THE EFFECT OF TEMPERATURE ON THE TWITCH DURATION OF THE DORSO-LONGITUDINAL FLIGHT MUSCLES OF *LOCUSTA MIGRATORIA MIGRATORIDES*

By G. Barnish and H. Ferris

Reprinted from:
Proceedings and Transactions
of the Rhodesian Scientific Association
Volume 51, 1966

THE EFFECT OF TEMPERATURE ON THE TWITCH DURATION OF THE DORSO-LONGITUDINAL FLIGHT MUSCLES OF *LOCUSTA MIGRATORIA MIGRATORIDES* (REICHE AND FAIRM.)

By G. BARNISH AND H. FERRIS

Department of Zoology, University College of Rhodesia, P. Bag 167 H, Salisbury, Rhodesia.

[Present address of G.B.: Research Laboratory, P.O. Box 8105, Causeway, Salisbury, Rhodesia.

Present address of H.F.: Tobacco Research Board of Rhodesia, P.O. Box 1909, Salisbury, Rhodesia.]

(Received: 30th August 1965)

INTRODUCTION

The aim of this project was to study the differences in twitch times of the dorso-longitudinal flight muscles of *Locusta migratoria migratorioides* (Reiche and Fairm.) with regard to changing temperatures and the consequent effect on the action of antagonistic muscles.

ANATOMY OF DORSO-LONGITUDINAL MUSCLE AND ITS INNERVATION

The dorso-longitudinal muscles run longitudinally along the dorsal surface of the three segments of the thorax. In these investigations only those muscles present in the metathorax were dealt with, as these were the largest and also the most convenient from the point of view of dissection and attachment. There is one pair of muscles in each segment, and they are attached to successive phragma. Each lies on either side of the midline. One muscle block consists of five syncytia, each syncytium innervated by one nerve, a branch of nerve 1B (Neville, 1963). The dorso-longitudinal muscles are the main depressor muscles of the wings, and their antagonists are the dorso-ventral or tergo-sternal muscles. These are nearer the sides of the thorax than the dorso-longitudinal muscles (see Figure 1a).

The muscles are innervated by nerve 1Ba, which arises from the ventral thoracic ganglia. One nerve goes to each muscle block, i.e., there is one pair of nerves in each segment. The nerve runs from a ventral ganglion, round the alimentary canal and dorsally up to the muscle, where it enters in the mid-line. The nerve can be seen, surrounded by a large layer of fat, lying on the ventral half of the muscle block, and also lying on the muscles which are lateral to the dorso-longitudinal muscles.

MATERIALS AND METHODS

A live locust was used and the abdomen cut off at the first abdominal segment. The wings and the legs were cut off as near to their position of attachment to the body as possible. The thorax was held and the head twisted off, the remaining portion of the alimentary canal was pulled through the thorax. The thorax was cut dorsally and ventrally slightly to one side of the midline, thus

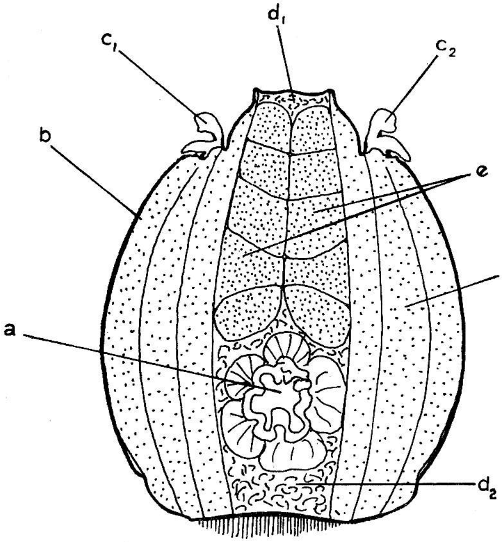

Figure 1a—Transverse section of metathorax of Locusta migratoria migratorioides (*R. & F.*).
a: gut lumen; b: cuticle; c_1 *and* c_2*: wing bases;* d_1 *and* d_2*: fat bodies; e: dorso-longitudinal flight muscles; f: dorso-ventral flight muscles.*

in a muscle bath to the bottom of which a rectangle of cork board had been fixed. The portion of thorax was pinned out at its corners and the nerve 1B could be seen running obliquely from the ventral surface to the dorso-longitudinal muscles, which were also clearly visible.

It must be pointed out that only one of the pair of muscles was used in the experiments, and this muscle was the one on the opposite side of the thorax to where the longitudinal dorsal and ventral cuts were made. A small amount of cleaning up was necessary, as most of the locusts had much yellow fat present around the alimentary canal; and also the phragma on to which the dorso-longitudinal muscle was attached had to be clearly visible.

