POWER OUTPUT FROM A FLIGHT MUSCLE OF THE BUMBLEBEE *BOMBUS TERRESTRIS*

III. POWER DURING SIMULATED FLIGHT

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Summary

1. The work loop approach was used to measure mechanical power output from an asynchronous flight muscle, the dorso-ventral muscle of the bumblebee *Bombus terrestris*. Measurements were made at the optimum muscle length for work output at 30 °C and at a muscle temperature (40 °C) and oscillatory frequency (141–173 Hz, depending on the size of the animal) characteristic of free flight. Oscillatory strain amplitude was adjusted to maximize power output.

2. There was much preparation-to-preparation variability in power output. Power output in the muscles with the highest values was slightly greater than $100\,\mathrm{W\,kg^{-1}}$. It is argued that there are many experimental factors which might reduce measured power output below that in the living bumblebee, and no obvious factors which might lead to overestimates of muscle power. The conclusion is that flight muscle in the intact bumblebee can produce at least $100\,\mathrm{W\,kg^{-1}}$.

Key words: bumblebee, *Bombus terrestris*, muscle, flight muscle, muscle work, power.

Introduction

Three complementary approaches have been used to determine the energetic costs of flying in insects, birds and bats: (1) measurement of the metabolic rate of animals flying in wind tunnels or hovering in respiratory chambers; (2) determination of the aerodynamic costs of flight based on animal shape and size and the kinematics of wing movements during flight; and (3) physiological measurement of the mechanical power available from the flight muscles, usually obtained from semi-isolated muscles but in one dramatic study (Biewener *et al.* 1992) determined from parameters measured from a wing muscle of a bird during free flight. Measurements of metabolic rate provide direct information on the total costs of flight and how this varies with animal size and speed (for examples, see Casey and Ellington, 1989; Ellington *et al.* 1990). Metabolic rate can be used to estimate muscle power output and the aerodynamic costs of flight, but to do this accurately requires information on the fraction of the total metabolic power which is expended by the flight muscles and on the efficiency of the muscles in converting chemical energy to work. In insects, in which resting metabolic rates are relatively small and in which the costs of respiration and circulation are likely to be negligible, it is reasonable to assume that the total metabolic rate during flight is assignable to the flight muscles, but this assumption is not equally valid for vertebrates. And, in general, information is sketchy on the

chemical-to-mechanical efficiency of muscles under normal operating conditions. Substantial progress has been made in the past few decades in developing aerodynamic theory for flapping flight and the practical application of this theory for analyzing the flight performance of animals (e.g. Pennycuick, 1968, 1989; Weis-Fogh, 1973; Rayner, 1979; Ellington, 1984*a–f*; Dudley and Ellington, 1990*a,b*). Several studies have used force–velocity characteristics determined from or assumed for flight muscle in order to estimate the mechanical power available from these muscles (Weis-Fogh and Alexander, 1977; Pennycuick and Rezende, 1984; Ellington, 1985). The method that gives muscle power output most directly and that requires the fewest assumptions is the work loop approach, which was first introduced in studies of insect asynchronous flight muscles (Machin and Pringle, 1959) and later extended to synchronous flight muscles (Josephson, 1985).

Comparing estimates of aerodynamic power and directly measured muscle power provides a means of confirming aerodynamic theory and of evaluating the adequacy, and freedom from experimental errors, of the approach used to measure muscle power. The aerodynamic power output of a given insect can be expected to be quite variable, changing with flight speed, whether the animal is in level flight, climbing or descending, and with the extent to which the animal's crop and

