J. exp. Biol. **159**, 419–431 (1991)
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NEURAL CONTROL OF FIBRILLAR MUSCLES IN BEES DURING SHIVERING AND FLIGHT

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Accepted 28 May 1991

Summary

The big indirect flight muscles in the thorax of honeybees and bumblebees show two modes of action: they contract with 'conventional' twitches in response to slowly repeated muscle potentials and go into tetanus at higher muscle potential frequencies. They can also contract much faster when quickly stretched (stretch-activation).

We observed contractions of DV (dorsoventral) and DL (dorsal longitudinal) muscles optically with the help of a tiny mirror glued to the scutellum. We noticed that DL muscles contracted much more than DV muscles during pre-flight warm-up. During warm-up, muscle potential frequencies in DL muscles were higher than in DV muscles (DL frequency/DV frequency=1.3), whereas during flight the ratio reversed (DL/DV=0.8). The scutal fissure was completely closed during shivering warm-up, apparently because the DL muscles shortened as much as they could. As a consequence, fast antagonistic stretching was not possible. However, the scutal fissure oscillated between wide open and closed during flight, and antagonists could stretch each other quickly. Flight was started by highly synchronized 'conventional' contractions of many muscle elements in DV muscles. Antagonistic stretch-activation during flight led to faster shortening than during shivering warm-up and synchronized all activated muscle elements to produce maximal contractions.

The indirect flight muscles of bumblebees were in tetanic contractions during shivering warm-up over the whole range of temperatures between 8 and 36°C. These tetanic contractions probably prevented other researchers from observing mechanical muscle activity. Our results, which for the first time allow us to detect tetanic contractions directly, make it very improbable that non-shivering thermogenesis occurs in bumblebees, as has been proposed previously.

Introduction

Bees use two pairs of big fibrillar muscles, the dorsal longitudinal (DL) and the dorsoventral (DV) indirect flight muscles in various behaviours. The most important behaviours include warming of individuals in preparation for flight (Esch, 1960; Heinrich, 1980), flight itself (Esch *et al.* 1975; Esch 1976), communal

Key words: flight muscle, neural control, bees, shivering, flight, *Apis mellifera ligustica*, *Bombus impatiens*.

hive heating (Himmer, 1932; Esch, 1960; Heinrich, 1987) and fanning during regulation of hive temperatures (Lindauer, 1954). Honeybees also buzz their wings while communicating in waggle dances (Esch, 1961; Wenner, 1962). Different contraction mechanisms must be used for different behaviours since thorax vibrations might not be noticeable (heating) (Esch, 1964; Surholt *et al.* 1990), wings might oscillate in short bursts of small vibrations (dance) or wings might continuously beat up and down with considerable amplitudes (fanning and flight).

It is difficult to understand how motor neurones can control the fibrillar muscles in such diverse behaviours, since these muscles are asynchronous. Earlier researchers could not find one-to-one relationships between muscle potentials and contractions (Roeder, 1951; Pringle, 1957). It was assumed that asynchronous muscle potentials turn on a 'stretch-activation mechanism', and that quick stretches by antagonists during oscillations initiate contractions of muscles at the molecular level (reviewed in Hoyle, 1983). How oscillations start in the very beginning, however, and why one does not see muscle movement during warm-up remained an unsolved problem. This led to unjustified speculations, for instance as to how flight starts (Nachtigall, 1985) or how heat might be generated through non-shivering thermogenesis (Newsholme *et al.* 1972).

'Asynchronous' muscles can also perform 'conventional' twitches in which one muscle potential causes one synchronous contraction, as we show in the following observations. A series of muscle potentials leads to tetanic contractions. Wing or thorax movements might be barely noticeable during these contractions (Bastian and Esch, 1970; Surholt *et al.* 1990). These twitches can be used to produce meaningful behavioural responses, such as heating, 'buzzing' or initiation of flight.

Materials and methods

Honeybees (*Apis mellifera ligustica* Spinola) were taken from the apiary in the Department of Biological Sciences at the University of Notre Dame. Bumblebee (*Bombus impatiens* Cresson) queens and workers were caught on flowers in front of the Biology building. Individuals were held in a refrigerator at near 5 °C prior to an experiment until they cooled and became motionless. They were quickly cemented to a small wooden rod by the notum using tacky wax. A small, very light plastic mirror was glued to the middle of the scutellum. A horizontal bar of light was focused on the center of this mirror from behind and above. This bar was reflected onto the surface of a triangular photovoltaic cell. DV or DL contractions moved scutellum and mirror about a horizontal elastic hinge across the notum (Pringle, 1957). These reflected the projected light bar up (DV) or down (DL) the triangular photocell. More or less surface area was illuminated, and photocell output voltage represented angular movement of the scutellum and thus muscle contractions (Fig. 1).

