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CONTRACTION KINETICS OF THE FAST MUSCLES USED IN SINGING BY A KATYDID

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INTRODUCTION

The fastest muscles known in terms of contraction frequency are found in insects. The myogenic (asynchronous) flight muscles of some small dipterans can contract at more than 1000 Hz during flight (Sotavalta, 1947, 1953). Neurogenic (synchronous) muscles can also reach impressive frequencies during flight, approaching or exceeding 100 Hz in several Lepidoptera. The highest contraction frequencies yet reported for muscles known to be or thought to be neurogenic occur during singing; reaching 250 Hz in a cicada (Young, 1972) and 280 Hz in a bush cricket (Walker, 1969). Katydids (Orthoptera, Tettigoniidae) produce songs by scraping specialized areas on the right and left forewings against one another. During singing the katydid, *Neoconocephalus robustus*, rubs its forewings together at frequencies up to 212 Hz (mean = 187 Hz; Josephson & Halverson, 1971). There is a muscle action potential associated with each contraction, indicating that the muscles are neurogenic. A song from an individual animal may continue with only brief interruptions for more than an hour (Heath & Josephson, 1970). The rapidity of contraction is achieved in part by operating the muscles at elevated temperature. Singing is preceded by a period of warm-up during which antagonistic muscles are contracted synchronously, producing heat but no obvious movement. Singing begins when the thoracic temperature reaches about 33 °C and during singing the thoracic temperature rises another degree or so (Heath & Josephson, 1970).

The singing muscles of *N. robustus* are clearly extraordinary in terms of their contraction frequency. A wing frequency of 200 Hz indicates that the fusion frequency of the wing muscles is greater than this and therefore that the rise time of the muscle contraction is less than 5 msec. More detail about the contraction kinetics of these muscles is not available from measurements made with intact animals. It might be pointed out that for maximum efficiency the tension generated by the wing opener and closer muscles should be confined to their half-cycles; otherwise, muscles must do additional work in stretching antagonists which are still actively generating tension. Thus from the singing frequency in intact animals one can state that the contraction rise time of the singing muscles must be less than 5 msec and suggest that for reasons of efficiency there has probably been selective pressure pushing the total twitch duration toward the half-cycle duration of 2·5 msec.

The work described in this paper extends the observations on *N. robustus* to a tropical species with a similar song, *Euconocephalus nasutus*, and provides direct

measurement of contraction parameters for the singing muscles. For convenience the presentation will be divided into two parts, the first dealing with thoracic temperature and sound-pulse frequency in intact animals, the second treating muscle contraction kinetics.

(1) SONG PULSE FREQUENCY AND THORACIC TEMPERATURE

Euconocephalus nasutus is native to Southeast Asia but recently it has been inadvertently introduced to Hawaii. It now flourishes on drier parts of the island of Oahu where my experiments were carried out. As with *N. robustus* only male animals sing, and singing occurs in the early evening, generally beginning shortly after sundown. Songs were recorded from animals in the field with a portable tape recorder. The recorded songs were later displayed on an oscilloscope and photographed. The song, like that of *N. robustus*, consists of a regular series of sound pulses (Fig 1). Each pulse is presumably produced on the closing stroke of the wings (cf. Josephson & Halverson, 1971). The mean pulse frequency for eight animals was 157 Hz (s.e., 2 Hz).

Thoracic temperatures were also measured in these animals from which sounds were recorded. The animals were caught by hand while singing. They were rapidly placed on a cork board, and a small thermistor mounted in a hypodermic needle (Heath & Adams, 1969) was inserted into the thoracic muscles. The thermistor output was displayed on a Y.S.I. telethermometer. During temperature measurements the animals were held only by being impaled on the thermistor probe. The thoracic temperature in each case was monitored for several minutes as the animals cooled following singing. The delay between the cessation of singing and the first recorded temperature ranged from 11–18 sec (mean, 14·5 sec). Since the body temperature changes rapidly immediately at the end of singing when the thermal gradient is greatest, this delay means that the first recorded temperature is certainly lower than that during singing.

To estimate the thoracic temperature during singing it was assumed that the animal produces no heat when it stops singing and that the rate at which it loses heat, and hence the rate at which its temperature changes, is proportional to the temperature gradient between the thorax and the environment. Specifically:

$$dT_B/dt = -K(T_B - T_A).$$

dT_B/dt = rate of change in body temperature (deg./sec),

T_B = body temperature (deg.),

T_A = ambient temperature (deg.),

K = rate constant (sec^{-1}).

From this it follows that:

$$\log(T_B - T_A) = -(K/2{\cdot}3)t + \log(T_B^\circ - T_A).$$

T_B° = body temperature when t = 0, i.e., at the cessation of singing.

Thus a plot of $\log(T_B - T_A)$ against t should give a straight line which could be extrapolated to the ordinal intercept $[\log(T_B^\circ - T_A)]$ from which T_B° can be determined. T_A was assumed to be the temperature measured in the vicinity of the animal after its cooling curve had been determined. An example of this plot is seen in Fig. 2. The

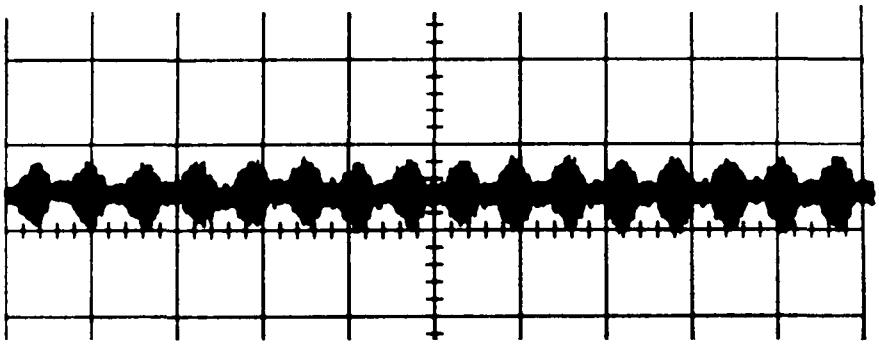

Fig. 1. The song of *E. nasutus*. The sweep speed is 10 msec per major grid division.

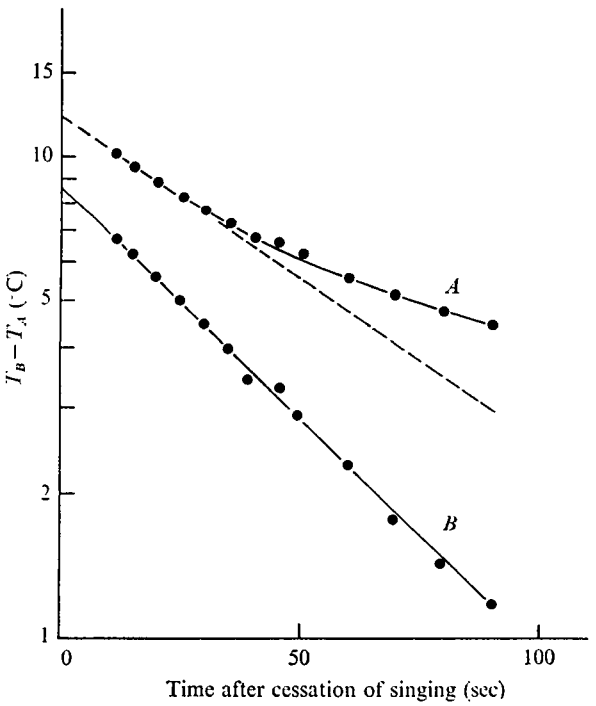

Fig. 2. Thoracic cooling at the cessation of singing. In *A* the data is plotted as the difference between the thoracic temperature and the measured environmental temperature. The dotted line is drawn through the first few points of this curve. The ordinal intercept of this line was used to estimate the thoracic temperature gradient during singing. In *B* the data is plotted as the difference between the thoracic temperature and a constant temperature chosen to give the best fit to a straight line. See text for details.

initial temperature ($T_B°$), determined by the ordinal intercepts of eye-fit lines through the measured points, averaged 36·0 °C (s.e., 0·7 °C). For comparison, the average of the first thoracic temperatures actually measured from each animal was 34·0 °C (s.e., 0·6 °C). The measured environmental temperatures averaged 24·4 °C (s.e., 0·4 °C), indicating that during singing the animal maintains a temperature gradient of about 12 °C between its thorax and its environment.

