High-Speed Video Cinematographic Demonstration of Stalk and Zooid Contraction of *Vorticella convallaria*

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ABSTRACT Stalk contraction and zooid contraction of living *Vorticella convallaria* were studied by high-speed video cinematography. Contraction was monitored at a speed of 9000 frames per second to study the contractile process in detail. Complete stalk contraction required approximately 9 ms. The maximal contraction velocity, 8.8 cm/s, was observed 2 ms after the start of contraction. We found that a twist appeared in the zooid during contraction. As this twist unwound, the zooid began to rotate like a right-handed screw. The subsequent stalk contraction steps, the behavior of which was similar to that of a damped harmonic oscillator, were analyzed by means of the equation of motion. From the beginning of stalk contraction, the Hookean force constant increased, and reached an upper limit of 2.23×10^{-4} N/m 2–3 ms after the start of contraction. Thus, within 2 ms, the contraction signal spread to the entire stalk, allowing the stalk to generate the full force of contraction. The tension of an extended stalk was estimated to be 5.58×10^{-8} N from the Hookean force constant of a stalk. This value coincides with that of the isometric tension of a glycerol-treated *V. convallaria*, confirming that the contractile system of *V. convallaria* is well preserved despite glycerol treatment.

INTRODUCTION

The *Vorticellid* ciliates, such as *Vorticella*, *Carchesium*, and *Zoothamnium*, are composed of zooids and long stalks. The coiling of the stalks and the simultaneous change in shape of the zooids, namely, zooid contraction, generally occur in an all-or-none fashion. The stalk coiling is produced by the contraction of spasmoneme, an intracellular fibrous organelle that resides in a helical form inside the elastic, cylindrical outer sheath of the stalk. In glycerinated *Vorticella*, spasmoneme contraction is induced by Ca^{2+} without hydrolysis of ATP (Hoffmann-Berling, 1958; Amos, 1975; Asai et al., 1978). Zooid contraction is also induced by Ca^{2+} (Katoh, 1995). Reversible extension occurs in the presence of Ca^{2+} chelators such as EDTA and EGTA (Hoffmann-Berling, 1958; Ochiai et al., 1983). This contraction differs fundamentally from other forms of cell motility. In an effort to clarify this unique system of contraction, numerous studies using glycerinated *Vorticellid* ciliates have been conducted by Asai et al. (1978) and by Amos et al. (1972, 1975).

In 1970, Jones et al. studied the contraction of *V. difficilis*, *V. campanula*, and *Carchesium* sp. by means of high-speed cinematography. In their studies, no turning of the zooid was detected during the intermediate stalk contraction steps, although rotation was observed after the contraction had been completed.

We used a high-speed video system to perform this study. This system has a maximal speed of 40,000 frames per second, and each frame produces a very clear image. Therefore, in contrast to the studies of Jones et al. (1970), we were able to observe a twist that appeared in the zooid during contraction and then unwound such that the zooid began to rotate like a right-handed screw. The subsequent stalk contraction steps, which resembled the behavior of a damped harmonic oscillator, were analyzed by means of the equation of motion for a particle subject to a linear restoring force (Hookean spring force) and a frictional force proportional to the transitional velocity of the zooid. This analysis confirmed that the tension developed in living *V. convallaria* is equal to that of the glycerinated model.

MATERIALS AND METHODS

Organisms

V. convallaria were cultivated at 20°C in 0.025% Vita-shrimp infusion, which had been inoculated with *Escherichia coli* (Vita-shrimp is a tropical fish food) (Asai et al., 1978). A fishing line served as the substrate for *V. convallaria* attachment.

Contraction of ciliates

The spontaneous contraction of *V. convallaria* was monitored in an artificial solution containing 0.1 M KCl, 4 mM EGTA, 20 mM MOPS, and 10 μM (estimated) free calcium ions, at pH 6.8. In this artificial solution, the metachronal wave of *V. convallaria* peritrich cilia, which hinders focusing on the full length of the stalk, ceases.

Microscopic images of the contraction of *V. convallaria* were recorded with a high-speed video system (FASTCAM-ultima, Photron Co.). The contraction was recorded at a speed of 9000 frames per second. With this system, every frame was saved in IC memory, rather than on video film. This allowed the recording of spontaneous contractions.