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gut are full or empty. Most of the available values for aerodynamic power output are for animals hovering or in steady, level flight, and do not represent the maximum capacity of the animal. In studies of muscle power, however, it is usual to vary the controlling parameters systematically so as to find optimal values and maximum power levels. The appropriate comparison, therefore, is between maximum values of aerodynamic power output and reported values for muscle power. In an important set of experiments, Marden (1987, 1990) estimated the maximum lift force generated by a number of insects and birds by determining the maximum loading at which take-off was just possible. Ellington (1991) used these values to determine the maximum induced power output of the wing muscles; induced power being that part of the aerodynamic power involved in imparting momentum to the air flowing around the wings and in this way generating lift and thrust on the animal. The values for approximately 150 insects, distributed among seven orders, tended to cluster between 50 and 80 $W\,kg^{-1}$. When allowance was made for profile power, the power needed to overcome drag on the wings, the values for maximum mechanical power output of the wing muscles were generally 85–98 $W\,kg^{-1}$. These estimates of aerodynamic power agree reasonably well with measured values for maximum power output under conditions mimicking those of flight obtained from synchronous muscles of insects: 76 $W\,kg^{-1}$ for the wing muscle of a tettigoniid (Josephson, 1985), 72–75 $W\,kg^{-1}$ for locust flight muscle (Mizisin and Josephson, 1987) and 90 $W\,kg^{-1}$ for a flight muscle from the sphingid moth *Manduca sexta* (Stevenson and Josephson, 1990).

The relationship between aerodynamic power and muscle power is far less satisfactory for asynchronous flight muscles than for synchronous ones. Although aerodynamic power output based on load-lifting capacity seems to be similar in insects with synchronous and with asynchronous flight muscles (Ellington, 1991), some of the estimates of maximum aerodynamic power from insects using asynchronous muscles are substantially higher than values obtained from insects with synchronous muscles. Because of the low density of helium, flying in oxygen–helium mixtures is more costly than flying in normal air. Dudley (1995) found the aerodynamic power output of orchid bees, which use asynchronous muscles, reached 130–160 $W\,kg^{-1}$ muscle in different species when the animals hovered in helium–oxygen mixtures. In a study of the aerodynamic output of maximally laden bumblebees in climbing flight, the calculated muscle power output reached 170–190 $W\,kg^{-1}$ (Cooper, 1993). Although the expected mechanical power output is high, actually measured values of mechanical power output from asynchronous muscle are generally quite low. Machin and Pringle (1959) report maximal mechanical power outputs of 30 $W\,kg^{-1}$ for flight muscle of the beetle *Oryctes rhinoceros* and 60 $W\,kg^{-1}$ for bumblebee flight muscle. Subsequent to the work of Machin and Pringle (1959), nearly all studies on the physiology of asynchronous muscles have been carried out with glycerinated or demembranated fibres from the bugs of the genus *Lethocerus*. In glycerinated or demembranated fibres, the diffusion rate of fuel (ATP) into

the fibres can limit performance (Jewell and Rüegg, 1966). Most studies of asynchronous muscle have been performed using very low values of oscillatory strain and temperatures much below those of normal flight; conditions deliberately chosen to limit work output and ATP usage and thereby to avoid the effects of ATP deficiency. In one of the few investigations in which biologically relevant values of strain were used (Pringle and Tregear, 1969), the maximum power output of glycerinated *Lethocerus* fibres was found to be 7.6 $W\,kg^{-1}$, which is much lower than the expected power output during flight even if allowance is made for the subnormal temperature (20 °C) at which the work measurements were made. It is only in the studies of Gilmour and Ellington (1993*a,b*), using bumblebee fibres, that the maximum power output obtained from glycerinated fibres approached that expected *in vivo*. The power output from the bumblebee fibres was quite variable from preparation to preparation, and generally less than 70 $W\,kg^{-1}$, but it did exceed 110 $W\,kg^{-1}$ (at 40 °C) in two of the many fibres examined. The optimal oscillatory frequency for power output in the glycerinated bumblebee fibres was approximately 50 Hz at 40 °C and the optimal strain approximately 5 %; one assumes that the power output of the glycerinated fibres would have been smaller had the measurements been made at the frequency and strain characteristic of flight (approximately 150 Hz and 2.5 %; Josephson and Ellington, 1997).

The following is a study of the maximal mechanical power output from living bumblebee flight muscles operating under conditions quite comparable to those of flight. This work was begun to try to reconcile the discrepancy between values for muscle power from asynchronous muscles estimated from aerodynamic considerations and those actually measured.