At this stage small hooks were inserted through the dorso-longitudinal muscle of the adjacent segment (mesothorax), the hooks passing over the phragma of the muscle concerned. These hooks provided a firm anchorage of one end of the muscle attachment without actually damaging the muscle itself (see Figure 1b). Through the cuticle (marked in Figure 1b) another small hook was passed, and this was attached by a piece of cotton to an isometric lever. Thus one end of the muscle was firmly affixed, while the other end was left free. The muscle was put under slight tension before recording the twitch contraction.

The apparatus was set up as in Figure 2. For the micro-electrodes a small length of platinum wire (5 to 6 mm.) was soldered

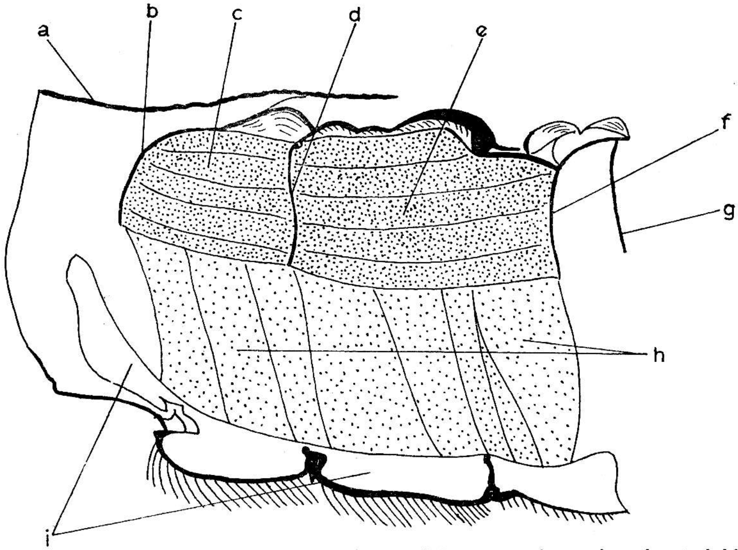

Figure 1b—Longitudinal section of thorax of Locusta migratoria migratorioides *(R. & F.) showing flight muscles.*

a: pronotum; b: mesothoracic phragma; c: mesothoracic dorso-longitudinal muscles; d: meso-metathoracic phragma; e: metathoracic dorso-longitudinal muscles; f: posterior metathoracic phragma; g: cuticle through which hook was passed; h: dorso-ventral flight muscles; i: fatty tissue.

electrodes were prepared. The active electrode (i.e., the one onto which the nerve was hooked) was then passed through a piece of fine glass tubing and fastened into the micro-manipulator. The other electrode was left free as this did not require precise positioning on the preparation. The copper wire was soldered onto two more robust conducting wires which led to the square wave stimulator. All the electrical joints were soldered to give optimal conduction.

A rectangle of cork (approx. 5 x 2.5 x 0.6 cm.) was affixed to the inside base of the Perspex muscle bath by the use of chloroform. Half-inch, thin, stainless steel, entomological pins were used to make the hooks. To make the hook which was attached to the isometric lever, the head of the pin was bent to form an eye, through which the cotton passed, and the opposite end bent into a hook. To make the hooks for anchoring the phragma, the heads of the pins were cut off and the blunt ends bent over.

For the micro-thermometer a thin-walled capillary tube about two and a half inches long was heated and a small bulb was blown at one end. The whole tube was then heated and the open end immersed in mercury which was drawn in as it cooled down. To calibrate the micro-thermometer it was placed in a muscle bath under a high powered binocular microscope, one eye-piece having a scale in it. Normal air temperature was measured on a standard Centigrade thermometer and then the number of divisions of the mercury meniscus from the end of the tube was noted. This number of divisions then represented the temperature shown by the Centigrade

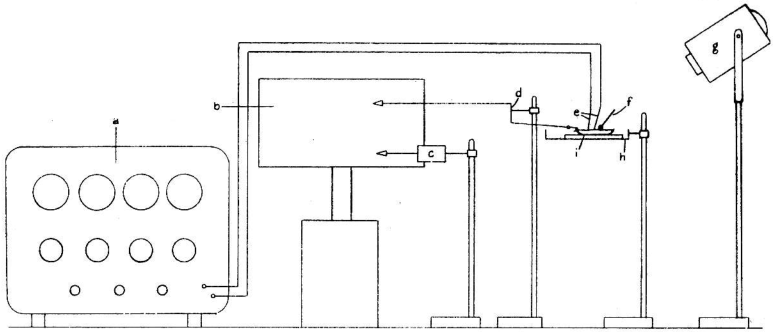

Figure 2—Diagram of apparatus.
a: Grass S4 square-wave stimulator; b: kymograph apparatus; c: 100 cycle-per-second time-trace marker; d: isometric lever; e: micro-electrodes; f: micro-thermometer; g: spot light; h: muscle bath; i: preparation.