Up to six wire electrodes (diameter 50 μm, insulated except at the tip) were inserted into dorsal longitudinal and dorsoventral flight muscles to observe as many muscle units as possible. Muscle units close to an electrode were represented

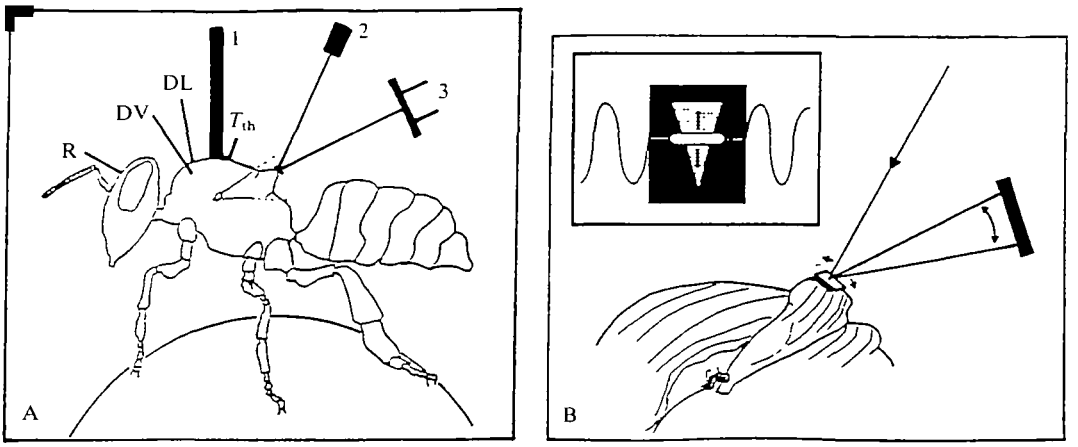

Fig. 1. Experimental set-up and preparation. (A) The honeybee is tethered to a rod (1) with a reference electrode (R) in the head, wire electrodes in the dorsoventral (DV) and dorsal longitudinal (DL) flight muscles and a thermocouple to determine muscle temperature (T_{th}). The light beam from the lamp (2) is reflected from a mirror attached to the scutellum onto a photocell (3). (B) Enlarged view of the angular mirror movement at the scutellum. Inset, the light bar moves up and down over the triangular photocell producing an output voltage proportional to position.

by muscle potentials with large amplitudes, neighbouring units were represented by much smaller muscle potentials. A reference electrode was pushed through a small hole in the middle of the front of the upper head. Electrodes were differentially connected to UFI model 2122 bio-amplifiers (input impedance>10 MΩ). Outputs of these amplifiers were connected to an IBM DACA input board in a Tandy 1000A computer. The board was used with UNKELSCOPE level 2+ data acquisition software. Samples were taken with sampling rates of either 0.5 or 1 ms for 2–4 s and stored on floppy disks. The original analog signals were also recorded on an instrumental tape recorder (Vetter model B, in FM mode) and could be replayed for additional analysis. Data were evaluated with UNKELSCOPE editing features or transferred to LOTUS123 worksheets for further processing.

A copper–constantan thermocouple (diameter 50 μm), waxed to the middle of the frontal dorsal thorax, determined muscle temperature (Esch, 1960). A second thermocouple 1 cm above the animal recorded environmental temperature. A BAT-12 digital thermometer (Sensortek) was used to read thoracic temperature. A Baily BAT-4 thermometer was used to determine air temperature. In the measurements in which temperature differences between the two muscle pairs were determined, the Baily thermometer was used in differential mode.

Results

Warm-up and flight

Shortening of DV and DL muscle units caused appropriate movements of the

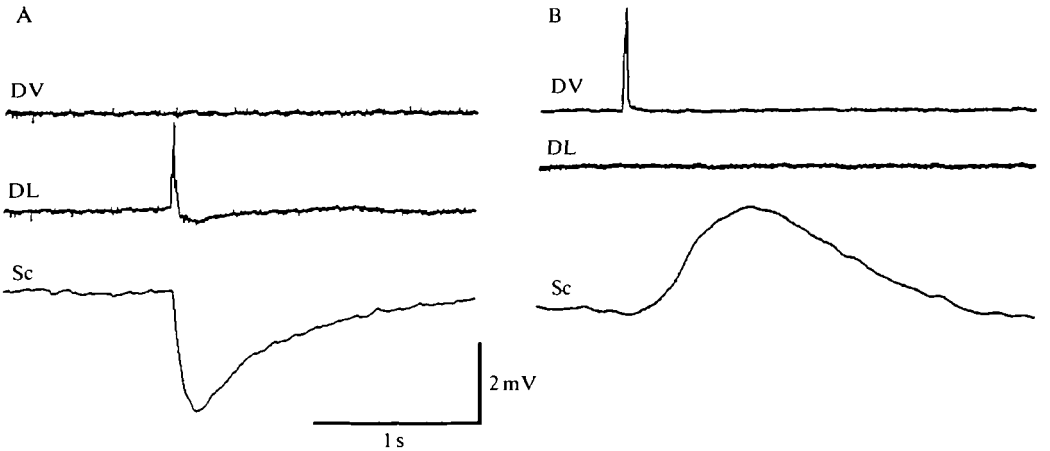

Fig. 2. Recordings from a bumblebee showing single muscle potentials without antagonistic action and the corresponding twitch (*in situ* muscle shortening) (Sc). (A) Shortening of the dorsal longitudinal muscle at T_{th}=13°C (DL); (B) shortening of the dorsoventral muscle at 13°C (DV).