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RESULTS

Contraction of *V. convallaria*

The spontaneous contraction of *V. convallaria* was monitored in the aforementioned artificial solution. Fig. 1 shows two series of frames from a contraction sequence. The stalk begins to coil near the zooid, and the coiling spreads distally. Complete stalk contraction required approximately 9 ms. In Figs. 1 and 2, a twist in the zooid observable from 3 to 5 ms after the start of stalk contraction is illustrated. The rotational forces of helical contraction produced this twist in the zooid. As the twist unwound, the zooid began to rotate like a right-handed screw. It is well known that the helix of contracted stalk is counterclockwise (Amos, 1972; unpublished observation). This means that the stalk elongation by uncoiling induces the counterclockwise rotation of the zooid to an observer looking down the stalk from the zooid toward the point of attachment. Conversely, the stalk contraction induces the clockwise rotation of the zooid, as in Fig. 2. Even after contraction of the stalk had been completed, the rotation continued. The number of rotations per contraction

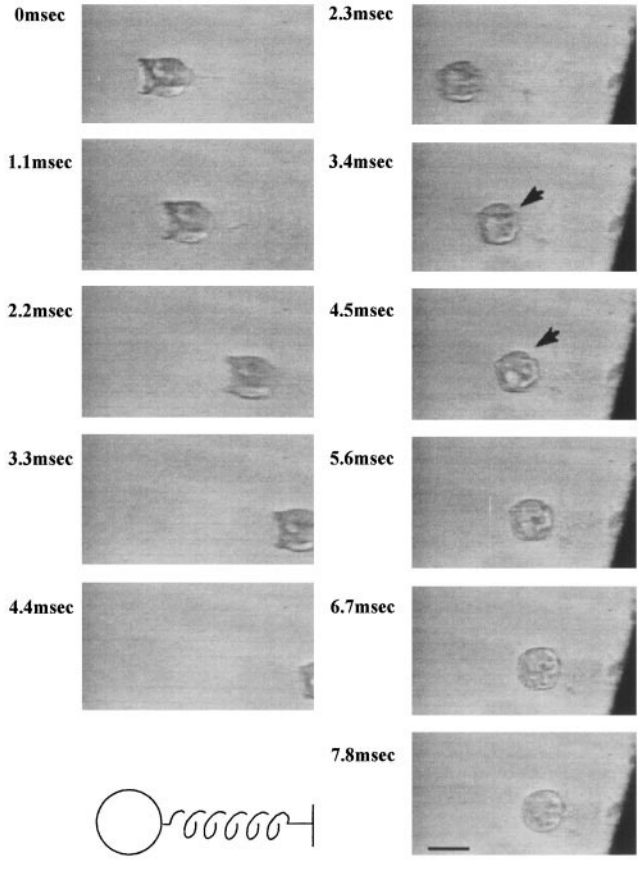

FIGURE 1 Two series of frames from the contraction sequence of *V. convallaria*. The times from the start of contraction are indicated on the left. Arrows indicate the twist in the zooid. Bar, 50 μm. A schematic illustration of the mechanical model of the damped harmonic oscillator is presented at the lower left. In this model, the zooid is regarded as a spherical ball, the stalk as a spring. The helix of contracted stalk is counterclockwise.

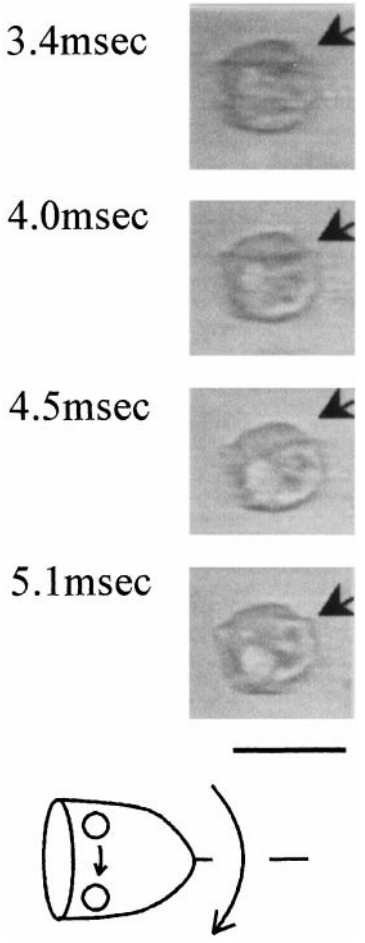

FIGURE 2 A series of frames illustrating the change in the twist in the zooid. The twist in the zooid in Fig. 1 is shown at higher video rates. Bar, 50 μm. The direction of zooid rotation during contraction was illustrated. Long arrow indicates the direction of zooid rotation (like a right-handed screw). A light vacuole in the zooid, which is located in the surface near the observer, moved down by the rotation of the zooid.

was approximately three. Along with this rotation, the zooid also changed from a bell to a globular shape. The zooid appeared entirely globular by 7 ms after the start of stalk contraction.