It is apparent in Fig. 2*A*, which is typical of all animals, that the data points do not lie on the anticipated straight line but rather form a curve which is concave upwards. The model is apparently inadequate. If the animal is cooling exponentially it is approaching not the environmental temperature but an asymptote somewhat above the measured environmental temperature. The reasons for this are not known. It may be that the insect produces sufficient heat by struggling while impaled upon the thermistor to hold its body temperature a few degrees above that of its environment. The effects of the value chosen for the temperature asymptote on the estimated body temperature during singing were investigated by assuming again that the cooling was exponential, but this time toward an unknown asymptote rather than the measured environmental temperature. A computer program was written which systematically varied T_A until the measured data points, when plotted as in Fig. 2, gave the closest fit to a straight line. An example of the fit produced with this procedure is shown in Fig. 2*B*. The apparent asymptote and the extrapolated ordinal intercept were used to calculate the body temperature at $t = 0$, that is, at the cessation of singing. The thoracic temperature during singing, determined in this way, averaged 36·2 °C (s.e., 0·8 °C). This thoracic temperature does not differ significantly from that obtained when it is assumed that the animal cools exponentially toward the measured environmental temperature. The time constants of the cooling curves are affected by the choice of the asymptotic temperature (compare the slopes of *A* and *B* in Fig. 2). This casts some doubt on the use of cooling curves obtained in the field to estimate metabolic rates during singing.

In summary, the wing frequency of *E. nasutus*, judged by the sound-pulse frequency, is about 160/sec and the thoracic temperature at which this performance is achieved is about 36 °C.

(2) CONTRACTION KINETICS OF THE SINGING MUSCLES

METHODS

Isolated muscle preparations

To evaluate performance of the forewing muscles during singing their contraction kinetics need to be determined at the thoracic temperature during singing, approximately 36 °C. Initially this was attempted with an isolated forewing muscle warmed by bathing it in heated saline. The muscle was the first tergocoxal of the mesothorax (see Fig. 3 in Josephson & Halverson, 1971). This is a wing elevator during flight, a wing closer during stridulation, and probably a coxal protractor during walking.

The animal was anaesthesized with CO_2 and the gut was removed by cutting it between the head and thorax and pulling it out through a slit in the abdomen. The animal was hemisected and the dorsal surface of one side was pinned to an inclined wax surface. The tergocoxal muscle was exposed, the mesothoracic leg was cut distal to the coxa, and the coxa was freed from the thorax except for the insertion of the tergocoxal muscle. A short thread connected to the coxa by a small hook was attached to the pin of an RCA 5734 transducer tube to record tension. The transducer was mounted on a manipulator so that the length and tension of the muscle could be altered. Tension recording was essentially isometric and the resonant frequency of the transducer without the coxal hook and connecting thread was greater than 2 kHz. The muscle was stimulated by a pair of stainless steel wires, 100 μm in diameter and

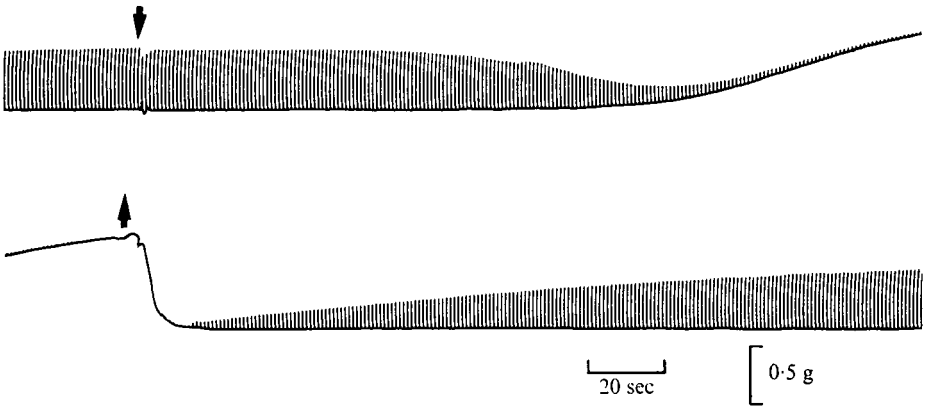

Fig. 3. The response of an isolated katydid muscle to immersion in locust saline. The muscle was stimulated once each second with supramaximal shocks. Before the downward arrow the muscle was in air, at the arrow it was immersed in saline. The twitches soon became smaller and the muscle went into contracture. The muscle relaxed and regained its responsiveness shortly after the saline was removed (upward arrow).

insulated except at the tip, inserted into the dorsal part of the muscle. The saline used was Usherwood's (1968) modification of Hoyle's locust saline except that I found it necessary to double the sodium bicarbonate concentration (from 4 mM to 8 mM) to raise the pH to 6·8.

This preparation was not satisfactory. The muscle would give reasonable twitches if the saline were totally withdrawn but when it was surrounded by saline the twitches gradually became smaller and the muscle went into contracture (Fig. 3). The contracture does not seem to be due to the ionic composition of the saline for it occurred with essentially the same time course in saline with no potassium or with twice the normal potassium, in saline with twice the normal calcium concentration, and in isosmotic sucrose. It seems likely that contracture is a result of anoxia, oxygen diffusion from the saline being inadequate to meet the metabolic requirements of the muscle. However, even perfusing air or oxygen through the mesothoracic spiracle did not prevent contracture in submerged muscles. Tracheal air perfusion has been successful in maintaining responsiveness in locust flight muscle (Weis-Fogh, 1956). The difficulty encountered with katydid muscles may reflect a high metabolic rate by the muscle tissue. In *N. robustus* 44% of the volume of the singing muscles is mitochondria (Elder, 1971). Although care was taken not to cut the trachea directly supplying the muscle, the tracheal system is disrupted when the animal is hemisected and the muscle is exposed, which may reduce the effectiveness of the trachea in delivering oxygen to the respiring tissue. Because of these problems a preparation was devised in which the tracheal system is essentially intact. The results to be considered have all come from this preparation.