mirror attached to the scutellum. The mirror was considered to be in 'zero position' when DL and DV muscles were inactive (no muscle potentials). The light beam moved upwards from zero (dorsal side) when muscle potentials occurred only in DV muscles. Muscle potentials restricted to DL muscles deflected the beam downwards (ventral side) (Fig. 2). Shortening restricted to DL muscles alone occurred much more frequently than shortening of DV muscles alone.

Trains of muscle potentials with different amplitudes could often be seen using one electrode. Potentials with different amplitudes must represent separate muscle units because recruitment of potentials with discrete amplitudes in the middle of existing recordings always led to additional shortening. Most recordings reflect more or less synchronized shortening of more than one muscle unit. If we increased the number of electrodes in one muscle from one to three we were able to record 65 % more muscle potentials. Muscle potential frequencies of all detected elements were very similar in a particular recording, but units in different parts of the muscle were not necessarily synchronized. When using only one electrode, we occasionally observed mirror movements, but no corresponding potentials.

Shortening times in non-flight recordings were much longer than those during flight (Fig. 3). Summation became very marked at muscle potential frequencies above a few hertz. Shortening time, and thus extent of summation, was also very sensitive to changes in muscle temperature (Fig. 3). In a few cases where only one muscle group was active, the time from maximal shortening to return to rest length could also be seen, and it was significantly longer than shortening time (Fig. 2).

DL and DV muscles were often excited by dissimilar muscle potential frequencies. Since these muscle groups contracted against each other, the balance of contractions shifted to the side of the muscle pair (DL or DV) that had a higher

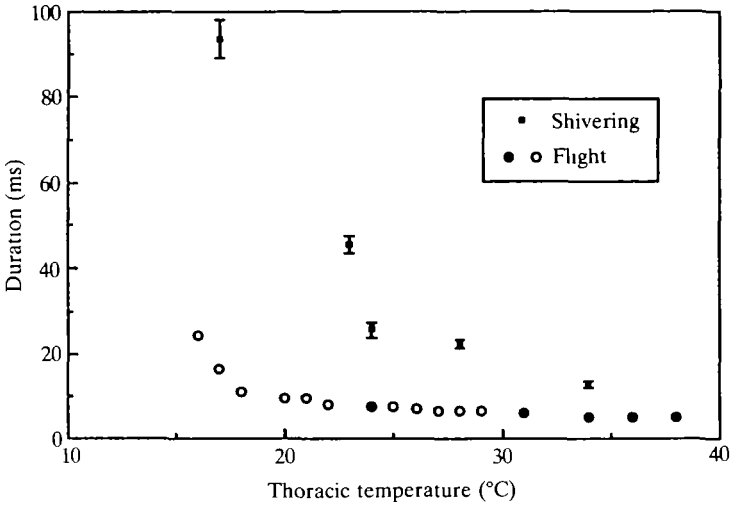

Fig. 3. Duration of wingbeat half-cycle (data from Esch, 1976, closed circles, and from F. Goller, unpublished results, open circles) and shortening time (±1 S.E.M) of conventional twitches (onset to peak displacement) as a function of muscle temperature in honeybees. Wingbeat half-cycles were determined from the time between minimum and maximum lift production of an animal tethered to a flight balance.

muscle potential frequency (see beginning of flight *vs* beginning of heating) (Figs 4 and 5). The extent of mirror movement reflected this balance between DV and DL muscle contractions.

These observations could be visually verified, and additional information could be gained. We looked at the lateral side of the thorax of a honeybee fixed in the apparatus through a stereo dissection microscope (25× amplification). The scutal cleft near the wings (Snodgrass, 1956) was nearly closed when DV and DL muscles were inactive (zero position=no muscle potentials). The scutal cleft closed further, probably until a skeletal protrusion finally stopped movement (morphological details in Pringle, 1957) when muscle potential frequency in DL muscles was higher than in DV muscles, such as during heating. The mesoscutellar arm had slid under the mesoscutum as far as it could and pushed the notum upwards. The mesoscutellar arm is moved through the mesophragma by contractions of dorsal longitudinal muscles. Contraction of DL muscles thus stretched the DV muscles. The scutellar fissure frequently opened briefly at low shivering rates, especially at body temperatures between 20 and 30°C. The ratio between DL and DV (DL/DV) muscle potential frequencies changed to values less than 1.0 at these instances.

