MOTION CHARACTERISTICS OF FLAGELLAR FRAGMENTS OF LONG INSECT SPERM

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ABSTRACT The flagellar length of cricket spermatozoa was reduced in steps from $\simeq 1{,}000\ \mu m$ (intact length) to 50 μm. In intact sperm the flagellar wave properties were largely independent of the viscosity of the external medium. When the flagellar length had been reduced to $<100\ \mu m$ the flagellar frequency was reduced at a raised external viscosity. Independent motion of different sections of a flagellum was not observed when its length is $<100\ \mu m$. It is concluded that in long thin flagella, transverse viscous forces cannot exert a moment beyond a lever length of $\sim 100\ \mu m$. It is shown that the existence of a maximum lever length, beyond which no moment can be transmitted, leads to the absence of a standing active contractile moment in the long insect sperm.

INTRODUCTION

In a recent paper (Rikmenspoel, 1978) a simple theory for the contractile coordination in sperm flagella was presented. It was found from the theory given, that the internal active contractile moments in sperm flagella can occur in two different forms. An active moment can exist which has the same phase over the length of the flagellum. The magnitude of this "standing" moment decreases linearly from the point of attachment of the flagellum toward the distal tip. Alternatively, an active moment can occur which travels with the wave crest along the flagellum. This "traveling" moment has a more or less constant magnitude along the flagellum.

It was concluded in the earlier paper (Rikmenspoel, 1978) that if the standing moment is the main component of the active moment, the flagellar wave has a wavelength comparable to the flagellar length. Sea urchin and bull sperm are examples of such flagella which are driven mainly by a standing moment.

It was also found in the theory that the absence of a standing active moment is only possible in flagella which display waves with a wavelength much smaller than the flagellar length. The long sperm of certain species of insects (e.g. cricket, drosophila) are examples of this case. In these sperm there appears to be only a traveling active moment, which moves with the wave crest along the flagellum.

The theory predicted that in the sperm with a wavelength, λ, much smaller than the flagellar length, ℓ, the motion should be independent of the external viscosity. It was indeed observed that in cricket sperm ($\ell \simeq 1{,}000\ \mu m$, $\lambda \simeq 20\ \mu m$) the frequency and amplitude of the flagellar waves did not change over a range of external viscosities of 1 – 70 cP. It was further derived from the theory that in the long insect sperm, sections of the same sperm should show independent motions with wave trains of different frequencies. This also was observed in the cricket sperm (Rikmenspoel, 1978).

The above motion characteristics of cricket sperm were all conditional upon λ being sufficiently smaller than ℓ. If, therefore, the length of a cricket sperm were reduced sufficiently, the theory predicts that the wave characteristics will change, and also that a dependence of the wave properties on the external viscosity should be observed, as it is in other sperm (sea urchin, bull) in which the condition $\lambda \ll \ell$ does not hold.

In this paper experiments are described in which the length of cricket sperm was reduced by dissection. The results indicate that when $\ell < 100$ μm, the wave properties of the shortened sperm indeed become similar to those of bull sperm.

EXPERIMENTAL METHODS

Spermatozoa were obtained by dissecting out the vas deferens of healthy mature male crickets. A ball of semen was then extracted. The semen was put in a small Petri dish in a basic suspension medium (BEM) described below. By gentle easing, a number of spermatozoa were separated from the clumped semen. One drop of this sperm suspension was transferred to a microscope slide chamber described previously (Lindemann and Rikmenspoel, 1971) and gently mixed into 1 ml BEM. The final concentration was such that approximately one sperm occurred in the field of view of a X10 microscope objective.

The BEM consisted of 160 mM sucrose, 72 mM KCl, 2 mM Na pyruvate, 1 mM $MgSO_4$, and 2% of 0.1 M $NaHPO_4$ buffer in quartz double-distilled water. The pH was adjusted to 7.4. ATP from Boehringer Mannheim Biochemicals (Indianapolis, Id.) was added to the BEM in a 4-mM concentration to maintain the motility of the sperm after dissection.