Change in contraction velocity

We analyzed the distance traveled as a consequence of zooid movement (Δx) in each video frame (every 0.11 ms). We found 0.11 ms (Δt) to be a short enough time to allow determination of the contraction velocity ($\Delta x/\Delta t$). We analyzed four sets of video data illustrating contractions. These sets of video contraction data had a similar appearance. A typical example is shown in Fig. 3. Fig. 3 *B* shows the velocity of stalk contraction. The maximal contraction velocity, 8.8 cm/s (SD = 0.48; $N = 4$), was observed 2 ms (SD = 0.12; $N = 4$) after the start of stalk contraction. This rate of contraction is high, ~1800 times zooid length per second. A car moving at this rate would be traveling at a speed of 260,000 km/h.

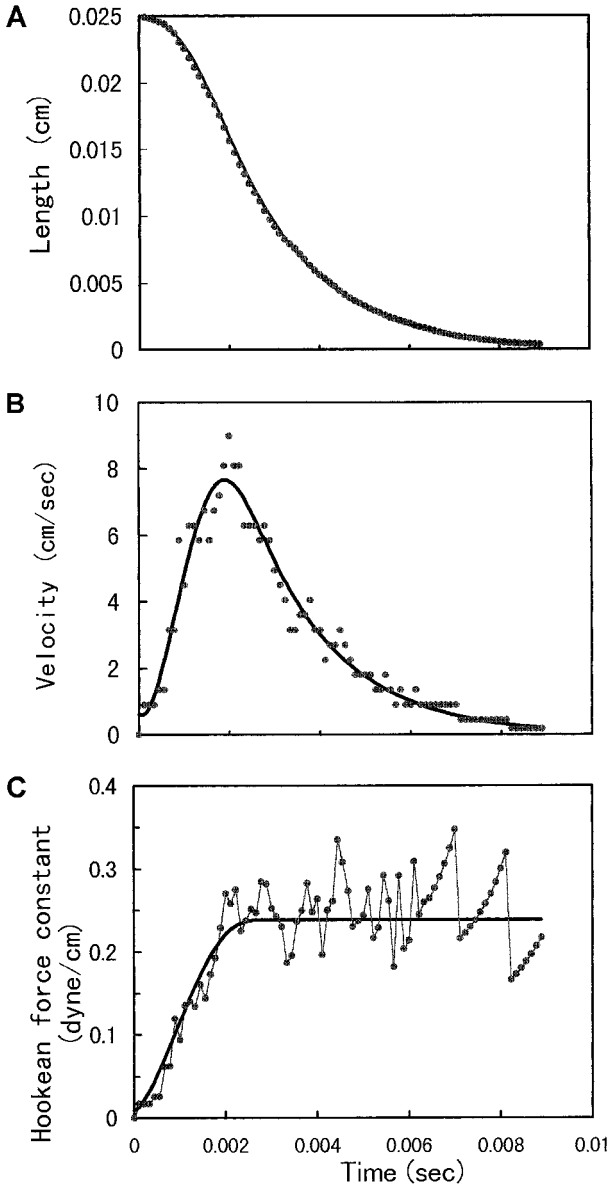

FIGURE 3 : (*A*) Time course of stalk length change. Plots indicate the length of stalk. The stalk length change is determined by summing the distance of zooid movement in all video frames; the fully contracted stalk length is considered to be zero. Lines have been estimated by the method of least squares. The lines are $x = (-1.56 \times 10^{11}t^5) + (1.33 \times 10^9t^4) - (3.26 \times 10^6t^3) + 529t^2 - 0.620t + 0.025$ ($0 \le t \le 0.0028$) and $x = 0.049\exp(-546t)$ ($0.0028 \le t \le 0.009$). (*B*) Time course of stalk contraction velocity. Plots indicate the stalk contraction velocity determined by the distance of zooid movement in each video frame (every 0.11 ms). Lines are determined by differentiating the above function. (*C*) Hookean force constant versus time. Plots indicate the Hookean force constant roughly estimated using the position data (x) in *A* and the velocity data (dx/dt) shown in *B*. The actual Hookean force constant was determined using the estimated curves shown in *A* and *B*. For details, see text.