The scutellar cleft opened wide at the beginning of flight when oscillations started. The DV muscles stretched the DL muscles and the cleft oscillated between wide open and closed during flight. The cleft stayed open briefly as the amplitude of oscillations diminished at the end of flight and closed immediately afterwards.

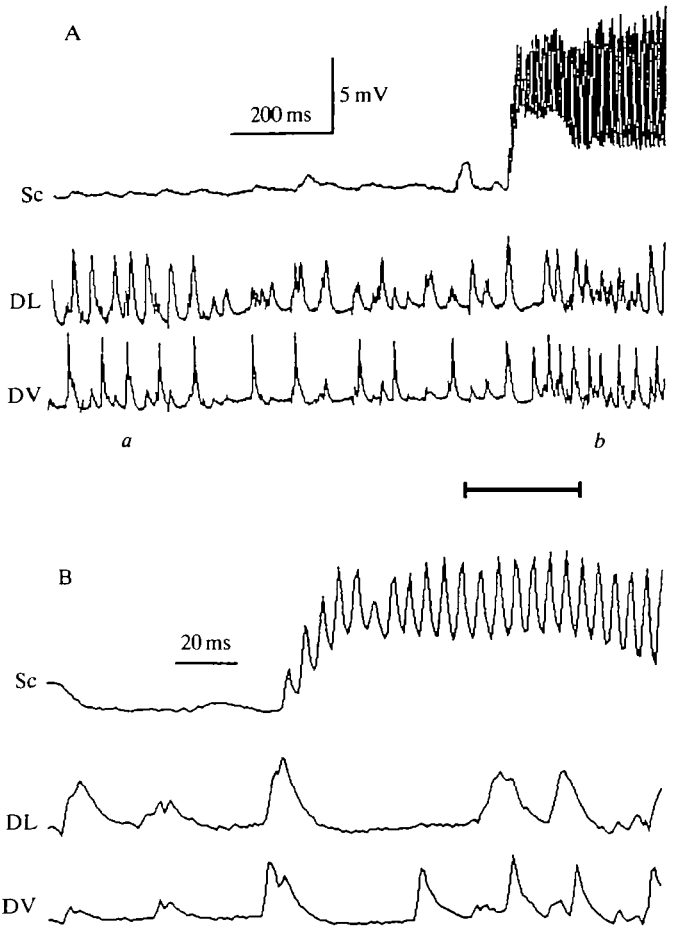

Fig. 4. (A) Muscle potentials in dorsoventral (DV) and dorsal longitudinal (DL) flight muscles of honeybees and *in situ* muscle movement (Sc) during the transition from warm-up to flight (thoracic temperature=33°C). Note different values of the ratio of DL/DV potential frequencies in *a* (1.6) and *b* (0.7). (B) Expanded view of the very beginning of flight (marked with a bar in A) showing the synchronized stimulation of both muscle groups corresponding to the beginning of oscillations.

Recordings of mirror movements and muscle potentials, as well as visual inspection, provide the following explanation for the events during shivering. The mirror is in zero position when DV and DL muscles are inactive, and the mesonotal suture is nearly closed. The mirror subsequently tilts downwards at the beginning of heating (mesonotal suture closed to stop), and the balance of muscle shortening tips towards contraction of DL muscles (Fig. 5).

Muscle potential frequencies in DL muscles are higher than those in DV muscles during shivering (ratio of DL/DV frequencies near 1.3). This high DL/DV ratio persists for various muscle potential frequencies observed during shivering at temperatures between 10 and 40°C in honeybees and between 8 and

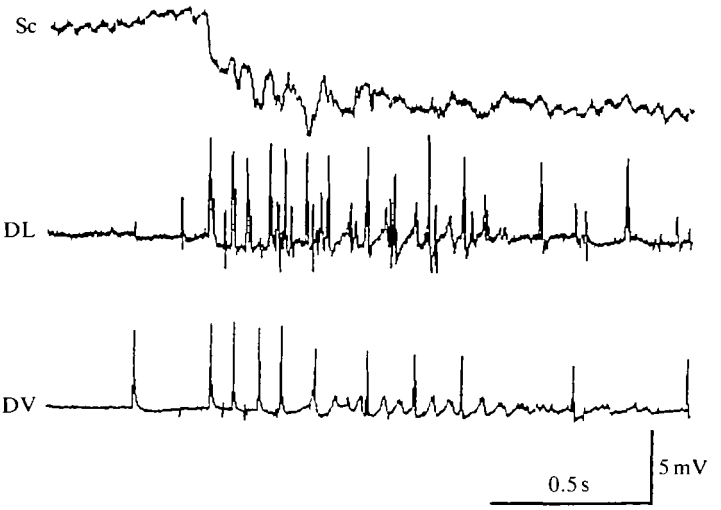

Fig. 5. Beginning of a heating episode. Muscle potential frequency in dorsal longitudinal (DL) muscles is higher than in dorsoventral (DV) muscles. Note the downward trend of the scutellar trace (*in situ* muscle response) (Sc), indicating shortening of the DL muscle. Synchronized muscle responses of lower amplitude than individual potentials can also be seen.