The above medium has been used in this laboratory to maintain motility in impaled bull spermatozoa (Rikmenspoel et al., 1978; O'Day and Rikmenspoel, 1979). The cricket spermatozoa appeared to show good motility after dissection in the BEM. No attempt was made to optimize the medium for the case of cricket sperm.

The viscosity of the BEM was raised when desired by the addition of Ficoll (Sigma Chemical Co., St. Louis, Mo.) as described previously (Rikmenspoel, 1976). The viscosity of the experimental samples was measured with a falling ball microviscometer (Lindemann and Rikmenspoel, 1972*a*). All experiments were preformed with a temperature of the preparation of 18.5 ± 0.5°C.

During experiments the sperm preparations were in an open fluid layer of a few tenth of 1-mm thickness in the slide chamber mentioned above. The preparations were illuminated in dark field and viewed by a X10 Zeiss microscope objective (Carl Zeiss, Inc., New York). Cutting of the cricket sperm flagella was done using a glass microelectrode as a microknife as previously described (Lindemann and Rikmenspoel, 1972*b*). The microelectrode was mounted at a 15° angle to the slide surface. The penetration point of the top water-air interface of the preparation by the electrode was sufficiently far from the optical axis of the microscope so as not to disturb the viewing of the preparation.

The length of intact cricket sperm, measured with an eyepiece micrometer, was found to be fairly uniform; 980 ± 30 μm (average and standard deviation over 15 sperm). This figure includes the head and a stiff nonmotile terminal piece of 110 ± 10 μm length (average and standard deviation). When cuts were made in the sperm flagella the aim was to have the total length (including the head) of the proximal fragment 500, 250, 150, 90, or 50 μm. The actual length of the proximal piece achieved was measured on the moving films (see below) made during the experiments. The measured lengths of the proximal fragments in each group showed a standard deviation of $\sim$15%.

Moving films at 100 frames/s of the sperm and the proximal fragments were made on Kodak Plus X film (Eastman Kodak Co., Rochester, N.Y.) using a Millikan DMB-5C camera. Analysis of the wave frequency and amplitude was performed with the aid of a Vangard motion analyzer (Vangard Systems, Inc., Irvington, N.Y.) as previously described (Rikmenspoel, 1965, 1978).

RESULTS

Live cricket sperm display wave trains of different frequency and amplitude on different parts of the flagellum (Rikmenspoel, 1978). All data reported below refer to the wave train starting at the proximal junction of the flagellum because in the shortest dissected proximal fragments (50 or 90 μm length) this was the only wave train retained. The more distal parts of long insect sperm flagella often have waves of a low frequency and high amplitude. The average flagellar frequency reported below was therefore higher than that found previously as an average over all waves on the flagellum. At a normal external viscosity of 1.2 cP the average frequency of the proximal wave train of the intact cricket sperm was 13.3 ± 3.4 Hz (average and standard deviation over 18 sperm).

For the dissection experiments sperm were generally chosen that showed good flagellar motility. This implies that our data may have a slight selective bias toward sperm with a higher flagellar frequency. After a cut was made in a flagellum a 2-min. interval was taken to allow the sperm to attain a (new) steady-state motion. The motion of the proximal wave train of the flagellar fragment was then recorded on moving film. It was often possible to perform the dissection more than once on the same sperm, resulting in successively shorter proximal fragments. In the data presented below no separation was made between fragments obtained after a single or a multiple dissection. It proved difficult, however, especially at the higher external viscosities to perform the dissection to a 50-μm long fragment. All results reported on 50-μm long fragments were the result of a single cut made on an intact sperm. Fig. 1 A is an enlargement of a part of a 16-mm film frame showing the proximal part of an intact cricket sperm. Fig. 1 B shows a dissected fragment ~50-μm long of a cricket sperm.