In vivo *muscle preparation*

The animal was fastened, ventral side up, to a block of wax by pins and staples which encircled but did not penetrate the body. To avoid spontaneous muscle contraction the sternum was removed from the appropriate segment and the ganglion

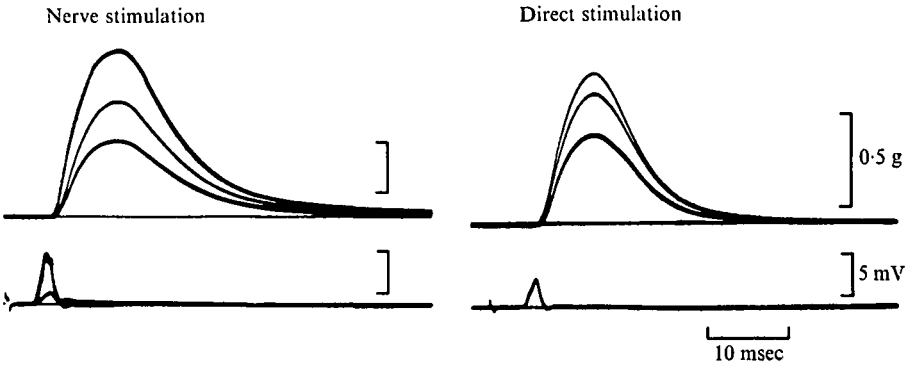

Fig. 4. Twitch responses of one singing muscle to nerve stimulation and of another singing muscle to direct stimulation, 25 °C. In each case the stimuli were a series of shocks of gradually increasing intensity. The upper traces are tension, the lower traces are the recorded muscle action potentials.

was exposed and removed after its nerves and tracheal supply were cut. The leg was transected distal to the coxa and the coxa was freed from all thoracic attachments except the insertion of the first tergocoxal muscle. The transducer was attached to the coxa in the same way as in the isolated muscle preparations. Except when muscle length was a deliberately varied parameter, twitch parameters were measured with the muscle stretched 10–20% above the slack length, that length at which tension first appears in an unstimulated muscle. The muscle was usually stimulated through a pair of stainless steel wires, 100 μm in diameter and insulated except at the tip. The wires were inserted into the origin of the muscle through holes in the dorsal exoskeleton and waxed in place. The stimuli were 0·1–0·5 msec voltage pulses. In some preparations a glass suction electrode was used to stimulate the nerve carrying motor neurones to the muscle. In these cases some of the ventral trachea were removed to expose the nerve. Even this seemingly minor damage affected the muscle performance; twitch rise times and relaxation times were slower than when the muscle was stimulated directly in less damaged preparations (Table 1). Even when using electrodes imbedded in the muscle, the muscle was almost certainly activated by the motor neurones coursing through it. In other insect muscles, using similar stimulating techniques, the nerve threshold has been found to be lower than that of the muscle (Roeder & Weiant, 1950; Weis-Fogh, 1956). In the katydid muscle this is seemingly also the case. If a series of single shocks of gradually increasing intensity is given to the motor nerve, muscle twitch tension occurs in three discrete steps (Fig. 4). This indicates that there are three fast axons, axons which evoke twitch responses, in the motor nerve. When the muscle is stimulated directly, tension again occurs in three distinct steps (Fig. 4). Were the muscle fibres activated directly by the stimuli, one would expect tension to be smoothly graded with stimulus intensity. The tension steps indicate that the muscle is activated by the same fast axons as those excited when the nerve is directly stimulated. In some instances, with repetitive stimulation, there was evidence for slow motor axons as well, axons which require a number of stimuli at moderately high frequency to initiate appreciable contraction. The threshold of the slow axons was found to be higher than that of the fast axons, both when stimulating

e nerve directly and when using electrodes inserted into the muscle; apart from this, slow axon responses were not examined.

Muscle action potentials were recorded with a pair of silver wires, 50 μm in diameter and insulated except at the tip, which were inserted into the muscle near the entry of the motor nerve at the coxal end. With this method of recording the onset of a potential signals the arrival of activity at the recording site but the time of the action potential peak is not useful since it depends on electrode separation and orientation. With direct stimulation the muscle was activated near its dorsal origin while the action potential was recorded near the ventral insertion of the muscle. There is therefore a delay between the initiation of an action potential and the time when it is recorded, the delay being due to conduction through the muscle. Since contraction presumably begins near the stimulating electrode, the delay between the recorded action potential and the onset of contraction is shorter than the actual electrical-to-mechanical latency by an amount equal to the conduction time through the muscle.

When a series of stimuli of varying intensity are given to the muscle, the recorded potential changes discontinuously, as does the tension, but usually there are potential increments corresponding to only one or two of the tension increments (Fig. 4). Apparently the three twitch motor units are not distributed uniformly through the muscle but are segregated so that a pair of closely spaced electrodes may record from only one or two of them. Similar discrete distributions of motor units have been reported for other insect muscles by Neville (1963), Elsner (1968), and Pearson & Iles (1971) among others.

The thoracic temperature in the intact animal preparations was altered by warming the whole animal with a heat lamp. The thoracic temperature was monitored with a thermistor bead, 200 μm in diameter, inserted into the musculature of the same segment but on the side opposite the tergocoxal muscles being studied. The heat lamp was moved closer to the animal as it warmed so as to keep the warming rate about 1 °C/min.

The cross-sectional area of the muscles was determined by dividing the weight of a muscle, measured after surface liquid had been lightly blotted off, by the length of the muscle measured *in situ* (e.g., Close, 1972). Unfortunately the importance of determining the cross-sectional area was not realized until most of the physiological measurements had been made. The area determinations were made with a different set of muscles. It is assumed that the mean cross-sectional area is the same as would have been obtained from the experimental group.

RESULTS

Singing muscles

At 25 °C the twitch rise time of the singing muscle is about 7 msec. and the twitch duration, measured from onset to 90% relaxation, is about 20 msec. This is rapid as compared with most muscles but it would be quite inadequate to account for the contraction frequencies reached during singing. At 25 °C the muscle produces a nearly smooth tetanus when stimulated at 150/sec, approximately the singing frequency (Fig. 5). When warmed to 35 °C the muscle becomes faster and the tension rises and falls appreciably following each stimulus at 150/sec. Thus thoracic warming

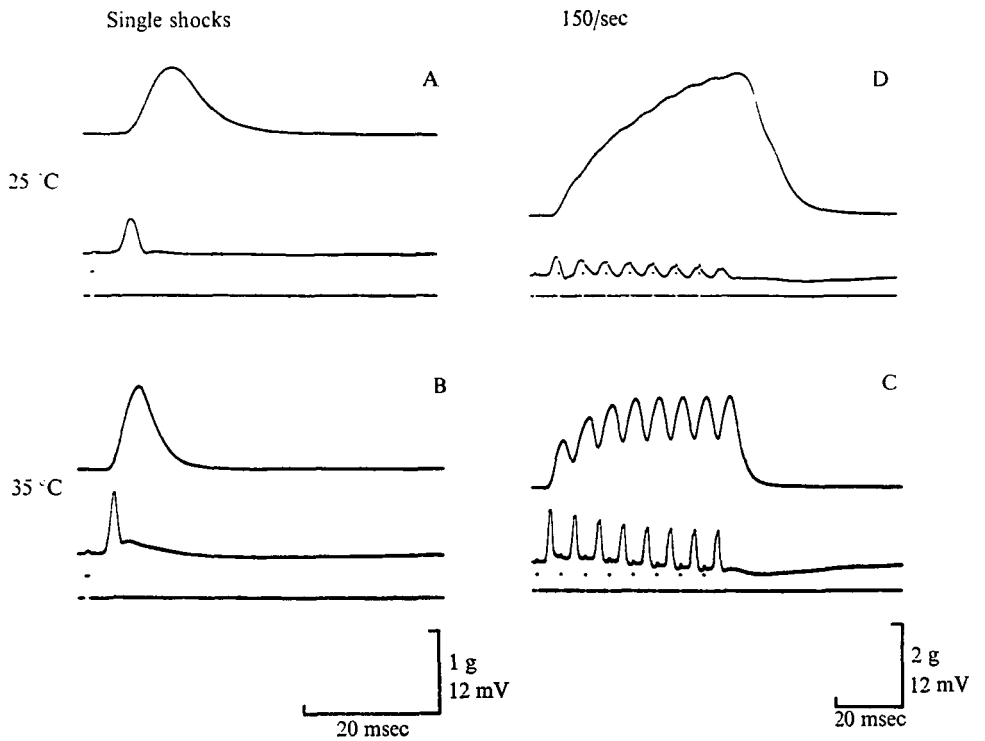

Fig. 5. Response of a singing muscle to single and repetitive stimuli at 25 °C and 35 °C. The upper traces are tension, the middle traces are recorded muscle action potentials, and the lower traces monitor stimuli. By the time of D the action potential had begun to decline, perhaps because of muscle movement and resulting damage around the recording electrode.