Materials and methods

The general procedures for measuring work output from bumblebee flight muscle are described in the preceding paper (Josephson, 1997). In preliminary experiments, it became apparent that the capacity for work output generally declined during the course of an experiment and that performance declined much more rapidly at 40 °C, which is within the range of normal thoracic temperatures during flight (Joos *et al.* 1991; Heinrich, 1993), than at 30 °C, which was the usual experimental temperature. The goal of this portion of the study was to obtain an estimate of the maximal power available from the flight muscles of the bumblebee under the conditions at which they normally operate. Because of the progressive decline in muscle performance, it was necessary to obtain measurements of power output as soon as possible after setting up the preparation, and especially as soon as possible after warming it to the flight temperature. The following protocol was used in obtaining measurements of power output at 40 °C.

The preparation was mounted on the apparatus and the thorax was warmed to 30 °C. The stimulus intensity needed to obtain maximal tetanic contractions was determined in a series of trials using stimulus bursts (0.5 ms pulses at 50 Hz for 0.5 s) whose
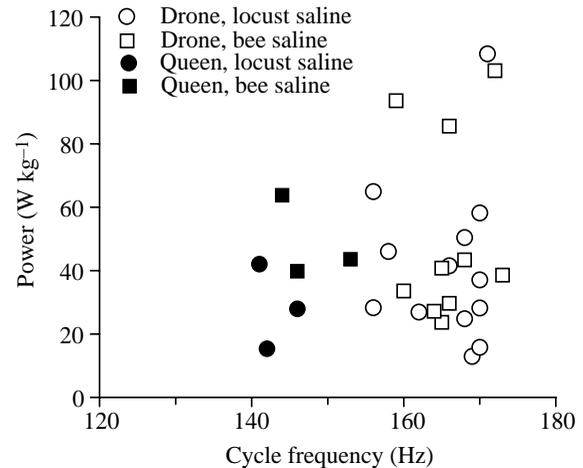
intensity was increased from trial to trial in steps of 50 %. The intertrial interval was 60 s. Next, the optimum muscle length for work output was determined. The muscle was stimulated tetanically (0.5 ms pulses, 0.5 s bursts at 50 Hz) and sinusoidal strain (0.15 mm peak-to-peak amplitude, 64 Hz) was imposed on the muscle during the plateau of the tetanic contraction. The muscle length on the first trial was set to be less than the expected optimal length, and the length was increased in steps of 0.05 mm from trial to trial until an optimum length was clearly established. The intertrial interval was 60 s. It typically took about five trials to establish the optimal muscle length. Often a few twitches were then obtained from the muscle to determine the effect of temperature on muscle contraction kinetics (see Josephson and Ellington, 1997).

Next, the muscle temperature was increased to 40 °C by resetting the control point on the temperature controller, and work output was measured at the higher temperature. It took approximately 60 s for the thoracic temperature to increase from 30 °C to 40 °C. The first work measurement at the higher temperature was made 60 s after the thorax reached 40 °C. The duration of the stimulus bursts in the trials at 40 °C were 0.3 or 0.5 s, and the intertrial intervals were 60 s. A few twitches were recorded in the intertrial intervals for comparison with those at 30 °C. It was found necessary to increase the stimulus frequency to 100 Hz to obtain fully fused tetanic contractions consistently at 40 °C. The oscillation frequency in the work measurements at 40 °C was the expected wingbeat frequency during flight as calculated from the regression equation provided by Cooper (1993) ($f=429.5m_b^{-0.17}$, where f is wingbeat frequency in Hz and m_b is body mass in mg). The oscillatory length change was initially set at 0.15 mm (approximately 3 % of the muscle length), and it was subsequently varied systematically from trial to trial in steps of 0.01 or 0.02 mm until an optimum strain was established, or until muscle performance had declined until it was obviously no longer representative of maximal performance. Muscles were moistened as necessary with bee saline or with locust saline (compositions given in Josephson and Ellington, 1997).

At the time of these measurements, only drones and queens were available from the colony. Thirty-nine preparations were used in this series. Of these, two were discarded early in the course of the experiments because the maximum isometric tetanic force measured while determining an appropriate stimulus intensity was unusually weak (less than 70 mN, equivalent to approximately 23 kN m^{-2} for muscles of average size), two were discarded because the maximum power at 30 °C was very low (less than 200 μW = approximately 13 W kg^{-1}) and four were discarded because they became quite weak when warmed to 40 °C and their maximum work output at the expected wingbeat frequency was close to zero or negative.