Table 1. *Ratio of muscle potential frequencies in DL and DV flight muscles (DL/DV) for honeybees (recordings from five individuals) and bumblebees (three individuals)*

	DL/DV ratio			
	Honeybee		Bumblebee	
Mode	Mean	*N*	Mean	*N*
Shivering	1.34±0.25	17	1.38±0.34	13
Flight	0.86±0.13	27	0.70±0.08	6
Buzzing	1.08±0.12	11	–	–

Data for bumblebees were determined from recordings in Heinrich and Kammer (1973).
N refers to the number of determinations from recordings that lasted approximately 1 s.
Values are mean±S.D.
DL, dorsal longitudinal; DV, dorsoventral.

40 °C in bumblebees (Table 1). The shift in DL/DV frequency ratio is illustrated in Fig. 4A, where a transition from heating (DL/DV=1.6) to flight (DL/DV=0.7) is shown for one individual. The higher activity of DL muscles during heating is also reflected in a higher muscle temperature. At an ambient temperature of 24 °C the temperature of DL muscles was about 0.6 °C higher than the temperature of DV muscles at the beginning of shivering. The difference amounted to 2.6 °C when the DV muscles reached 36 °C. This occurred in all three honeybees that we tested.

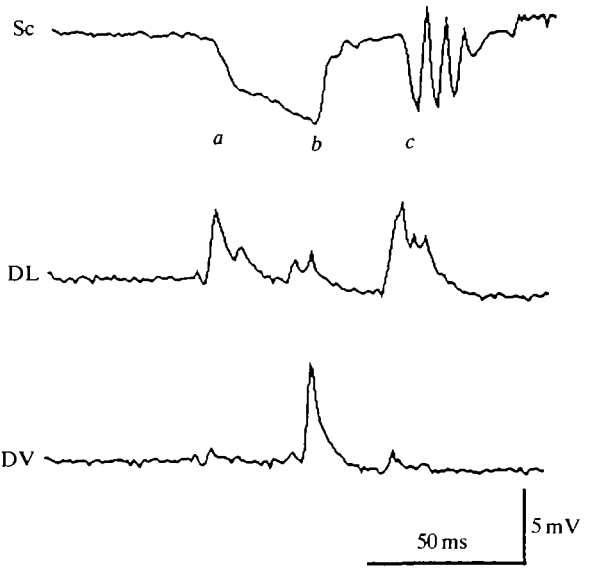

Fig. 6. Muscle activation in dorsal longitudinal (DL) and dorsoventral (DV) muscles leading to a short buzz (*in situ* muscle response in Sc). A DL muscle potential causes a 'conventional' shortening (slow) indicated by downward movement (Sc, *a*); a DV muscle potential causes faster upward movement (*b*); the following DL potential causes still faster shortening and stretch activation of the DV muscle (*c*).

Buzzing in honeybees and bumblebees

During shivering, and often immediately following flight, short bursts of fast contractions could be observed. These bursts were similar to bursts of 'buzzing' sounds during wagging runs in honeybees dances (Esch, 1961). They consisted of between one and several fast DV/DL contractions with amplitudes somewhat smaller than those in flight, as judged by mirror movements. Wings were held in a V-shaped position and did not beat up and down far. Fig. 6 shows the beginning of such a buzzing phase. At *a*, DL units became active and DL muscles contracted. At *b*, DV units fired and the summed contraction of DL muscles was very quickly counteracted. Muscles were pulled back to near zero position. Another burst of at least three groups of DL units at *c* contracted the DL muscle faster than in *a*. The velocity was high enough to start oscillations through 'stretch-activation'. The average DL/DV ratio during buzzing was 1.08, indicating equal activation of both muscle groups (Table 1).

Discussion

Flight muscle contractions and scutellar movement

Snodgrass (1956) explained the connection between contractions of fibrillar DV and DL flight muscles and scutellar movements. The DL muscles are attached frontally to the notum of the second thoracic segment and rostrally to the

postphragma of the mesothorax. Their contraction tilts the scutellum downwards. The DV muscles are affixed dorsally to the same notum as the DL muscles. They connect ventrally directly to the sternum. The scutellum tilts upwards when they contract. Esch and Bastian (1968) and Bastian and Esch (1970) connected transducers directly to the attachment sites of DL and DV muscles and demonstrated that potentials in DV and DL muscles led to shortening of the respective muscles. Shortening of muscles monitored with the mirror on the scutellum is indistinguishable from direct transducer observations.