is not only a byproduct of the intense activity during singing, it is necessary for the performance achieved. In six preparations stimulated at 150/sec the tension minimum between peaks averaged 47% of that at the peaks (s.e., 2·4%); the muscle was able to relax about half-way between contractions. It is this tension fluctuation which presumably drives the wings during singing. Since the tension never drops to zero, the antagonistic muscle sets must do considerable work on one another during singing so the efficiency of singing is probably low. The effects of temperature on some twitch parameters are shown in Figs. 5, 6 and summarized in Tables 1, 2. All of the temporal characteristics of the twitch become shorter with increasing temperature. The Q_{10} values of the temporal parameters listed in Table 2 all lie between 1·5 and 2 for the temperature range 25–35 °C. The maximum twitch tension in the singing muscle was little affected by temperature change. This suggests that the decay rate of muscle activation and the shortening speed of the contractile component have similar temperature coefficients (cf. Hill, 1951).

The time from the onset of contraction to 50% relaxation for a single twitch at 35 °C is 7·7 msec. As mentioned above, at 35 °C a muscle is able to relax about half-way between tension peaks when stimulated at 150/sec. This corresponds to 6·7 msec between the onset of a new contraction and 50% relaxation. The shorter time to 50% relaxation with repetitive stimulation is a result of tension summation; at the higher

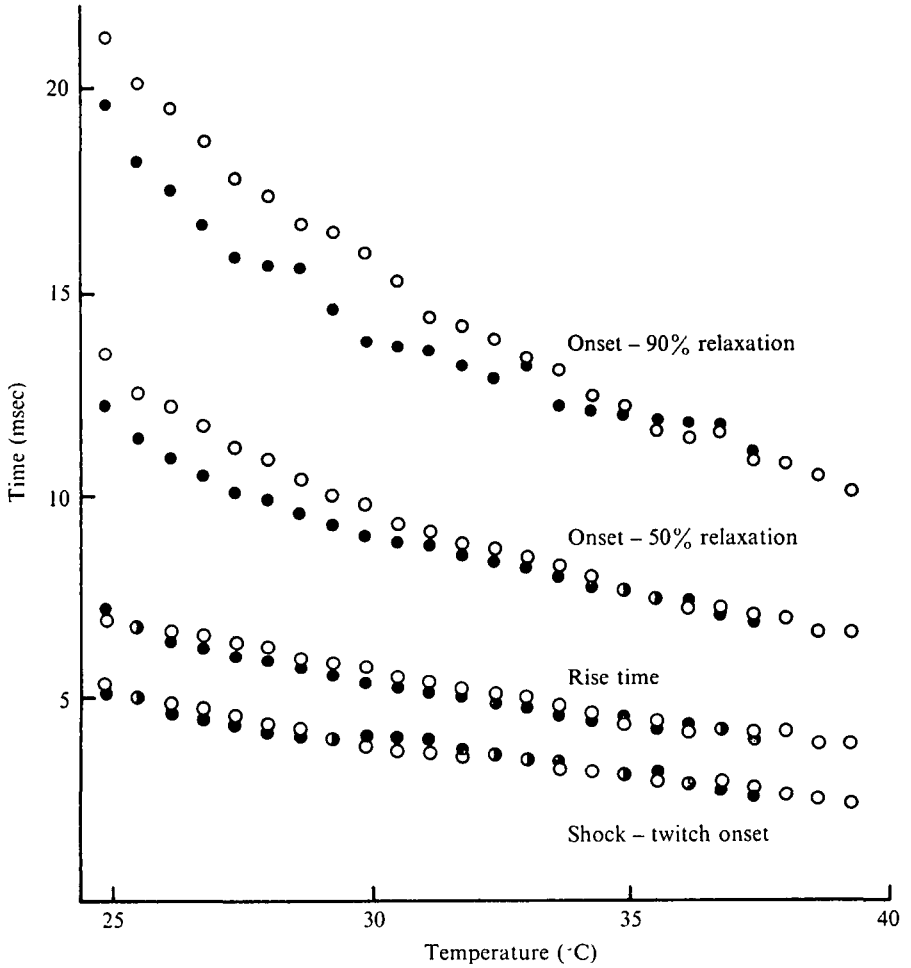

Fig. 6. The effect of temperature on some twitch parameters from a singing muscle. The closed circles are from an increasing temperature series and the open circles from a subsequent decreasing temperature series.

tension reached during repetitive stimulation, relaxation begins a bit sooner and is initially faster than in single twitches.

Homologous non-singing muscles

Although female katydids do not sing, they do contain the same set of mesothoracic muscles as do males. The female muscles are somewhat smaller than those of males (Table 2) and are white rather than the pink of male mesothoracic muscles. The difference in colour probably reflects greater amounts of mitochondria and their contained cytochromes in the male muscles. It was of interest to know whether female muscles are capable of the same performance as male muscles, even if this capability is never used.

Muscles homologous to those in the mesothorax occur in the metathorax of males and females. In males the metathoracic wings do not participate in singing but are used, together with the mesothoracic wings, in flying. The metathoracic wing muscles

Table 1. *Twitch contractions of singing muscle to direct stimulation and nerve stimulation, mean (s.e. in parentheses)*

	Direct stimulation, $n = 6$			Nerve stimulation, $n = 3$			*Statistically significant difference ($P < 0.05$)
	25°	30°	35°	25°	30°	35°	
Shock to action potential onset (msec)	3·27 (0·23)	—	—	2·73 (0·22)	—	—	—
	—	2·55 (0·19)	—	—	2·13 (0·07)	—	—
	—	—	2·18 (0·09)	—	—	1·73 (0·07)	*
Action potential onset to twitch onset (msec)	1·30 (0·15)	—	—	2·27 (0·09)	—	—	*
	—	0·98 (0·13)	—	—	1·87 (0·12)	—	*
	—	—	0·65 (0·13)	—	—	1·47 (0·12)	*
Twitch rise time (msec)	7·05 (0·24)	—	—	8·67 (2·01)	—	—	—
	—	5·30 (0·22)	—	—	7·70 (1·75)	—	—
	—	—	4·58 (0·28)	—	—	7·13 (1·18)	—
Twitch onset to 50% relaxation (msec)	12·15 (0·65)	—	—	15·70 (3·17)	—	—	—
	—	8·98 (0·46)	—	—	12·50 (2·18)	—	—
	—	—	7·73 (0·51)	—	—	11·87 (0·91)	*
Twitch onset to 90% relaxation (msec)	20·03 (2·04)	—	—	22·70 (4·38)	—	—	—
	—	14·48 (1·23)	—	—	20·03 (1·83)	—	*
	—	—	12·18 (0·88)	—	—	17·87 (1·29)	*
Twitch tensions (g)	0·81 (0·09)	—	—	1·25 (0·19)	—	—	—
	—	0·83 (0·10)	—	—	1·34 (0·22)	—	—
	—	—	0·88 (0·10)	—	—	1·49 (0·21)	*