Results

Muscle preparations proved quite variable in their power output under conditions chosen to mimic those of flight (Fig. 1). In most muscles, the power output was 20–50 W kg^{-1},

Fig. 1. The distribution of muscle power output at 40 °C measured from different preparations under conditions chosen to mimic those of flight.

but in some it was close to 100 W kg^{-1}. The greatest power output in this series was 108 W kg^{-1}. Maximum power output did not seem to depend on whether bee saline or locust saline was used to moisten the muscles, and mass-specific power was similar in muscle of drones and in the much larger muscles of queens. The optimum strain for power output at 40 °C was 3.1±0.3 % (mean ± S.D., $N=27$).

The relationships between stress and strain during sinusoidal oscillation at 40 °C, and the shapes of the work loops, were generally similar to those described in the preceding paper for muscles at 30 °C (Fig. 2). In unstimulated muscles, force increased non-linearly with length during sinusoidal oscillation. For passive materials, resilience, the ratio of the work done during shortening to the work required to relengthen the muscle, is a measure of the capacity for energy storage. The net area of the work loops from unstimulated muscle was generally small, indicating high resilience. In fact, in one preparation, the work done during shortening by the unstimulated muscle was slightly greater than that required to relengthen the muscle, and there was positive net work, albeit small, from the unstimulated muscle over a full cycle (Fig. 2D). This strongly confirms the suggestion made in Josephson (1997) that the mechanical properties of unstimulated muscle are in part due to active cross bridges, and that unstimulated muscle is not a passive material in the usual sense of the word. In stimulated muscles, stress and strain were nearly in phase during the shortening portion of the imposed oscillatory strain cycle, but muscle stress lagged considerably behind strain during muscle lengthening. For the cycle as a whole, there was an average phase lag between stress and strain and therefore anticlockwise (positive work output) work loops were obtained.

The mean maximum power for muscles in this series was 22.9±6.6 W kg^{-1} at 30 °C (±S.D., $N=29$) and 44.6±25.4 W kg^{-1} at 40 °C (±S.D.). It should be pointed out that values for power output at 30 °C and 40 °C are not exactly comparable, so it may not be appropriate to calculate Q$_{10}$ values. The power
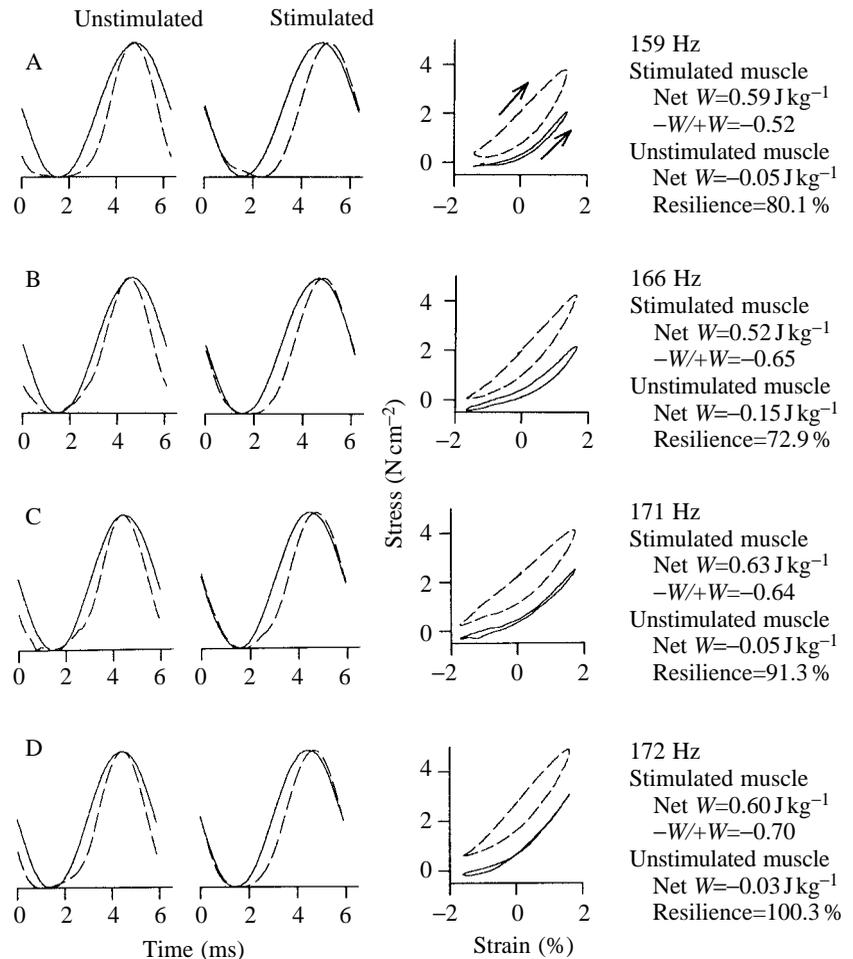
Fig. 2. Muscle length and force, and work loops, from the four preparations giving the highest values of power output. Normalized values of length and force are shown as continuous and broken lines respectively in the first two columns. Work loops (third column) were anticlockwise for stimulated muscles (loops drawn with dashed lines) and clockwise for unstimulated muscles (solid lines) except that of the lowest row in which the unstimulated loop was also anticlockwise. The length and force traces and the work loops in each row are the fifth cycle of oscillation. The numerical values in the right-hand column are the mean values for cycles 3–7. W, work; $-W/+W$, ratio of negative work to positive work.