In muscles directly attached to a transducer, muscle potential frequencies between 5 and 10 Hz cause tetanus-like fusion of single contractions. Similar observations were made by Ikeda and Boettiger (1965). They attached the DL muscles of bumblebees directly to a transducer and recorded their contractions in response to stimulation of the motor nerve. The DL muscles went into smooth tetanus at stimulus frequencies near 10 Hz.

Failure to see scutellar movement at high shivering rates, where muscle potential frequencies are above 10 Hz, is probably the result of tetanic fusion of contractions. It could also be caused by the pull of the DL muscles against a mechanical stop.

Various types of muscle contraction

The big fibrillar flight muscles can produce two types of muscle contractions – the conventional twitch and the stretch-activated fast contraction (Esch and Bastian, 1968; Bastian and Esch, 1970). These muscles respond to motor neurone discharge with conventional twitches when they are not stretch-activated. The shortening time in these twitches is much smaller than the time required to return to rest length (Fig. 5; Ikeda and Boettiger, 1965). When activated muscles are stretched quickly, development of tension lags behind length change (as first described by Boettiger, 1957*a*,*b*). Under these conditions, fast stretches result in fast contractions. Mutual stretching of antagonists then leads to the well-known oscillations (for a review see Hoyle, 1983). One should note, however, that during oscillations lengthening following the contraction of a muscle pair is not 'relaxation', but stretching by the antagonist. Thus, shortening and lengthening times are very similar. Because contractions of various muscle elements within a muscle during conventional twitches are normally not synchronized (Fig. 5), the amplitude of contractions is not big. Stretch activation in DV and DL muscles during oscillations ensures that *all* muscle units are synchronized to contract at the same time. Thus, maximal contraction amplitude can be achieved. It would be very difficult to obtain such a degree of synchronization by strictly neuronal, as opposed to mechanical, means in these muscles.

Shivering versus *flight*

Snodgrass (1956) gave a detailed description of the mechanical movements of the thorax capsule and the scutellum in honeybees resulting from contractions of DV and DL muscles, and he also discussed the potential role of smaller direct

muscles in the flight apparatus. However, Pringle (1961) pointed out that Snodgrass's description of the role of smaller direct and accessory muscles during flight must be largely incorrect, since only fibrillar muscles can contract at the high frequency of the wingbeat.

Bees heat by shivering with the large fibrillar flight muscles but these muscle movements cannot easily be detected (Esch, 1964; Bastian and Esch, 1970; Kammer and Heinrich, 1972; Heinrich and Kammer, 1973; Surholt *et al.* 1990). Heinrich and Kammer (1973) proposed that bumblebees contract antagonists synchronously and thus largely eliminate oscillations. Such synchronous muscle potential patterns are commonly seen in bumblebees. Frequently, however, the antagonistic muscles are *not* activated synchronously, and muscle potential patterns alone would not then explain why the muscles still do not oscillate. Similarily, muscle potential patterns in honeybees never show synchrony as in bumblebees. Observations in the present study of intact bees and in previous studies of muscle preparations (Ikeda and Boettiger, 1965) show 'conventional' twitches during shivering, lasting much longer than contractions during flight, specifically in their return to rest length. Shivering muscles go into smooth tetanus at fairly low muscle potential frequencies. Furthermore, during shivering, DL muscles contract more than their antagonists. Unequal excitation of DL and DV muscles pushes the structures connected to the mesophragma (which is moved by DL muscles) against a skeletal stop. Oscillations are thus avoided as muscle contractions increase. No muscle movements are seen at higher muscle potential frequencies, both because summation keeps the DL muscles in tetanus and because the muscles are contracted against a mechanical stop. There is not enough summation at low muscle potential frequencies, however, and muscle movements can then be detected.

Whenever DV muscles are pre-stretched by DL muscles, and a greater number of DV muscle units contract simultaneously, faster ('stretch-activated') twitches of DL muscles can occur. Sometimes stretch-activated twitches are big and fast enough to stretch-activate the antagonists sufficiently, and a few big oscillations can be seen, as for instance during buzzing. The increasing intensity of mutual stretch-activation can be observed as the velocity of the contractions increases over a few oscillations.

Non-shivering thermogenesis

Tetanic contractions during heating were found in all of the ten bumblebees we observed over the temperature range from 8°C (where muscles stop functioning) (Goller and Esch, 1990) to 40°C. Thoracic heating without muscle potentials and resulting contractions never occurred. This observation is particularly important, since a number of reports suggest that bumblebees might produce heat by non-shivering thermogenesis (NST) (Newsholme *et al.* 1972; Greive and Surholt, 1990; Surholt *et al.* 1990). According to this model, flight muscles use phosphofructokinase and fructose-1,6-diphosphatase reactions in 'futile cycling', thus generating heat without contractions by splitting ATP. The model suggests further that Ca^{2+}

release during muscle contractions shuts NST off by inhibiting the fructose-1,6-diphosphatase reaction (Clark *et al.* 1973; Greive and Surholt, 1990). This hypothesis must be incorrect since flight muscle contractions, and thus Ca^{2+} release, can *always* be seen when heat is produced (Esch *et al.* 1991).