Fig. 2 shows the flagellar frequency as a function of the external viscosity for intact cricket sperm and for fragments of various length. It can be seen in Fig. 2 that at normal viscosity the

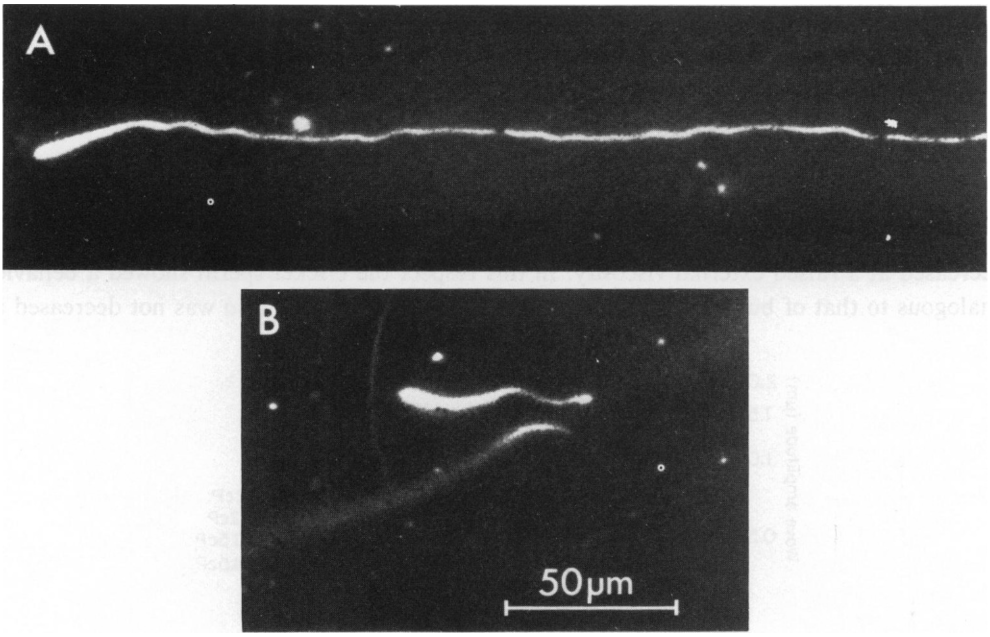

FIGURE 1 (A)Proximal part of an intact live cricket sperm showing several wave trains. 1(B) Short (50 μm), proximal fragment of a dissected cricket sperm. The tip of the flagellum was stuck to the slide after the dissection. A part of the cutoff distal section of the sperm can be seen below and to the left of the proximal fragments. The external viscosity for both Fig: 1 A and B was 94.5 cP.

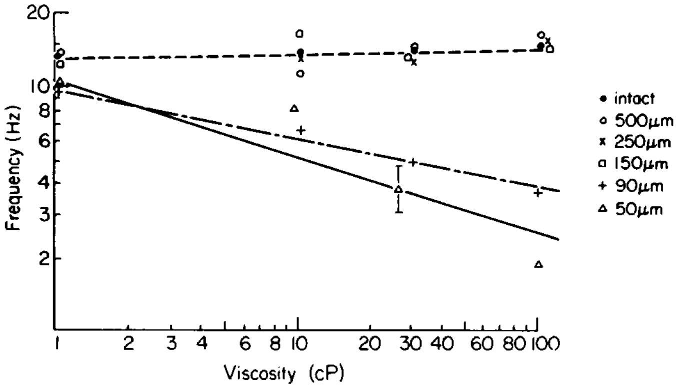

FIGURE 2 Flagellar frequency as a function of the external viscosity for intact cricket sperm and for dissected flagellar fragments of various length. The lines in this figure and in Figs. 3–5 were drawn by eye for heuristic purpose only. Each point in this figure and in Figs. 3 and 5 represents an average over 6–18 sperm, except a few points for 500-μm fragments which were derived from four or five sperm. The vertical bars in this figure and in Fig. 3 represent typical standard deviations. The standard errors in the averages were typically three times smaller than the standard deviations shown.

flagellar frequency changed little after dissection. Only the short fragments of 90 and 50 μm length show a slight reduction in frequency, from ~13 to ~10 Hz. The dissection itself is thus not causing a large change in contractile activity.