measurements at 30 °C were at a common oscillation amplitude (0.15 mm) and frequency (64 Hz); those at 40 °C were at a frequency which varied from preparation to preparation, depending on the size of the animal containing the muscle, and at the strain that was determined to be optimal for work output for each preparation.

Discussion

What is the maximum power output?

How should the results of Fig. 1 be interpreted, given the large preparation-to-preparation variability and power values which are substantially lower than those expected from the muscle on the basis of the flight performance of the bumblebee? There are several reasons why the maximum muscle power, measured as described, might be lower than that available to the animal during flight. (1) The muscle may have become somewhat distorted when the preparation was mounted and the force transducer attached to the ventral exoskeletal plate upon which the muscle inserts. If this plate were not positioned correctly, some fibres in the muscle would have been longer than their *in vivo* (and presumably optimum) length and others shorter than their *in vivo* length when the muscle as a whole was at the length that maximized power output. Muscle performance is strongly dependent on muscle length

(Josephson, 1997), and a relatively small deviation of fibre length from the optimal value can be expected to reduce power output significantly. The importance of avoiding muscle distortion – and the difficulty of doing this with muscles which are, like most asynchronous flight muscles, rather short and broad – was recognized by Darwin and Pringle (1959) in one of the first studies on the physiology of asynchronous muscle. It seems likely that incorrect positioning of muscle origins and insertions is the principal cause of the low and variable performance of the muscles shown in Fig. 1. (2) All the muscle fibres were subjected to the same absolute length change during the cyclic contractions. However, not all the fibres in the muscle are the same length (see Josephson and Ellington, 1997). The strain, therefore, was greater for short fibres than for long ones. In the bumblebee, the distortion of the thorax may be such that all fibres operate at their optimal strain; this was probably not the case in the work loop determinations. (3) The strain trajectory, which was sinusoidal, may not have been that which maximized power output. Gilmour and Ellington (1993b) found that inclusion of a second harmonic in the strain trajectory increased the work output from some glycerinated muscle preparations from bumblebees. (4) The salines used to moisten the muscle may have lacked modulatory factors needed for maximal muscle performance. (5). Damage to the tracheal system during dissection may have rendered parts of the muscle

anoxic. Such damage may account for the rapidity with which performance declined at 40 °C in most preparations.