into the muscle bath and the procedure repeated as for air, the number of divisions of the eye-piece scale representing the temperature of the water as shown by the Centigrade thermometer. Calibrations were made for temperatures between 8°C. and 38.5°C. and a calibration curve was plotted for rapid conversion of divisions to degrees Centigrade. This type of micro-thermometer was used as the bulb could be placed under the tissue being tested, giving an accurate reading of the tissue temperature.

When the preparation was firmly fixed on to the cork base of the muscle bath, and cleaned, recordings of twitch contractions were made. Nerve 1B was picked up on the electrode attached to the micro-manipulator at a point as far from the muscle as possible. The nerve was then lifted up above the layer of tissue to prevent any short circuiting and the indifferent electrode was hooked into another part of the tissue. The square-wave stimulator was adjusted to give repetitive stimuli and the voltage was increased from 0.15 volts up to the lowest voltage at which the muscle gave maximal twitch. This was usually somewhat over 0.25 volts.

When it was observed that the muscle was responding to the stimuli, the muscle bath was filled with locust Ringer solution, the temperature was taken and recordings of the twitch responses made. Between three and eight recordings were made at each temperature. The temperature in the muscle bath was altered by adding either cold (down to 10°C.) or hot (up to 35°C.) locust Ringer solution. When cold Ringer solution was added it was allowed to warm up naturally, readings and tests being made at each micro-thermometer division. Hot Ringer solution was allowed to cool to normal air temperature while readings were taken.

Several locusts were used to obtain the readings but it was very seldom that a complete range of readings was obtained from a single locust. The results were used to give a mean contraction time for all the locusts at each temperature.

It must be pointed out that the preparation was handled as little as possible during the whole procedure in order to prevent any until it was hooked on to the electrode. The preparation, from the living locust to the hooking up of the nerve and the testing, took an average time of about ten minutes. Speed was found to be an essential factor in this experiment, as any undue delay or rough handling caused the muscle to cease functioning.

RESULTS

When sufficient recordings had been made the kymograph records for each locust at each temperature were examined, and the height of each contraction was measured in millimetres. The time taken for the complete twitch was calculated in hundredths of a second, using the 100 cycle per second time trace, and later converted into milliseconds. By means of the calibration curve the micro-thermometer reading in each case was converted into degrees Centigrade. Between three and eight twitches had been recorded for each muscle at each temperature and the average twitch time and contraction height was calculated for each locust at each particular temperature. Table 1 shows the averages of these readings for each temperature.

The object of measuring the contraction height was to see if there was any correlation between this and the twitch time. If there was a close correlation all the readings would have to be worked out in relation to some standard height of contraction before any graph of twitch time against temperature could be plotted. If however, there was little or no correlation, then the twitch times for the dorso-longitudinal muscles of all the locusts at any particular temperature could be averaged and plotted against the temperature. To ascertain which was the case Table 2 was drawn up in which the twitch time and contraction height averages of the different dorso-longitudinal muscles recorded at a particular temperature were calculated as a percentage of the contraction height and twitch time

TABLE 1

Average of 328 readings of twitch times and contraction heights with nine dorso-longitudinal flight muscles of *Locusta migratoria migratorioides* (R. & F.) at different temperatures.

°C.	Muscle Number	Average Twitch Time in Milliseconds	Average Contraction Height in Millimetres
10.2	6	106	0.74
10.6	6	115	1.15
11.2	6	116	0.81
11.8	7	76	1.05
12.4	7	68	1.05
13.2	3	59	1.25
13.2	8	109	2.95
14.0	8	108	2.55
14.8	3	58	1.47
14.8	4	50	0.68
14.8	8	103	1.98
15.4	4	58	1.10
15.4	7	59	1.78
15.4	8	78	3.04
16.4	3	57	1.40
16.4	4	50	0.91
16.4	8	67	1.10
17.0	1	52	1.23
17.0	3	55	0.72
17.0	6	44	0.80
18.0	8	72	3.11

