t)$, where η_a is a positive constant [7]. Note that if $\eta = \eta_p - \eta_a > 0$, there is net dissipation of energy in the tissue; if $\eta < 0$, there is net generation of energy in the tissue. For live nematodes, we expect $\eta < 0$ since the net energy produced in the (muscle) tissue is needed to overcome the drag from the surrounding fluid.

We simplify Eqs. (1) and (2) by noting that the nematode's deflections off its centerline remain small. In such case, $s \approx x$ and $\cos(\varphi) \approx 1$. This results in a set of linearized equations for $\vec{F}$ and M which ultimately lead to [8]

$$\frac{\partial^2 M}{\partial x^2} + C_n \frac{\partial y}{\partial t} = 0 \cdot \qquad (3)$$

Experiments (Fig. 2a) reveal that (i) *C. elegans* swims at a single frequency f and (ii) the curvature κ has non-zero amplitudes at the head (x=0) and tail (x=L). Boundary conditions are matched to be consistent with experimental observations (details in [8,9]). We solve Eq. (3) for $y(x,t)$ and obtain the curvature κ $(x,t) = \partial^2 y/\partial x^2$. The solution for $y(x,t)$ is a superposition of four traveling waves (details in [8,9]).

V. Material Properties of *C. elegans*

The predicted nematode body position $y(x,t)$ is fitted to experimental data to compute κ [9]. The fitting method yields estimates of the bending modulus (K_b) and the phase angle $\psi = \tan^{-1}(\eta\omega/E)$. An example of a theoretical curvature contour plot is shown in Fig. 2(b). While not perfect, the model reveals both the traveling bending wave and the characteristic decay in κ from head to tail (Fig. 2a).

The effective material properties (E and η) are estimated from K_b=$I(E^2 + \eta^2\omega^2)^{1/2}$ and ψ. Here, the nematode is modeled as an idealized cylinder, where the effects of internal hydrostatic pressure are neglected [4]. Namely, we consider the second moment of inertia (I) of the nematode cross section as represented by its "shell" which comprises the cuticle, hypodermis, and longitudinal muscles [4].

The estimated values of E and η are shown on a log-log phase diagram in Fig. 5. Results show that for nematodes swimming in buffer solution E=0.62±0.05 kPa and η = -177.1±15.2 Pa-s. Estimated values of E lie within the wide

range of values of elasticity measured for soft tissues, including brain (0.1-1 kPa) and muscle cells (8-17 kPa) [10]. The values of η for live *C. elegans* are negative because the organism's tissues are generating rather than dissipating energy [8]. We note, however, that the absolute values of tissue viscosity η are within the range (10^2-10^4 Pa-s) measured for living cells [11].

The estimated magnitudes of E and $|\eta|$ increase monotonically over an order of magnitude with increasing mechanical load (Fig. 5). The data suggests an increase in the nematode's muscle tonus as it senses the fluidic environment and responds with dynamic changes in its material properties.

Next, we tested if our methods are sensitive to changes in muscle functional properties. We quantified material properties of three distinct mutant muscular dystrophy (MD) strains [8]: one with a well characterized muscle defect (*dys-1;hlh-1*); one with a qualitatively subtle movement defect (*dys-1*), and one mutant that has never been characterized with regards to motility phenotypes but is homologous to a human gene that causes a form of MD expressed in nematode muscle (*fer-1*). While both *fer-1* and *dys-1* genes are expressed in *C. elegans* muscle, they exhibit little, if any, change in whole motility under standard lab assays [12].

We find that all three mutants swimming in pure buffer solution (n=7-25) exhibit significant changes in tissue properties (Fig. 5). Mutants have a lower E when compared to wild-type nematodes. In other words, *dys-1*, *dys-1;hlh-1*, and *fer-1* mutants are softer than their wild-type counterpart. Values of $|\eta|$ for *fer-1* mutants are similar to wild-types, within experimental error. However, the values of $|\eta|$ for *dys-1* mutants are lower than wild-type *C. elegans*. Since muscle fibers are known to exhibit visible damage for *dys-1;hlh-1* mutants [13], we hypothesize that the deterioration of muscle fibers may be responsible for the lower values of E and η found for *dys-1* and *dys-1;hlh-1* mutants.

VI. Conclusions

We have characterized the swimming behavior of *C. elegans* at low *Re* number. Over the range of fluid viscosities (μ) investigated, the nematodes' robust motility supports *C. elegans*' locomotive adaptability to its surrounding environment [5,6]. By coupling experiments with a simple model, we are able to estimate, non-invasively, the nematode's material properties such as Young's modulus (E) and tissue viscosity (η). Results show that *C. elegans* behaves effectively as a viscoelastic material. Both E and $|\eta|$ increase over an order of magnitude as μ is increased. We speculate that the increase in E may be associated with the shortening of sarcomeres and higher density of muscle cells typical in muscle contraction [14]; usually muscle stiffens as larger mechanical loading is applied. We suspect that the increase in $|\eta|$ is associated with the larger amount of energy necessary to overcome the increased fluid drag. By quantifying tissue properties associated with muscular dystrophy mutations in *C. elegans*, our method also sheds new light on our understanding of muscle function and animal locomotion.

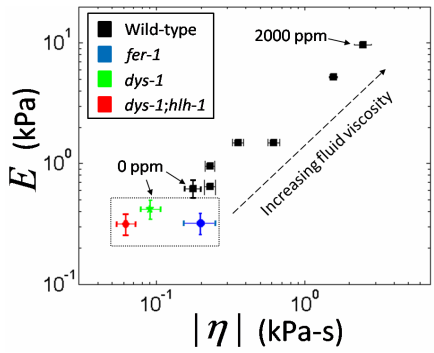

Fig. 5 Wild-type *C. elegans*' material properties as a function of fluid viscosity. Also included are examples of dystrophic strains at 0 ppm

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