The factors listed above would all tend to reduce mechanical power output and make the power measured from semi-isolated muscle preparations less than that of intact muscles within the bumblebee. There are no obvious features of the experimental approach which might make measured power output greater than that in the intact animal, other than errors in calibrating the equipment, which I think to be unlikely, or in measuring the size of the muscle. The effects of possible errors in the measurement of muscle size are discussed below and are dismissed as being insignificant. It can be reasonably concluded that the measured values of muscle power in Fig. 1, even the highest values presented, underestimate the true power. It is concluded that the power available from bumblebee flight muscle under flight conditions is greater than $100 \, \mathrm{W \, kg^{-1}}$, but how much greater is uncertain.

The power output obtained from living bumblebee muscles was quite similar to that found in glycerinated fibres from bumblebee flight muscle (Gilmour and Ellington, 1993*a*). In both studies, there was large variability from preparation to preparation. The power output was 116 and $113 \, \mathrm{W \, kg^{-1}}$ in the two glycerinated fibres with the highest values. In experiments with glycerinated fibres, as in experiments with living, whole muscles, there are many factors which might reduce power output to a level below that in intact bumblebees (damage to fibres during preparation, non-optimal composition of bathing solutions, non-optimal strain trajectory, etc.) and no obvious factors which might lead to overestimates of the available power. The conclusion from the work with glycerinated fibres, as with that from living muscles, is that the power output of bumblebee flight muscle is greater than $100 \, \mathrm{W \, kg^{-1}}$.

On errors in measured muscle mass

The data in Fig. 1 are mass-specific power: realized power divided by muscle mass. It is conceivable that the four preparations in Fig. 1 for which maximum power was greater than $80 \, \mathrm{W \, kg^{-1}}$ were all instances in which the muscle mass was mismeasured and was inaccurately low, giving an overestimate of mass-specific power. The possibility of measurement errors can be examined by comparing the measured muscle mass with that expected on the basis of the size of the animal from which the muscle came. A linear regression analysis gave the following relationship between muscle mass (m_{m} in mg) and animal mass (m_{b} in mg) for the 64 preparations for which information on both parameters was available: $m_{\mathrm{m}}=0.0398m_{\mathrm{b}}+3.14$ ($r^2=0.864$, S.E.M.$=\pm0.78$ and ±0.002 for intercept and slope respectively).

For the four preparations with the highest power output in Fig. 1, the measured muscle mass averaged 98 % of the mass predicted from the animal's size and the regression equation linking animal mass and muscle mass. The measured muscle mass of the preparation giving the highest power output differed by less than 1 % from the predicted value. Thus, it is quite unlikely that the larger values for mass-specific power in Fig. 1 were a consequence of errors in weighing muscles.

Variability in fibre length and strain

The consequences of varying fibre length within a muscle, and therefore of varying strain among the fibres, were examined in the following way. The fibre length distribution was taken to be that shown in Fig. 2 of Josephson and Ellington (1997). It was assumed that curves relating work output and strain in whole muscles are also applicable to individual fibres within the muscle. The strain–work curve shown as Fig. 9 in Josephson (1997) is a reasonable representative of the strain–work curves, and it was used to estimate the extent to which the work output from fibres which are longer or shorter than the average length within the muscle is reduced because these fibres do not operate at optimal strain. The optimal strain in the strain–work curve (3.14 %) is slightly longer than the average optimum strain found in all preparations (3.0 %), so the strain–work curve was rescaled by multiplying all strain values by a constant so as to make the optimum strain equal to 3.0 %. The work output for the fibres in the individual bins of the length distribution, relative to that which would have been obtained from these fibres at the optimal strain, was estimated from the strain–work curve. For example, consider the bin centred on 110 % length in the fibre length distribution of Fig. 3. This bin contains 13.5 % of the total number of fibres. Because the fibres in this bin are longer than average, a strain which is optimal for the average fibre length in the muscle will
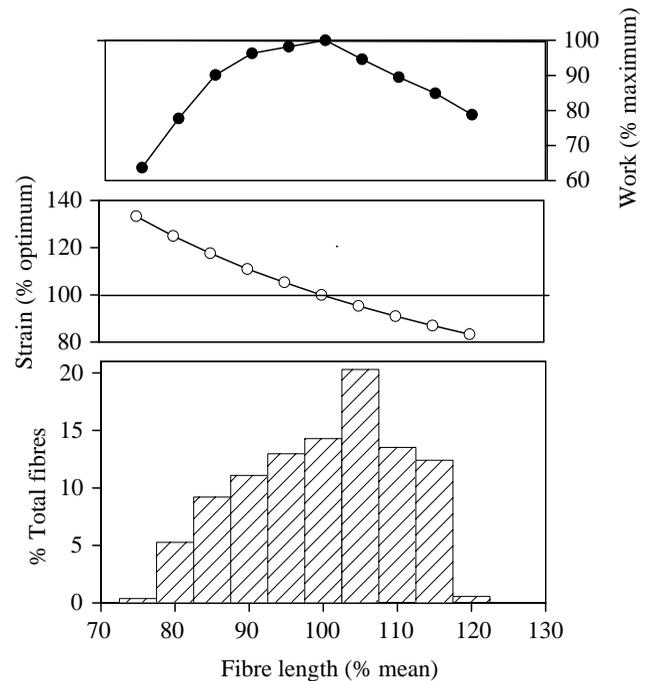