Table 2. *Twitch characteristics of first tergocoxal muscles from different sources, mean (s.e. in parentheses)*

	Male mesothoracic			Female mesothoracic			Male metathoracic		
	25°	30°	35°	25°	30°	35°	25°	30°	35°
Action potential onset to twitch onset (msec)	1·30 (0·15)	—	—	2·22 (0·23)	—	—	2·23 (0·49)	—	—
	—	0·98 (0·13)	—	—	1·48 (0·22)	—	—	1·45 (0·42)	—
	—	—	0·65 (0·13)	—	—	1·23 (0·14)	—	—	1·28 (0·36)
Twitch rise time (msec)	7·05 (0·24)	—	—	15·0 (0·59)	—	—	17·4 (0·82)	—	—
	—	5·30 (0·22)	—	—	12·2 (0·39)	—	—	13·5 (0·84)	—
	—	—	4·58 (0·28)	—	—	9·9 (0·36)	—	—	11·5 (0·70)
Twitch onset to 50% relaxation (msec)	12·15 (0·65)	—	—	23·4 (1·08)	—	—	26·8 (0·69)	—	—
	—	9·03 (0·46)	—	—	18·6 (0·77)	—	—	20·7 (0·88)	—
	—	—	7·70 (0·51)	—	—	15·5 (0·50)	—	—	17·5 (0·70)
Twitch onset to 90%' relaxation (msec)	20·03 (2·04)	—	—	33·1 (1·72)	—	—	39·2 (0·76)	—	—
	—	14·48 (1·23)	—	—	26·0 (1·26)	—	—	30·1 (1·33)	—
	—	—	12·18 (0·88)	—	—	21·8 (0·95)	—	—	25·3 (1·00)
Twitch tension (g)	0·81 (0·09)	—	—	1·67 (0·22)	—	—	2·41 (0·30)	—	—
	—	0·83 (0·10)	—	—	1·90 (0·27)	—	—	2·60 (0·39)	—
	—	—	0·88 (0·10)	—	—	2·08 (0·30)	—	—	3·03 (0·43)
Tetanic tension (g)	3·01 (0·27)	—	—	4·19 (0·50)	—	—	6·23 (0·88)	—	—
Tetanic tension/twitch tension	3·92 (0·45)	—	—	2·79 (0·33)	—	—	2·79 (0·22)	—	—
Cross-sectional area (mm^2)*	1·10 (0·05)	—	—	0·92 (0·10)	—	—	1·22 (0·07)	—	—
Tension/cross-sectional area ($g\ cm^{-2}$)	273 (87)	—	—	455 (74)	—	—	511 (78)	—	—

* Determined from different sets of muscles.

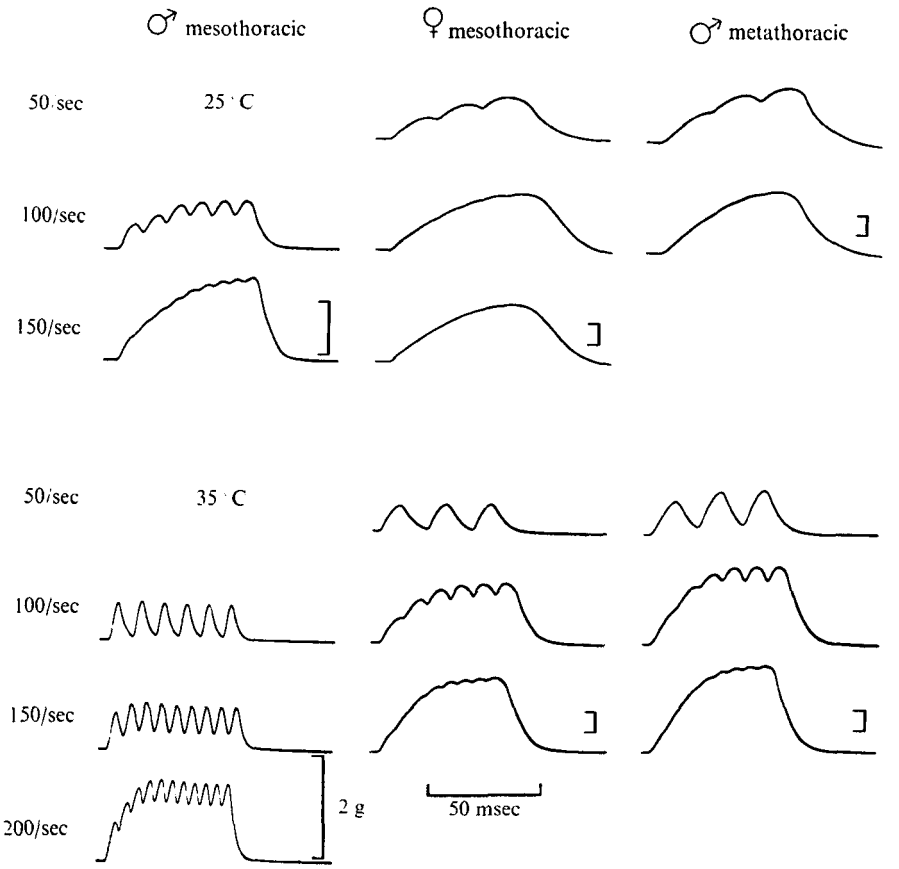

Fig. 7. Contractile responses of singing muscle and purely flight muscles to repetitive stimuli at 25 °C and 35 °C.

are white like female mesothoracic muscles. Wing frequencies during flight were determined in three male animals by attaching the animals to a phonograph cartridge (Roeder, 1951). The wing frequency in the three averaged 19·1 Hz (range, 17·5–20·3, 25 °C). Thus the frequency at which the metathoracic muscles operate during flight is nearly an order of magnitude smaller than that reached by their mesothoracic homologs during singing.

The performance of first tergocoxal muscles from the mesothorax of females and from the metathorax of males was determined in the same way as for mesothoracic muscles of males. These results are summarized in Table 2. The tetanic tensions were determined at 25 °C with a stimulus frequency of 150/sec for the male mesothoracic muscles and 100/sec for the others. The stimulus trains were sufficiently long to allow the tension to reach steady state. The values listed must be regarded as underestimates. At these frequencies there was some ripple in the tension, especially with the singing muscle (Fig. 7). Increasing the stimulus frequency sometimes resulted in somewhat greater mechanical summation and higher tension but often the muscles would not follow stimuli in a one-to-one fashion at higher frequencies. The stimulus frequencies used were the highest tried at which muscles would reliably follow each stimulus.

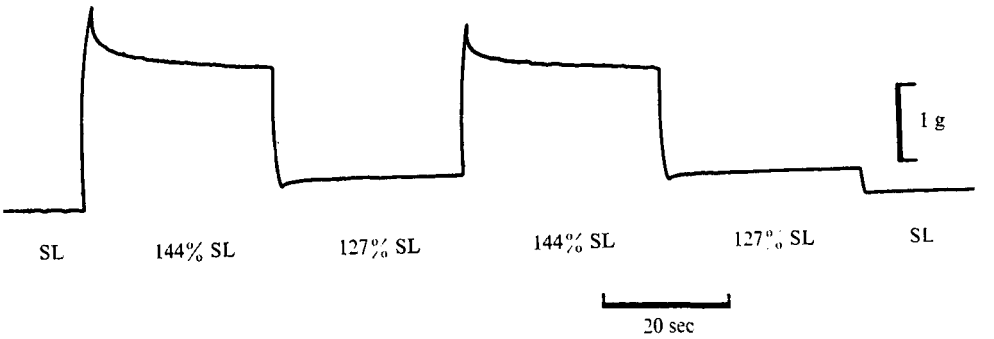

Fig. 8. Tension changes following stretch and release of an unstimulated singing muscle, 25 °C. The tension was just zero at slack length (SL).