When the flagellar length was at least 150 μm, the flagellar frequency did not change appreciably with viscosity as Fig. 2 shows. 90-μm long fragments and those of 50μm length showed a clear decrease of flagellar frequency at higher viscosity. The slope of the solid line in Fig. 4, drawn as an approximation of the relation between the frequency and the viscosity, η, for the 50-μm fragments, is about -0.4, indicating a decrease not very far from proportional to $1/\sqrt{\eta}$.

Fig. 3 shows the wave amplitude in the proximal wave train, plotted as a function of the flagellar length, for the four values (1.2, 9.2, 28.5, and 94.5 cP) of the viscosity studied. It can be seen in Fig. 3 that at all values of the flagellar length the average wave amplitude was not decreased at a raised external viscosity. In this respect the cricket sperm showed a behavior analogous to that of bull sperm, in which the the wave amplitude also was not decreased at

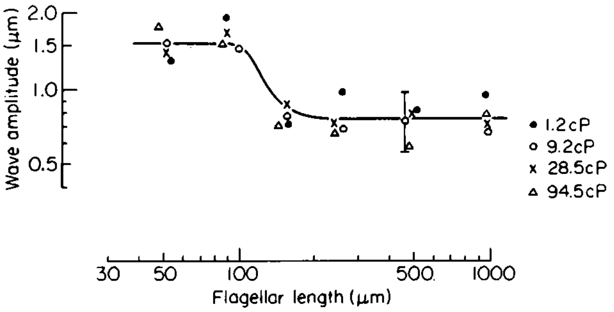

FIGURE 3 Flagellar wave amplitude as a function of the flagellar length of cricket sperm at four different viscosities.

TABLE I

FLAGELLAR FREQUENCY AND AMPLITUDE OF 50-μm LONG FRAGMENTS OF CRICKET SPERM, WITH THE TIP EITHER FREE OR ATTACHED TO THE SLIDE

Viscosity	Condition of tip	No. of sperm	Frequency	Amplitude
cP			*(av ± SD) Hz*	*(av ± SD) μm*
1.2	Free	5	11.5 ± 3.1	1.2 ± 0.3
	Fixed	7	10.0 ± 3.7	1.4 ± 0.4
94.5	Free	2	2.1 ± 0.2	2.3 ± 0.7
	Fixed	6	1.6 ± 0.7	1.7 ± 0.6

raised viscosity (Rikmenspoel et al., 1973). Sea urchin sperm, however, show a clear reduction of wave amplitude at higher external viscosity (Brokaw, 1966).

At all values of the external viscosity the wave amplitude of the cricket sperm showed an abrupt increase when the flagellar length had become smaller than 100 μm, as Fig. 3 shows. This indicates that a change in wave character occurs when the flagellar length is <100 μm.

After dissection of a cricket sperm the tip of the dissected proximal fragment frequently stuck to the slide. Especially when short fragments (50 or 90 μm) were cut in media with high viscosity it proved to be nearly impossible to free the flagellar tip (by micromanipulation with the microelectrode) without severly damaging the flagellar fragment. Table I illustrates that for the short fragments the frequency and amplitude of the motion depend only little on whether the tip of the flagellar fragment was free or fixed to the slide. When the tip of the flagellar fragment was fixed to the slide, it was usually able to pivot at the point of attachment. This represents a "hinged" boundary condition. That hinged cricket sperm compared to free ones showed no significant difference in wave properties represent a further analogy with bull sperm, in which this was also observed (Rikmenspoel, 1978). Sea urchin sperm, in the contrary, show a change in wave motion when hinged compared to free swimming (Gibbons, 1975).