TABLE 1—Continued

°C.	Muscle Number	Average Twitch Time in Milliseconds	Average Contraction Height in Millimetres
18.8	7	51	1.54
18.8	8	60	1.42
19.6	8	62	2.92
20.4	1	51	2.75
20.4	3	35	0.45
20.4	5	34	1.20
20.4	7	48	1.68
20.4	9	51	2.55
21.4	1	50	2.78
21.4	2	51	1.30
21.4	8	57	2.63
21.4	9	57	3.60
23.0	3	41	0.85
23.0	7	45	1.58
23.0	9	55	2.83
24.0	1	49	2.80
24.0	2	55	1.50
24.0	5	46	1.68
24.0	7	37	1.80
25.0	2	47	1.82
25.0	3	40	1.05
25.0	5	47	1.48
25.0	9	48	1.68
26.0	5	34	0.88
26.0	6	39	1.88
26.0	9	53	3.02
26.4	2	46	1.48
26.4	6	42	1.38
26.4	9	50	4.80
27.0	2	48	2.42
27.0	5	39	0.65
27.0	9	50	4.55
27.6	6	49	1.50
27.6	9	47	2.85
28.2	2	39	2.12
28.2	6	39	1.28
28.2	9	46	2.48
29.0	9	49	2.98
29.4	2	41	2.05
29.4	6	40	1.40
29.4	9	47	2.68
30.0	6	38	1.08
30.0	9	47	2.60
30.6	2	42	2.12
30.6	5	40	1.72
30.6	6	38	0.70
30.6	9	46	2.22
31.4	9	46	1.98
32.0	9	40	2.25
32.6	5	40	1.85
32.6	9	43	2.00
33.6	5	43	1.94
33.6	9	40	1.82
34.4	5	39	0.90

of the muscle with the largest average contraction height at that temperature. This was done for several different temperatures over the range examined.

From Table 2 it can be seen that the twitch time is only slightly dependent on the contraction height if the two are at all

TABLE 2

Average contraction heights and twitch times at 5 temperatures as a percentage of the averages for the muscle with the largest contraction height at that temperature.

°C.	Muscle Number	% Contraction Height	% Twitch Time
14.8	8	100.0	100.0
	4	34.3	48.5
	3	74.2	56.3
18.8	1	100.0	100.0
	7	64.7	94.4
	8	59.7	111.1
21.4	9	100.0	100.0
	1	77.2	87.7
	2	36.1	89.5
	8	73.1	100.0
27.0	9	100.0	100.0
	2	53.2	96.0
	5	14.3	78.0
28.2	9	100.0	100.0
	2	85.5	84.7
	6	51.6	84.7

is correlated with a decrease in twitch time of 52%, while a drop of only 26% in contraction height is correlated with a decrease in twitch time of 44%; at 18.8°C. two decreases in contraction height in the region of 40% are correlated with a decrease in twitch time of 6% and an increase of 11%. At 21.4°C. decreases in contraction height of 23% and 64% are both correlated with a similar decrease in twitch time. From these results and many others which can be taken from the averages in Table 1 it is concluded that there is no close relationship between contraction height and twitch time, and any relationship occurring, along with many other factors, accounts for the spread of the points in Figure 3.

Accepting this conclusion it was possible to sum all the twitch times from the muscles used at a particular temperature, find their average and plot this against the temperature. These average twitch times over the temperature range studied are shown in Table 1.

From these results the graph (Figure 3) of the twitch time in milliseconds against temperature in degrees Centigrade was drawn. It can be seen that an increase in temperature from 10 to 20°C. causes a substantial decline in twitch duration, which drops from about 120 to nearly 53 milliseconds. Above 20°C. the decrease in twitch duration with temperature increase is much less marked, the curve flattening out at about 37°C.

Comparison of this curve with that obtained with the desert locust, *Schistocerca gregaria,* Forskal, by Neville and Weis-Fogh (1963) shows a close agreement of the twitch durations at 25°C. (about 46 milliseconds in *Locusta* compared with 48 milliseconds in *Schistocerca.*) However at higher temperatures the twitch durations obtained with *Locusta* are much longer than in *Schistocerca* (at 35°C. Neville and Weis-Fogh report a duration of 33 milliseconds whereas the duration in these studies was found to be about 40