Fig. 3. The data used to calculate the effect of fibre length heterogeneity on work output. The top curve is the expected relationship between fibre length and work output based on the length–work curve obtained from whole muscles (Fig. 9 of Josephson, 1997). The middle curve is the fibre strain for an oscillatory length change which is optimum (=3 %) for the average fibre. The lower histogram is the expected fibre length distribution within bumblebee muscles constructed from Fig. 2 of Josephson and Ellington (1997).

be somewhat below optimum for the fibres in the bin. The expected strain in these fibres is 2.73 % (3/110 %). By linear interpolation between measured values in the strain–work curve, the expected work output at a strain of 2.73 % is 89.5 % of the maximum value. This work output, multiplied by the fraction of the total number of fibres represented by the bin, gives the collective work output of the fibres within the bin. The total work calculated by this procedure and summed over all the bins was 92.6 % of that which would have been obtained had all fibres operated at optimal strain. Imposing strain in such a way that the length change is the same for all fibres within the muscle, as in these experiments, is likely to reduce the work output by approximately 7 % relative to the output possible were all the fibres to operate at their optimal strain.

References

BIEWENER, A. A., DIAL, K. P. AND GOSLOW, G. E. (1992). Pectoralis muscle force and power output during flight in the starling. *J. exp. Biol.* **164**, 1–18.

CASEY, T. M. AND ELLINGTON, C. P. (1989). Energetics of insect flight. In *Energy Transformations in Cells and Organisms* (ed. W. Weiser and E. Gnaiger), pp. 200–210. Stuttgart: Georg Thieme Verlag.

COOPER, A. J. (1993). Limitations of bumblebee flight performance. PhD dissertation, University of Cambridge, 205pp.

DARWIN, F. W. AND PRINGLE, J. W. S. (1959). The physiology of insect fibrillar muscle. I. Anatomy and innervation of the basalar muscle of lamellicorn beetles. *Proc. R. Soc. Lond. B* **151**, 194–203.

DUDLEY, R. (1995). Extraordinary flight performance of orchid bees (Apidae: Euglossini) hovering in heliox (80 % He/20 % O_2). *J. exp. Biol.* **198**, 1065–1070.

DUDLEY, R. AND ELLINGTON, C. P. (1990a). Mechanics of forward flight in bumblebees. I. Kinematics and morphology. *J. exp. Biol.* **148**, 19–52.

DUDLEY, R. AND ELLINGTON, C. P. (1990b). Mechanics of forward flight in bumblebees. II. Quasi-steady lift and power requirements. *J. exp. Biol.* **148**, 53–88.

ELLINGTON, C. P. (1984a). The aerodynamics of hovering insect flight. I. The quasi-steady analysis. *Phil. Trans. R. Soc. Lond. B* **305**, 1–15.

ELLINGTON, C. P. (1984b). The aerodynamics of hovering insect flight. II. Morphological parameters. *Phil. Trans. R. Soc. Lond. B* **305**, 17–40.