In the Introduction it was mentioned that in the theory presented in Rikmenspoel (1978) an equation of motion for the long insect sperm was derived in which the viscosity of the external medium did not occur. Correlations between wave properties (such as frequency and amplitude of the waves) should then not be affected by a change in external viscosity. Fig. 4 A shows the relation between the wave frequency and the wave amplitude observed in individual long cricket sperm flagella at 1.2 and 94.5 cP. It can be seen that the relation between frequency and amplitude in the long flagella is identical at 1.2 and 94.5 cP.

The above independence from viscosity effects was predicated on the condition that the flagellar length be sufficiently large compared to the wavelength of the wave in the flagellum. For "short" flagella the relation between frequency and wavelength can be expected to be dependent on the external viscosity. Fig. 4 B shows that for the 50-to 90-μm flagellar fragments long indeed the relation between wave frequency and amplitude was different at 1.2 and 94.5 cP.

The long cricket sperm display wave trains of different frequency and amplitudes in different parts of the flagella (Rikmenspoel, 1978). In Fig. 5 are shown the percentage of sperm and sperm fragments which displayed more than one wave train, as a function of the

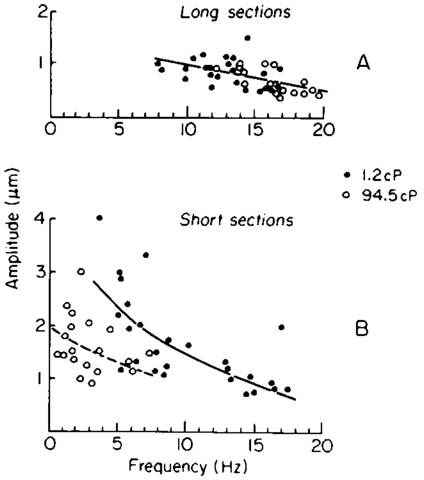

FIGURE 4

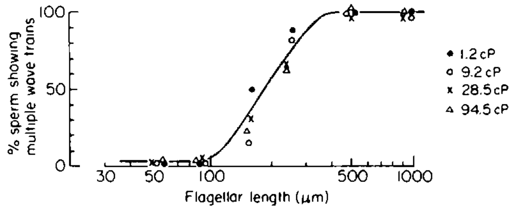

FIGURE 5

FIGURE 4 (A) Relation between the flagellar frequency and wave amplitude for long cricket sperm (500 and 950 μm length), at 1.2 cP (●) and at 94.5 cP (○). (B) As in Fig. 4 A, but for short flagellar fragments (50 and 90 μm length). The average relation at 1.2 cP (solid line) is clearly different from that at 94.5 cP (broken line).

FIGURE 5. Percentage of cricket sperm displaying more than one wave train on its flagellum as a function of the flagellar length at four different viscosities.

flagellar length. It can be seen in Fig. 5 that for flagellar lengths of 100 μm or less, only one wave train occurs, and that then the flagellum appears to move as a unit. For flagellar lengths larger than 150 μm different parts of the flagella can move independently.

As was mentioned above, the tip of the short flagellar fragments was frequently stuck to the slide. Fig. 1 B above illustrated that in a short flagellar fragment with the tip fixed to the slide the length of a wave cannot be uniquely defined. A meaningful measurement of the wavelength in the short flagellar fragments could therefore not be performed and consequently no data on the wavelengths under the various conditions are reported. The qualitative impression conveyed in Figs. 1 A and B that the wavelength in short flagellar fragments (50 and 90 μm) is larger than in intact sperm, was found consistently, however.

DISCUSSION

The results presented above clearly indicate that a change in wave properties occurs when the length ℓ of a cricket sperm is reduced to <100 μm. When $\ell > 100$ μm the flagellar frequency is independent of the external viscosity (Fig. 2). The equation of motion which relates the flagellar frequency to the wave amplitude does not appear to contain a viscosity dependent term for $\ell > 100$ μm (Fig. 4 A). When $\ell < 100$ μm the flagellar frequency is viscosity dependent, the relation of wave frequency and amplitude is viscosity dependent (Fig. 4 B) and the flagellum behaves as one single coordinated unit. It seems therefore that a different equation of motion governs the flagellar movements depending on whether $\ell < 100$ μm or $\ell >$ 100 μm.