Change in stalk length during contraction

Fig. 3 *A* shows the change in stalk length during contraction. The change in the length of the stalk is determined by summing the distance of zooid movement in all video frames, and the fully contracted stalk length is considered to be zero. The subsequent stalk contraction steps, which resembled the behavior of a damped harmonic oscillator, were analyzed by means of the equation of motion. We regarded the zooid as a spherical ball and the stalk as a spring (Fig. 1). The length of the nonextended spring is the same as that of the contracted stalk. Next, the spring was extended such that its length approached that of the extended stalk. The spring contracts, dragging the ball, which is slowed due to the viscosity of water. During stalk contraction, the Reynolds number $N_{Re} \le 2.25$, such that the hydromechanical friction on the zooid is not the inertial force in Bernoulli's formula ($F = (\pi/2)\rho(rv)^2$), but rather, the viscous force in Stokes' formula ($F = 6\pi\eta rv$). The equation of motion for this contraction, as with a damped harmonic oscillator, is

$$m\frac{d^2x}{dt^2} = -kx - 6\pi\eta r\frac{dx}{dt} \quad (1)$$

or

$$k = \frac{1}{x}\left(-m\frac{d^2x}{dt^2} - 6\pi\eta r\frac{dx}{dt}\right) \quad (2)$$

where m is the mass of the zooid (6.5×10^{-8} g), r is the radius of the zooid (25×10^{-6} m), η is the viscosity of water (10^{-3} Ns/m^{-2}), and k is the Hookean force constant of the spring. In Eq. 2 we can ascertain the Hookean force constant k using the position data (x), the velocity data (dx/dt), and the acceleration data (d^2x/dt^2). Moreover, in the case of motion of a microorganism, the inertia term ($-m(d^2x/dt^2)$) is very small except in the first stage of the movement because the Reynolds number is small. The roughly estimated value of the Hookean force constant k is plotted in Fig. 3 *C*. This rough estimate was obtained by substituting the position data (x) and the velocity data (dx/dt) in Fig. 3, *A* and *B*, for Eq. 2 and ignoring the inertia term. As shown in Fig. 3 *C*, k is fixed after 2–3 ms. If the value of k is constant, Eq. 1 is that of an overdamped harmonic oscillator. The solution is

$$x = a_1e^{-(\mu+\sigma)t} + a_2e^{-(\mu-\sigma)t}$$

$$\mu = \frac{6\pi\eta r}{2m}, \quad \sigma = \sqrt{\frac{(6\pi\eta r)^2}{4m^2} - \frac{k}{m}}$$

where a_1 and a_2 are constants determined by the initial condition of motion. In actuality, in Fig. 3 *A*, the contraction curve after 2.8 ms is an exponentially decreasing curve. Thus, the position data (x) were estimated by the method of least squares for an exponential curve. From 0 to 2.8 ms, if the Hookean force constant k is a function of time or position, we cannot solve Eq. 1. Therefore, the position data (x) from 0 to 2.8 ms were estimated based on an algebraic function of degree five by the method of least squares. The estimated position data (x) are plotted in Fig. 3 *A*. Using these estimated functions of position data, we can ascertain the velocity (dx/dt), as plotted in Fig. 3 *B*, and the acceleration (d^2x/dt^2) by differentiating the two functions. Thus,

the Hookean force constant k was determined from Eq. 2. The determined k is plotted against time in Fig. 3 *C*. From the start of contraction, the Hookean force constant increased, reaching an upper limit of 0.238 dyne/cm at 2–3 ms after the start of contraction. The other three sets of video data were also analyzed in this manner. The mean of the saturated Hookean force constant was $k = 0.223$ dyne/cm (SD = 0.011; $N = 4$), and saturation occurred at 2–3 ms after the start of contraction.