The twitch time course is about twice as long in purely flight muscles (female muscle and the male metathoracic muscle) as in the singing muscle. On the other hand the tension generated, expressed as g tension per cm^2 area, is greater in the non-singing muscles. The twitch tension is also more temperature-dependent in the non-singing muscle, increasing appreciably with increased temperature. Not only is the tetanic tension lower for the singing muscle but also the twitch tension is a smaller fraction of the tetanic tension in the singing muscle than in the purely flight muscles. The latter is presumably a reflexion of a short active state so there is less time to stretch series elastic components during a twitch. This interpretation is strengthened by the comparison between singing muscles activated by direct stimulation and by nerve stimulation (Table 1). The time course of contraction is slower in the muscles exposed for nerve stimulation. This longer duration of contractile activity is associated with an increase in the twitch tension.

Muscle length and twitch parameters

An unstimulated wing muscle responds to length changes like a highly damped spring. Muscle tension rises during stretch and then falls with time if the new length is maintained (Fig. 8). The tension decline is at first rapid but later very slow. If a stretched muscle is allowed to partially shorten, the tension drops during the shortening but then rises again when the new length is reached and shortening stops. A classical passive tension-length diagram for the katydid muscle is somewhat arbitrary because the tension recorded at a given length varies with time and depends also on the pathway taken to this length; i.e., whether the muscle has been stretched to this length or allowed to shorten to it.

The effects of muscle length on passive tension and twitch tension were investigated by stretching the muscle through a series of length steps and then allowing it to shorten along the same path. The muscle was left at each length for 60 sec. The passive tension was taken as the tension 20 sec after each length change. Thirty seconds after each length change the muscle was stimulated with a single, supramaximal shock. The muscle length at which tension first appeared during stretch, defined as slack length (SL), was slightly shorter than the normal muscle length in an intact animal.

A single stimulus evokes little or no tension at muscle lengths shorter than the slack length. As the muscle is stretched above slack length, passive tension and the

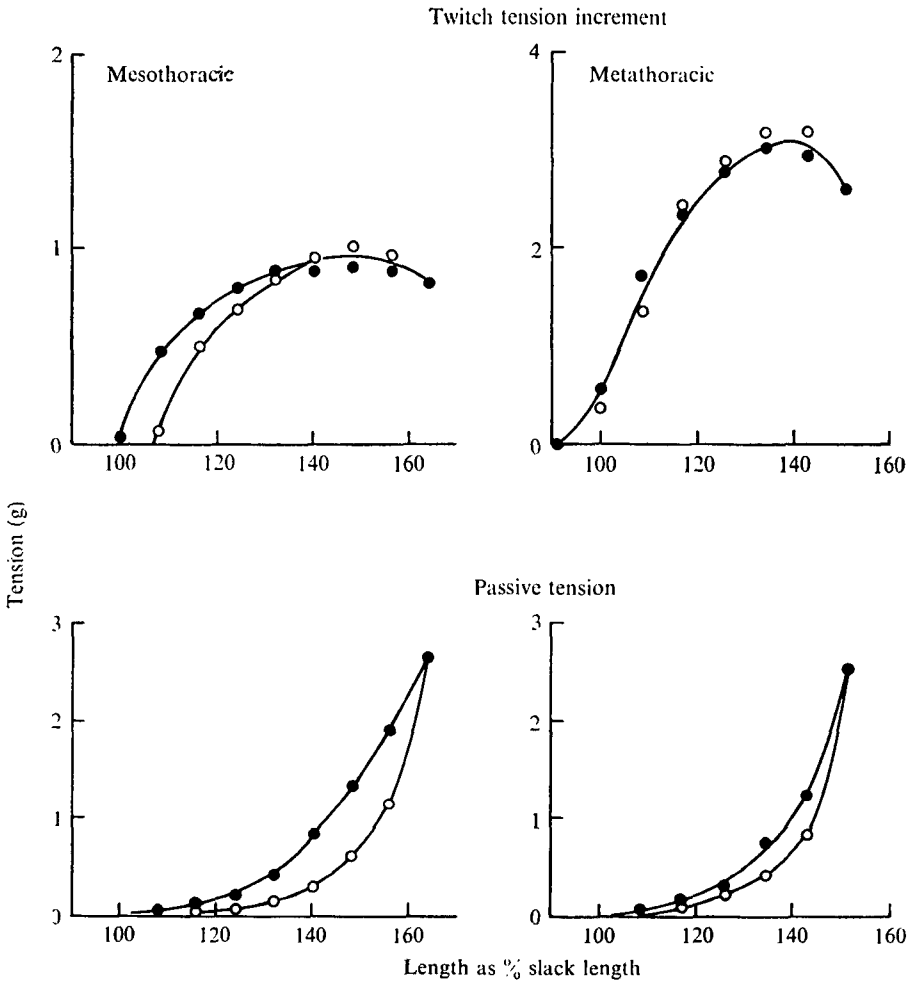

Fig. 9. Passive tension and the twitch tension increment as a function of muscle length, 25 °C. The closed circles are from an increasing length series, the open circles from a subsequent decreasing length series. The muscles were from male animals, the mesothoracic muscle a singing muscle and the metathoracic muscle a purely flight muscle. See text for experimental details.

tension increment during a twitch both at first increase (Fig. 9). The passive tension rises monotonically with muscle length; the twitch tension increment passes through a maximum and declines at longer lengths. The maximum twitch tension is reached only when the muscle is stretched far beyond lengths ever reached *in vivo*. At the length of maximum twitch tension (approximately 140% SL) about one-third of the muscle is pulled beyond its normal insertion and lies entirely outside the body.

The changes in length and tension during stretch also affect the time course of the twitch. The twitch rise time is nearly independent of muscle length but relaxation becomes appreciably slower as the muscle is stretched, both in singing and non-singing muscles (Figs. 10, 11). For this reason the time course of twitches (Tables 1, 2) were determined when the muscle was 10–20% longer than slack length, a length at which there is significant but not maximum twitch tension.

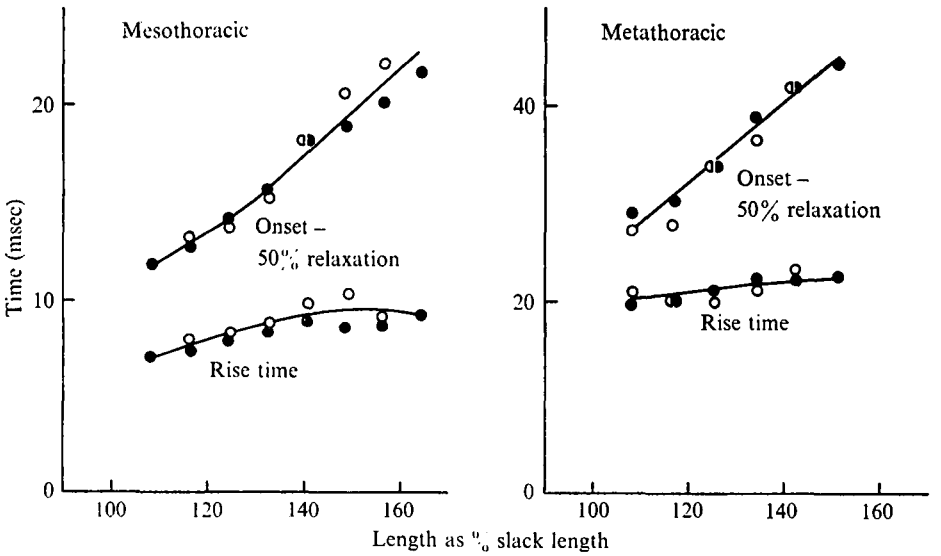

Fig. 10. The effect of muscle length on twitch rise time and duration. The points are from the same sets of twitches as Fig. 9.