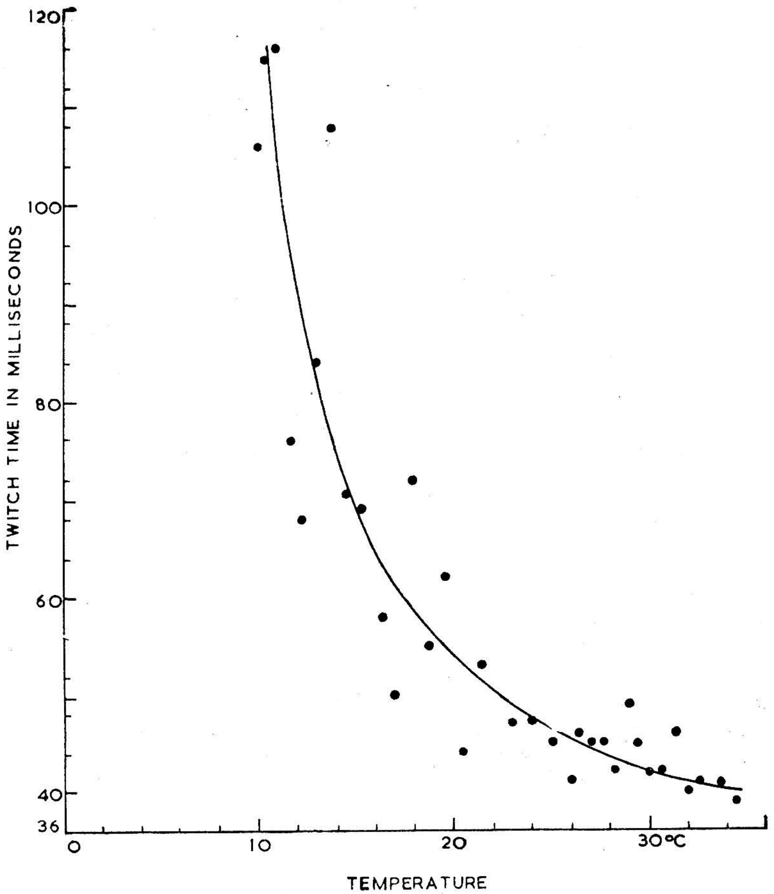

Figure 3—Relation between twitch time and temperature of the dorso-longitudinal flight muscles of Locusta migratoria migratorioides *(Reiche and Fairm.).*

DISCUSSION

The internal thoracic temperature of a locust in flight is 35° C. and the downstroke lasts for 60% of the entire wingstroke cycle (Weis-Fogh, 1956). The dorso-longitudinal muscles are responsible for the wing downbeat as they cause the buckling up of the tergum, thus forcing the wings downwards. Whether or not the insect can fly depends on how much the antagonistic tergo-sternal (dorso-ventral) muscles have relaxed before the dorso-longitudinal muscles contract and vice-versa, i.e. the degree to which the two sets of muscles are working against each other. Thus at 35°C. one would expect optimal conditions for flight and each set of muscles to have relaxed completely before the antagonists contracted so that they would not be working against each other.

From Figure 3 it can be seen that at 35°C. the twitch of the hence using the above assumption the twitch of the dorso-longitudinal muscles will last for about 27 milliseconds, and the complete wing-beat cycle for 67 milliseconds. This gives a wingbeat frequency of 15 per second. The normal wingbeat frequency of the desert locust, *Schistocerca gregaria* as found by Neville and Weis-Fogh (1963) is 17 per second in a normal female — giving a very favourable comparison. At 20°C. the downstroke last for 54 milli-seconds and consequently the upstroke for 30 milliseconds. Thus at 20°C. with a wingbeat frequency of 15 per second the antagonistic flight muscles are working against each other for 23 milliseconds of each beat, greatly increasing the difficulty of flight due to the added work involved.

At 18°C. the length of time that the muscles are working against each other is even greater (about 30 milliseconds) if flight is to take place, and hence at this temperature flight is presumably impossible, as at a wingbeat frequency of 15 per second the dorso-ventral twitch should only last for 27 milliseconds. Thus as the temperature is decreased flight becomes more and more difficult as the antagonists pull against each other for a greater length of time, until a temperature is reached when the relaxation of one muscle is still at a stage where the antagonist cannot pull against it, and hence flight is impossible. At very high and very low temperatures the muscles are probably physiologically unstable and this will impede flight if the antagonistic overlapping has not prevented it completely at this stage.

These conclusions are based on the assumption that the wingbeat frequency of the locust remains the same (15 per second) at different temperatures and that this is the optimal frequency allowing flight of the locusts. Actually there is a need for more work to be done on the interdependency of wingbeat frequency and also minimal frequencies which will lift the insects off the ground.

SUMMARY

1. Twitch times were found to be independent or only slightly dependent on contraction height.

2. Twitch times for different muscles at the same temperature were meaned and a graph plotted.

3. Results obtained compared very favourably with those of other workers for the desert locust.

4. Discussion is presented on the work involved during flight of the locust at different muscle temperatures.

5. There is need for further work on wingbeat frequencies of locusts in relation to internal and atmospheric temperatures.

ACKNOWLEDGMENTS

Dr. E. Bursell for his invaluable assistance, checking and criticism; Dr. P. A. Langley and Mr. K. Nott for technical assistance,

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