Initiation of oscillations

The uncoordinated twitches in DL and DV muscles during shivering are too small and too slow to provide mutual stretch-activation, as required for the fast muscle oscillations during flight. Our observations explain how oscillations begin. Synchronous contraction of a great number of muscle elements in the DV muscles results in a twitch of large amplitude (see synchronized multi-unit potentials in Fig. 4B). This contraction leads to stretch-activation in the DL muscle, which is also activated by a synchronized multi-unit potential. This multi-unit spike always occurs approximately half of a wingbeat cycle after the DV multi-unit potential. The DL muscle, in turn, stretches the DV muscle, and oscillations begin (see also Esch and Bastian, 1968). The mechanical synchronization provided by mutual stretching evidently increases the effectiveness of stretch-activation, since the shortening velocity of DV and DL muscles increases to a maximum within a few wingbeats (Fig. 7). This increase in velocity can be attributed to both an increase in amplitude and a faster shortening time. Once initiated, oscillations keep going as long as the muscles involved are activated sufficiently by motor nerves. Sometimes the general level of activation is just high enough to support a few

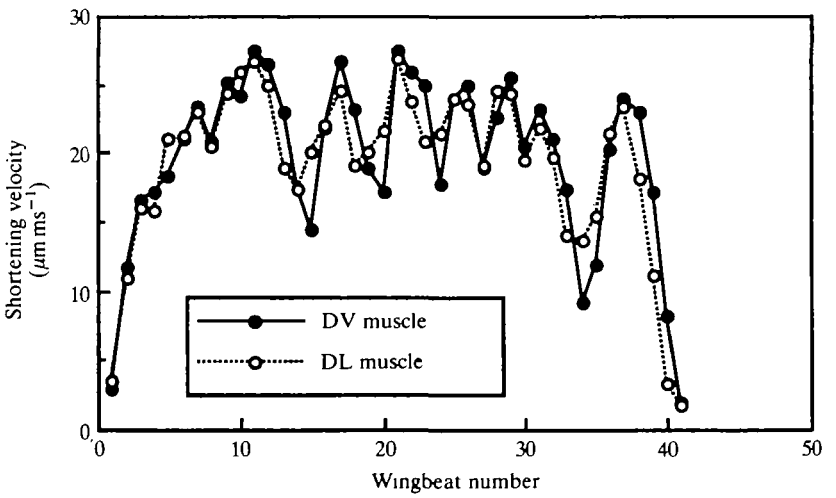

Fig. 7. Shortening velocity of dorsal longitudinal (DL) and dorsoventral (DV) muscles for all wingbeats in a representative recording of a flight episode. Amplitudes were measured as distances between the minimum and maximum light deflections caused by scutellar movement. They were calibrated against actual length changes of DV and DL muscles (see Esch and Bastian, 1968).

oscillations, as during buzzing. High muscle potential frequencies during flight ensure continuous oscillations. Oscillations stop only at the end of flight, when electrical activity in DV and DL muscles ceases for approximately 50–150 ms.

The possible role of 'starter muscles', direct muscles that could provide the first stretch of the DL or DV muscles in the initiation of flight, has been reviewed for *Calliphora* by Nachtigall (1985). But, even in *Calliphora*, removal of all presumptive starter muscles had no effect, since '[e]limination tests have not resulted in definite proof of the necessity or existence of such a starting muscle' (quote from Nachtigall, 1985). Such muscles could not exist in bees because all the direct and accessory indirect muscles of the mesothorax are of 'normal' (slow) structure. They contract tonically and not fast enough to stretch-activate fibrillar flight muscles and thus start oscillations (Pringle, 1961). We suspect that oscillations in the flight muscles of flies begin in a similar way to those in bees.

References

BASTIAN, J. A. AND ESCH, H. (1970). The nervous control of the indirect flight muscles of the honeybee. *J. comp. Physiol.* **67**, 307–324.

BOETTIGER, E. G. (1957*a*). The machinery of insect flight. In *Recent Advances in Invertebrate Physiology* (ed. B. T. Scheer), pp. 117–142. University of Oregon Publications.

BOETTIGER, E. G. (1957*b*). Triggering of the contractile process in insect fibrillar muscles. *J. cell. comp. Physiol.* **46**, 370–371.

CLARK, M. G., BLOXHAM, D. P., HOLLAND, P. C. AND LARDY, H. A. (1973). Estimation of the fructose diphosphatase–phosphofructokinase substrate cycle in the flight muscle of *Bombus affinis*. *Biochem. J.* **134**, 589–597.

ESCH, H. (1960). Ueber die Koerpertemperaturen und den Waermehaushalt von *Apis mellifica*. *Z. vergl. Physiol.* **43**, 305–335.