When the Hookean force constant was $k = 0.223$ dyne/cm (2.23×10^{-4} N/m), the force generated in the extended stalk was estimated to be 5.58×10^{-8} N ($F = kx$). This value is consistent with the isometric tension generated by glycerol-treated *V. convallaria* (4×10^{-8} N; SD = 3×10^{-8}; $N = 24$) (Moriyama et al., 1996; unpublished data), confirming that the contractile system of *V. convallaria* is well preserved in glycerol-treated specimens.

DISCUSSION

Forces generated by spasmoneme in vivo and in glycerinated models

Glycerol-treated models of *Vorticellid* ciliates were first used by Levine (1956), who observed that stalk contraction was induced by divalent ions and that reversible extension occurred with the addition of a Ca^{2+} chelator, EDTA. Hoffmann-Berling (1958) used a glycerol-treated preparation of *Vorticella* to demonstrate that ATP is not necessary for this in vitro contraction-extension cycle. Weis-Fogh and Amos (1972) used a glycerol-treated preparation of *Zoothamnium geniculatum* to analyze the mechanical and optical properties of spasmoneme. In 1978, Asai et al. improved the method of preparing glycerinated *V. convallaria*. The improved preparations were used for studies of the hysteresis of the contraction-extension cycle (Ochiai et al., 1979), contraction of spasmoneme and coiling of the sheath (Ochiai et al., 1983), contractility of spasmoneme in response to various divalent metal and lanthanide ions (Yokoyama and Asai, 1987), and Ca^{2+}-induced tension development (Moriyama et al., 1996).

None of these studies addressed the issue of whether the mechanism of spasmoneme contraction is adequately preserved after glycerol treatment. According to our analysis of a mechanical model composed of a ball with a spring, stalk contraction is the same as that of a spring for which the kinetic constant k is 2.23×10^{-4} N/m. Thus, the force generated by the extended stalk would be 5.58×10^{-8} N. This value is consistent with the isometric tension generated by glycerol-treated *V. convallaria* (4×10^{-8} N; SD = 3×10^{-8}; $N = 24$) (Moriyama et al., 1996; unpublished data). These observations confirm that the contractile system is well preserved in glycerol-treated *V. convallaria*. Thus, the early studies using glycerol-treated *Vorticella* accurately reflected in vivo contraction and force generation.

The contractile mechanism

Several studies have assessed the manner in which *Vorticellid* ciliates contract. Ueda (1954) described a maximal contraction rate of 21 cm/s for *Carchesium*. Sugi (1960) reported the contraction of *Carchesium* to be propagated down the stalk at a velocity of 20–50 cm/s. In *V. difficilis*, Jones et al. (1970) obtained a contraction rate of 1.4–5.6 cm/s and reported that no turning of the zooid was detected during stalk contraction and that rotation actually occurred after the completion of contraction. In the present study we obtained a maximal contraction rate of 8.8 cm/s (SD = 0.48; $N = 4$) for *V. convallaria*. The new findings in this report include not only the detection of zooid turning during contraction but also the detection of a twist in the zooid during contraction. Jones and colleagues (1970) did not detect either the turning or the twist because of the limited capacity of their measuring device. These investigators speculated that the rotational forces of helical contraction might be absorbed in the elasticity of the stalk and released after the completion of contraction. We found, however, that the zooid twist actually manifested during contraction. This twist in the zooid is derived from the rotational forces of helical contraction. A portion of these rotational forces is absorbed in the elasticity of the zooid, not only in that of the stalk. As the twist unwound, the zooid began to rotate like a right-handed screw. The total number of rotations was approximately three.

Propagation of contraction signal

From the start of contraction, the Hookean force constant increased, reaching an upper limit of 0.223 dyne/cm 2–3 ms after the start of contraction. Thus, within 2–3 ms, the contraction signal spread throughout the stalk such that the stalk generated the full contractile force. For the *Vorticellid* stalk, it is assumed that the contraction signal is calcium-induced calcium release (Katoh, 1995). Several membranous tubules, probably sites of Ca^{2+} storage, are present in the spasmoneme (Allen, 1973). These membranous tubules release stored Ca^{2+} when stimulated by an increase in the intracellular Ca^{2+} concentration. This release might produce a regenerative increase in the intracellular Ca^{2+} concentration. The released Ca^{2+} ions bind to Ca^{2+}-binding proteins, spasmins, which are components of spasmoneme. The binding of Ca^{2+} to spasmins results in spasmoneme tension development. All of these events take palace within 2–3 ms, allowing extremely rapid contraction of the *Vorticellid* stalk.

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