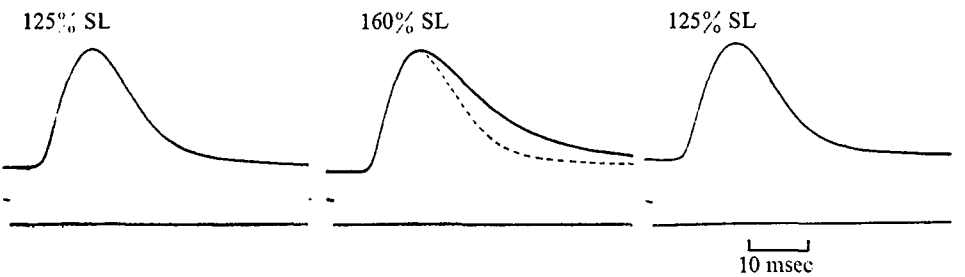

Fig. 11. Comparison of the twitch time course at two muscle lengths, singing muscle, 25 °C. The amplification was adjusted so that the height of the photographed twitch was the same in each case. The lower traces monitor the stimuli. The twitches were recorded in the sequence shown with the twitch at the longer length bracketed by the two at shorter length. The two twitches at 125% SL are superimposable. The dotted line in the middle trace shows the time course at 125% SL where this is different from that at 160% SL.

DISCUSSION

Muscle performance during singing

The above results suggest that the activity in the forewing muscles during singing is approximately that shown in Fig. 5C; the muscle operates in an unfused tetanus with the muscle relaxing about half-way between tension peaks. From this it follows that singing involves considerable internal work; muscles must shorten against antagonists which are themselves still generating tension. Some of this internal work may appear as heat and contribute to the elevated thoracic temperature during singing. These conclusions, however, should be accepted with some caution. The performance of the singing muscle seems to be easily impaired. The surgery necessary to isolate and stimulate a motor nerve apparently increases the twitch duration as compared to

that recorded from a more intact animal in which the muscle is stimulated by means of implanted electrodes. It is therefore possible that the relatively minor manipulations used to record tension from the muscles may have adversely affected their performance and that contraction and relaxation times in intact animals may actually be shorter than those reported here. There may also be changes in the time course of muscle activity when the muscle is *in situ* and shortens somewhat with each contraction. Glycerinated fibres from the neurogenic tymbal muscles of the cicada, *Fidicina*, give delayed tension changes following imposed stretch and release similar to those of myogenic flight muscles (Aidley & White, 1969). These delayed tension changes may accelerate relaxation and tension redevelopment in the muscle during normal activity. Frog muscle, too, gives oscillatory responses to appropriate loading (e.g., Sugi, 1972). It thus seems possible that in katydid muscles there may be phase shifts between changes in tension and length which shorten the twitch duration and thus decrease the amount of work done by antagonistic muscles upon one another.

Yet another factor which may have lengthened the twitch time course in these experiments is asynchrony between different parts of the muscle. When the muscle was directly stimulated it was excited near its dorsal origin. Activity in the excited motor neurone branches presumably travelled largely antidromically until it reached the primary axonal branches and then orthodromically to the ventral portions of the muscle. Because of conduction time within the muscle there would then be a lag between contractile activity in dorsal and ventral parts of the muscle, a lag which would tend to spread out the time course of a twitch. Given that there has been selection for rapid contraction, it would not be surprising if the normal innervation pattern was arranged to insure synchronous activation of all parts of the muscle. The extent of asynchrony can be estimated if it is assumed (1) that the electrical-to-mechanical latency at a given site is the same with direct stimulation and nerve stimulation, and (2) that the actual electrical-to-mechanical latency is that measured with nerve stimulation, when the recording electrodes were near the nerve's entry into the muscle and therefore presumably near the first portion of the muscle to be activated. At 35 °C the delay between action potential onset and twitch onset was 1·47 msec following nerve stimulation (Table 1). The measured latency with direct stimulation was 0·65 msec. This should be less than the actual latency by the conduction time from the dorsal to the ventral parts of the muscle. These numbers suggest that contraction began at the dorsal part of the muscle approximately 0·8 msec (1·47 − 0·65) before its onset in the ventral muscle. Since the muscle length is approximately 6 mm, this corresponds to an overall conduction velocity through the muscle of 7·5 m/sec which seems a bit fast, suggesting that the calculated delay may be an overestimate. This exercise indicates that asynchrony between dorsal and ventral parts of the muscle may be as much as 0·8 msec. This delay, while not numerically large, is an appreciable fraction of the twitch time course at 35 °C and may contribute to making the measured twitch duration somewhat slower than that which obtains during singing in intact animals.

Singing muscle and purely flight muscle

The wing frequency during flight in *E. nasutus* (17–20 Hz) is the same as that of the locust, *Schistocerca gregaria* (Neville & Weis-Fogh, 1963) and the contraction

time course of purely flight muscles from *E. nasutus* (the wing muscles of females and the metathoracic wing muscles of males) is very similar to that reported for the locust. In *S. gregaria* the rise time for a twitch at 36 °C is 14 msec (Neville & Weis-Fogh, 1963); in the katydid the twitch rise time at 35 °C is 10–11 msec. At 35 °C the twitch duration in *S. gregaria* is 33 msec (measured to 95% relaxation); in *E. nasutus* 22–25 msec (to 90% relaxation). In the singing muscles of *E. nasutus* the equivalent times are approximately half those of the purely flight muscles. The greater contraction speed of the singing muscle than that of the purely flight muscles is not achieved without some sacrifice; the tetanic tension per square centimetre from the singing muscle is only half that of the purely flight muscle.

The performance of the singing muscle is a rather clear example of the compromises frequently encountered in muscle design. The song of *E. nasutus* has an extraordinarily high pulse frequency and can last for tens of minutes. To produce this song requires rapidly contracting muscles with high endurance. In a neurogenic muscle the contractile activity of the myofibrils is controlled by the availability of calcium; calcium is released by the sarcoplasmic reticulum to initiate contraction and re-sequestered by the sarcoplasmic reticulum to terminate contraction (e.g., Ebashi & Endo, 1968). For rapid twitches the diffusion distances between the sarcoplasmic reticulum and the contractile proteins must be short and therefore there must be extensive sarcoplasmic reticulum in the muscle. Continued activity and the resulting high metabolic rate demands a good energy supply and therefore well developed mitochondria. But the greater the volume within a muscle given to sarcoplasmic reticulum and mitochondria the less space there is for contractile protein and the lower the tension which the muscle can develop.

Preliminary electron micrographs indicate that the structure of muscles in *E. nasutus* is very similar to that in the katydid *N. robustus* which produces a similar song (H. Y. Elder, unpublished observations). In *N. robustus* the sarcoplasmic reticulum and mitochondria are considerably more elaborate in singing muscles than in purely flight muscles (Elder, 1971; Josephson & Elder, 1968). In the singing muscles the mitochondrial volume and the volume of the sarcoplasmic reticulum are approximately 44% and 19% of the total fibre volume respectively. Thus only about one-third of the fibre volume is available for myofibrils. The investment in mitochrondria and sarcoplasmic reticulum precludes a major investment in contractile protein.