ESCH, H. (1961). Ein neuer Bewegungstyp im Schwaenzeltanz der Bienen. *Naturwissenschaften* **48**, 140–141.

ESCH, H. (1964). Ueber den Zusammenhang zwischen Temperatur, Aktionspotentialen und Thoraxbewegungen bei der Honigbiene (*Apis mellifica* L.). *Z. vergl. Physiol.* **48**, 547–551.

ESCH, H. (1976). Body temperature and flight performance of honey bees in a servomechanically controlled wind tunnel. *J. comp. Physiol.* **109**, 265–277.

ESCH, H. AND BASTIAN, J. A. (1968). Mechanical and electrical activity in the indirect flight muscles of the honey bee. *Z. vergl. Physiol.* **58**, 429–440.

ESCH, H., NACHTIGALL, W. AND KOGGE, S. N. (1975). Correlations between aerodynamic output, electrical activity in the indirect flight muscles and wing positions of bees flying in a servomechanically controlled wind tunnel. *J. comp. Physiol.* **100**, 147–159.

ESCH, H., GOLLER, F. AND HEINRICH, B. (1991). How do bees shiver? *Naturwissenschaften* **78** (in press).

GOLLER, F. AND ESCH, H. (1990). Comparative study of chill-coma temperatures and muscle potentials in insect flight muscles. *J. exp. Biol.* **150**, 221–231.

GREIVE, H. AND SURHOLT, B. (1990). Dependence of fructose-bis-phosphatase from flight muscles of the bumblebee (*Bombus terrestris* L.) on calcium. *Comp. Biochem. Physiol.* **97B**, 197–200.

HEINRICH, B. (1980). Mechanisms of body-temperature regulation in honeybees, *Apis mellifera*. *J. exp. Biol.* **85**, 61–87.

HEINRICH, B. (1987). Thermoregulation by individual honeybees. In *Neurobiology and Behavior of Honeybees* (ed. R. Menzel and A. Mercer), pp. 102–111. Berlin, Heidelberg, New York, London, Paris, Tokyo: Springer Verlag.

HEINRICH, B. AND KAMMER, A. E. (1973). Activation of the fibrillar muscles in the bumblebee during warm-up, stabilization of thoracic temperature and flight. *J. exp. Biol.* **58**, 677–688.

HIMMER, A. (1932). Die Temperaturverhaeltnisse bei den sozialen Hymenopteren. *Biol. Rev.* **7**, 224–253.

HOYLE, G. (1983). *Muscles and Their Neural Control*. New York, Chichester, Brisbane, Toronto, Singapore: John Wiley & Sons.

IKEDA, K. AND BOETTIGER, E. G. (1965). Studies on the flight mechanism of insects. II. The innervation and electrical activity of the fibrillar muscles of the bumble bee, *Bombus*. *J. Insect Physiol.* **14**, 779–789.

KAMMER, A. E. AND HEINRICH, B. (1972). Neural control of bumblebee fibrillar muscle during shivering. *J. comp. Physiol.* **78**, 337–345.

LINDAUER, M. (1954). Temperaturregulierung und Wasserhaushalt im Bienenstaat. *Z. vergl. Physiol.* **36**, 391–432.

NACHTIGALL, W. (1985). *Calliphora* as a model system for analysing insect flight. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 5 (ed. G. A. Kerkut and L. I. Gilbert), pp. 571–605. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press.

NEWSHOLME, E. A., CRABTREE, B., HIGGINS, S. J., THORNTON, S. D. AND START, C. (1972). The activities of fructose diphosphatase in flight muscles from the bumble-bee and the role of this enzyme in heat generation. *Biochem. J.* **128**, 89–97.

PRINGLE, J. W. S. (1957). *Insect Flight*. 133pp. Cambridge: Cambridge University Press.

PRINGLE, J. W. S. (1961). The function of the direct flight muscles in the bee. *Proc. XIth Int. Congr. Ent.* (Vienna 1960) **1**, 660.

ROEDER, K.D. (1951). Movements of the thorax and potential changes in the thoracic muscles of insects during flight. *Biol. Bull. Mar. biol. Lab., Woods Hole* **100**, 95–106.

SNODGRASS, R. E. (1956). *Anatomy of the Honey Bee*. 334pp. Ithaca, New York: Comstock Publishing Associates.

SURHOLT, B., GREIVE, H., BAAL, T. AND BERTSCH, A. (1990). Non-shivering thermogenesis in asynchronous flight muscles of bumblebees? Comparative studies on males of *Bombus terrestris*, *Xylocopa sulcatipes* and *Acherontia atropos*. *Comp. Biochem. Physiol.* **97**A, 493–499.

WENNER, A. M. (1962). Sound production during the waggle dance of the honey bee. *Anim. Behav.* **10**, 79–95.