In the earlier paper (Rikmenspoel, 1978) it was argued that the full equation of motion for a flagellum given in Eqs. 1–3 would change to the simplified one of Eq. 27 of that paper when the wavelength λ is sufficiently smaller than ℓ. The results presented in this paper indicate that the change in equation of motion takes place (with a wavelength $\lambda \simeq 20$ μm; [Rikmenspoel, 1978]), when $\lambda < 0.2\ell$.

The above has shown that in the long cricket sperm a critical length Λ exists with $\Lambda \simeq 100$ μm. A simple intuitive interpretation of the meaning of Λ can be given. In a flagellum the major part of the forces are in a transverse direction compared to axis of the flagellum. To exert a moment (as occurs in the equation of motion), the influence of a transverse force has to be transmitted over the distance of the lever of the moment. In a long, very thin, structure like a flagellum, the distance over which a transverse force can be transmitted is likely to be limited.

The critical length $\Lambda \simeq 100$ μm in cricket sperm can probably be interpreted as the distance over which the transverse viscous forces on a cricket sperm flagellum can transmit a moment. In the Appendix it is shown that the assumption only of such a limit Λ to the transmittal distance for the transverse forces leads to the conclusion that no standing moment is present in the long cricket sperm. The simplified equation of motion (Eq. 27 of Rikmenspoel, 1978).

$$M_{\text{el}} + M_{\text{act}} = 0, \tag{1}$$

can also be derived on the basis of the existence of $\Lambda \simeq 100$ μm, as shown in the Appendix.

The present results reinforce confidence that the simplified equation of motion (Eq. 1) is valid for long insect sperm. It was pointed out before (Rikmenspoel, 1978) that as consequence of Eq. 1, the active moments in long insect sperm flagella are directly displayed in the flagellar wave motion.

When the cricket sperm flagella were shortened to < 100 μm, the wave properties, as a function of the external viscosity, were similar to those of mammalian sperm: the wave amplitude was constant, the wave frequency decreased as $\eta^{-1/2}$. (Rikmenspoel et al., 1973) This is different from the behavior of sea urchin sperm, which show a strongly decreasing wave amplitude and a weakly decreasing frequency with increased η (Brokaw, 1966). The morphology of long insect sperm is more similar to that of mammalian sperm because both contain the set of nine auxilliary coarse fibers in addition to the normal axonemal structure (Kaye, 1964, Fawcett, 1966). It would appear therefore that the long insect sperm would be a better model for the study of the contractile system in mammalian sperm than sea urchin sperm.

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APPENDIX

The equation of motion for a flagellum balances the elastic bending moment, the active contractile moment, and the moment as a result of the viscous drag:

$$M_{\text{el}} + M_{\text{act}} = M_{\text{visc}}. \tag{A1}$$

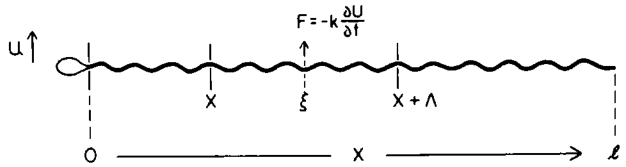

FIGURE A1 Coordinate system used in the calculation in this Appendix.