It is interesting that the investment in very fast muscles is such a selective one, restricted to sex and segment in homologous muscles. There is currently much interest in factors controlling intrinsic contractile properties of muscle, in particular trophic influences of nerves. The wing muscles of these katydids would seem to offer useful preparations for such studies; the differences between the ordinary flight muscles and the singing muscles are certainly obvious with either light microscopy (singing muscles are pink, purely flight muscles white), electron microscopy (Elder, 1971), or to physiological measurements such as those made here.

One wonders why *E. nasutus* and *N. robustus* have adopted such a metabolically expensive means of communicating between males and females. Would not a song with a lower pulse frequency have been adequate, a song requiring fewer muscle contractions per second? As an explanation it seems reasonable to suggest that in the evolution of some katydids females became preferentially responsive to high pulse

frequencies; given a choice a female would turn toward a male producing a high frequency and away from one with a lower frequency. The evolutionary outcome of the rule 'go to the source with the highest pulse rate' would be rapid selection for males able to produce high pulse frequencies; the eventual pulse rates reached being determined by physiological limitations and the availability of enough food to support such intense activity.

Electrical–mechanical coupling

Neville & Weis-Fogh (1963) report that in locust flight muscle the delay between the peak of the extracellularly recorded muscle action potential and the onset of contraction is about 1 msec and practically independent of temperature throughout the flight range. From this they infer that coupling processes between the action potential and contraction 'can hardly involve any time-consuming chemical reactions' (i.e., reactions with high temperature coefficients). For reasons explained above, with the recording methods used for katydids the time of the action potential peak is not a particularly useful parameter. The delay between the action potential onset and the onset of contraction is certainly temperature dependent in katydid muscle with a Q_{10} of approximately 2 for direct stimulation and 1·5 for nerve stimulation (Tables 1, 2). Because the stimulating and recording electrodes were widely separated, the electrical-to-mechanical latency following direct stimulation is affected by conduction time through the muscle (see above). Were there a constant delay between an action potential at a site and the onset of contraction at that site, the recorded electrical-to-mechanical latency for the whole muscle should actually increase as the muscle is warmed and the conduction velocity through the muscle is increased. This clearly does not happen. Further, with nerve stimulation, electrical activity was recorded near where the motor nerve entered the muscle and therefore at the part of the muscle presumably first excited. Here, too, the delay between action potential onset and contraction onset declined with increasing temperature. In katydid wing muscles the electrical to mechanical latency and hence the coupling processes are temperature dependent.

Muscle length and performance

In considering the relation between muscle length and tension it is appropriate to distinguish between passive tension, that tension resulting from stretch of an unstimulated muscle, and active tension, the tension increment due to stimulation. During isometric tetanic contraction the active tension of striated muscle varies with muscle length, typically being greatest at about the greatest length the muscle reaches in the body. At least in frog muscle the relation between muscle length and tetanic tension can be accounted for solely on the basis of the amount of overlap between the thick and thin filaments of the muscle (Gordon, Huxley & Julian, 1966). In frog muscle active twitch tension is greatest at muscle lengths often considerably longer than that which is optimum for tetanic contraction (e.g., Hartree & Hill, 1921; Close, 1972). In the katydid muscles the twitch tension is greatest at muscle lengths considerably longer than are ever reached in the body. Although the relation between tetanic tension and length was not determined for katydid muscle, the rather long length at which active twitch tension is maximum suggests that here, too, the optimum length for twitch tension is greater than that for tetanic tension. The difference

between optimum lengths for twitches and tetani has been taken to indicate that the tension developed in a twitch is a function not only of the overlap between thick and thin filaments but also of length-dependent changes in muscle activation (Close, 1972). The active state of frog muscle has been found to be shorter at shorter muscle lengths, and allowing a muscle to shorten during a twitch further accelerates the decay of the active state (Edman & Kiessling, 1971; Briden & Alpert, 1972). Changes in the activation process with length should be reflected in the time course of the twitch. Data from a number of sources indicate that in frog and toad muscle relaxation is slower the greater the muscle length (e.g., Hartree & Hill, 1921; Ritchie, 1954; Jewell & Wilkie, 1960; Close, 1972). This is also true for the katydid muscle. The data are less clear on the relation between muscle length and the contraction rise time. For frog and toad sartorius muscle the twitch rise time has been reported to be (1) relatively constant despite varied muscle lengths (Hartree & Hill, 1921), (2) increased with increasing muscle length (Jewell & Wilkie, 1960; Hill, 1970, p. 124), or (3) increased with increasing length in some length ranges and unaffected in others (Close, 1972). In katydid muscle the twitch rise time is only slightly increased with stretch even though relaxation is greatly delayed. Thus in frog muscle, and presumably also katydid muscle, the maximal twitch tension occurs at muscle lengths greater than those at which filament overlap is greatest, suggesting more intense or more prolonged activation at the longer lengths, yet this greater activation is not reliably reflected in the contraction time. But constancy in the twitch rise time does not mean constancy in the activation process. The tension rise ends when the tension in the muscle is just greater than that which can be generated by the contractile elements; this occurs at different tension levels and hence different activation levels at different lengths. Thus it may be only coincidental that the length-dependent intrinsic strength of the muscle associated with the amount of filament overlap and length-dependent changes in the intensity and time course of activation combine to terminate the rise of tension after a fixed time but at different tension levels (see also Close, 1972).

The effects of length on the performance of katydid muscle are rather different from those reported for locust flight muscle (Buchthal, Weis-Fogh & Rosenfalck, 1957). In the locust muscle the twitch tension increment was found to be maximal at about the longest length the muscle reaches *in situ*, and the time course of twitch contractions was independent of muscle length. The measurements on the locust muscle were made at a relatively low temperature (11 °C) so the differences may be temperature effects rather than species effects.

SUMMARY

1. The sound-pulse frequency, and by inference the wing-stroke frequency, in the song of the katydid, *Euconocephalus nasutus*, is 160 Hz. The thoracic temperature during singing is 36 °C, which represents a gradient between the insect's thorax and its environment of 12 °C.

2. Contraction kinetics were measured as a function of temperature for a singing muscle (first tergocoxal forewing muscle of males) and homologous, purely flight muscles (forewing muscles of females, hindwing muscles of males). Because the performance of the singing muscle was found to be easily degraded, the measurements were made from nearly intact animals.

3. At 35 °C the contraction rise time for an isometric twitch of a singing muscle is 7·7 msec. The twitch duration, measured from onset to 90% relaxation, is 12·2 msec. Purely flight muscles are about half as fast, but develop more tension per cross-sectional area, both in twitches and tetani. The temporal characteristics of twitch contractions, including the delay between the onset of the muscle action potential and the onset of contraction, have Q_{10} values between 1·5 and 2.

4. At 35 °C a singing muscle contracts in an unfused tetanus when stimulated at 150 Hz, relaxing about half-way between tension peaks. This result suggests that this is the way the muscle operates during singing. At 25 °C the muscle contracts in a smooth tetanus when stimulated at 150 Hz. A warm thorax is not only a consequence of the intense activity during singing but also necessary for the performance achieved.

5. Isometric twitch tension is maximum at muscle lengths more than one-third greater than the *in situ* length. Twitch contraction time is nearly independent of muscle length but relaxation time increases as the muscle is stretched.

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