With a coordinate system as illustrated in Fig. A1, where x is the running coordinate along the flagellum and U is the deviation from the equilibrium position we can write

$$M_{\text{el}} = IE\frac{\partial^2 U}{\partial x^2}, \tag{A2}$$

where IE is the stiffness of the flagellum. With a drag coefficient per unit length of the flagellum, k, the viscous drag force at location ξ is $F = -k(\partial U/\partial t)$. The moment of the force F exerted at x is $(\xi - x)\ F$. The total viscous moment at x is the sum of all moments exerted by the elements from x to $x + \Lambda$, where Λ is the cutoff length for the lever of the viscous forces proposed in the Discussion. We can thus write

$$M_{\text{visc}} = -k\int_x^{x+\Lambda}(\xi - x)\frac{\partial U}{\partial t}\,\mathrm{d}\xi. \tag{A3}$$

For a wave solution

$$U = Ae^{i(\omega t + \alpha x)}, \tag{A4}$$

the elastic moment, Eq. A2, becomes

$$M_{\text{el}} = -IE\ A\alpha^2 e^{i(\omega t + \alpha x)}. \tag{A5}$$

Evaluation of the integral of Eq. A3 by repeated partial integration yields after substitution of Eq. A4

$$M_{\text{visc}} = -\frac{k\omega A}{\alpha}e^{i(\omega t + \alpha x)}\,e^{i\alpha\Lambda}\left[\Lambda + \frac{i}{\alpha}(1 - e^{-i\alpha\Lambda})\right] \tag{A6}$$

Substitution of the expressions of Eqs. A5 and A6 into Eq. A1 and rearranging of the terms gives an expression for the active moment M_{act} which will result in the wave solution of Eq. A4:

$$M_{\text{act}} = A\alpha^2 e^{i(\omega t + \alpha x)}\left[IE - \frac{k\omega e^{i\alpha\Lambda}}{\alpha^3}\left\{\Lambda + \frac{i}{\alpha}(1 - e^{-i\alpha\Lambda})\right\}\right]. \tag{A7}$$

It can be seen in Eq. A7 that the term in the square brackets is not dependent on x. Eq. A7 therefore describes an active moment which is traveling along the flagellum with the flagellar wave.

Eq. A7 is valid for that part of the flagellum for which $0 < x < \ell - \Lambda$. With a value of $\ell = 950\ \mu\text{m}$ and $\Lambda = 100\ \mu\text{m}$ this represents ~90% of the flagellum. It should also be noted that the distal section $\ell - \Lambda < x < \ell$ in which Eq. A7 is not valid coincides with the inert terminal piece of 110 μm long. This may represent an example of mechanical tuning of the sperm flagellum to avoid being governed by different equations of motions at different locations.

The wavelength Λ in a cricket sperm has been found as $\lambda \simeq 20\ \mu\text{m}$ (Rikmenspoel, 1978), leading to $\alpha = 2\pi/\lambda \simeq 3 \times 10^3\ \text{cm}^{-1}$ and to $1/\alpha \simeq 3 \times 10^{-4}$ cm. The magnitude of the second term in the curved parentheses in Eq. A7 can therefore be neglected compared to $\Lambda(=10^{-2}\ \text{cm})$ and Eq. A7 can be reduced to

$$M_{\text{act}} = A\alpha^2 e^{i(\omega t + \alpha x)}\left[IE - \frac{k\omega\Lambda e^{i\alpha\Lambda}}{\alpha^3}\right]. \tag{A8}$$

The value of the stiffness IE of a cricket sperm flagellum is probably around $IE \simeq 10^{-12}$ dyn cm^2 (Rikmenspoel, 1978; Phillips, 1972). With $k = 2 \times 10^{-2}$ dyn cm^{-2} s, $\omega = 30$ s^{-1}, $\Lambda = 10^{-2}$ cm, and $\alpha = 3 \times 10^3$ cm^{-1} (Rikmenspoel, 1978) the magnitude of $k\omega\Lambda/\alpha^3$ is $\sim 2 \times 10^{-13}$ dyn cm^2. Within an accuracy of ~20% the second term in the square brackets of Eq. A8 can thus be neglected compared to the first one and Eq. A8 becomes $M_{act} = A\, IE\alpha^2 \exp i(\omega t + \alpha x)$. With Eqs. A2 and A5 we can thus write

$$IE \frac{\partial^2 U}{\partial x^2} + M_{act} = 0, \tag{A9}$$

which is identical to Eq. 27 of Rikmenspoel (1978).

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