ELLINGTON, C. P. (1984c). The aerodynamics of hovering insect flight. III. Kinematics. *Phil. Trans. R. Soc. Lond. B* **305**, 41–78.

ELLINGTON, C. P. (1984d). The aerodynamics of hovering insect flight. IV. Aerodynamic mechanisms. *Phil. Trans. R. Soc. Lond. B* **305**, 79–113.

ELLINGTON, C. P. (1984e). The aerodynamics of hovering insect flight. V. A vortex theory. *Phil. Trans. R. Soc. Lond. B* **305**, 115–144.

ELLINGTON, C. P. (1984f). The aerodynamics of hovering insect flight. VI. Lift and power requirements. *Phil. Trans. R. Soc. Lond. B* **305**, 145–181.

ELLINGTON, C. P. (1985). Power and efficiency of insect flight muscle. *J. exp. Biol.* **115**, 293–304.

ELLINGTON, C. P. (1991). Limitations on animal flight performance. *J. exp. Biol.* **160**, 71–91.

ELLINGTON, C. P., MACHIN, K. E. AND CASEY, T. M. (1990). Oxygen consumption of bumblebees in forward flight. *Nature* **347**, 472–473.

GILMOUR, K. M. AND ELLINGTON, C. P. (1993a). Power output of glycerinated bumblebee flight muscle. *J. exp. Biol.* **183**, 77–100.

GILMOUR, K. M. AND ELLINGTON, C. P. (1993b). *In vivo* muscle length changes in bumblebees and the *in vitro* effects on work and power. *J. exp. Biol.* **183**, 101–113.

HEINRICH, B. (1993). *The Hot-blooded Insects*. Cambridge, MA: Harvard University Press.

JEWELL, B. R. AND RÜEGG, J. C. (1966). Oscillatory contraction of insect fibrillar muscle after glycerol extraction. *Proc. R. Soc. Lond. B* **164**, 428–459.

JOOS, B., YOUNG, P. A. AND CASEY, T. M. (1991). Wingstroke frequency of foraging and hovering bumblebees in relation to morphology and temperature. *Physiol. Ent.* **16**, 191–200.

JOSEPHSON, R. K. (1985). Mechanical power output from striated muscle during cyclic contraction. *J. exp. Biol.* **114**, 493–512.

JOSEPHSON, R. K (1997). Power output from a flight muscle of a bumblebee. II. Characterization of the parameters affecting power output. *J. exp. Biol.* **200**, 1215–1226.

JOSEPHSON, R. K. AND ELLINGTON, C. P. (1997). Power output from a flight muscle of a bumblebee. I. Some features of the dorso-ventral flight muscles. *J. exp. Biol.* **200**, 1227–1239.

MACHIN, K. E. AND PRINGLE, J. W. S. (1959). The physiology of insect fibrillar muscle. II. Mechanical properties of a beetle flight muscle. *Proc. R. Soc. B* **151** 204–225.

MARDEN, J. H. (1987). Maximum lift production during takeoff in flying animals. *J. exp. Biol.* **130**, 235–258.

MARDEN, J. H. (1990). Maximum load-lifting and induced power output of Harris' hawks are general functions of flight muscle mass. *J. exp. Biol.* **149**, 511–514.

MIZISIN, A. P. AND JOSEPHSON, R. K. (1987). Mechanical power output of locust flight muscle. *J. comp. Physiol.* A **160**, 413–419.

PENNYCUICK, C. J. (1968). Power requirements for horizontal flight in the pigeon *Columbia livia*. *J. exp. Biol.* **49**, 527–555.

PENNYCUICK, C. J. (1989). *Bird Flight Performance. A Practical Calculation Manual*. Oxford: University Press.

PENNYCUICK, C. J. AND REZENDE, M. A. (1984). The specific power output of aerobic muscle, related to the power density of mitochondria. *J. exp. Biol.* **108**, 377–392.

PRINGLE, J. W. S. AND TREGEAR, R. T. (1969). Mechanical properties of insect fibrillar muscle at large amplitudes of oscillation. *Proc. R. Soc. Lond. B* **174**, 33–50.

RAYNER, J. M. V. (1979). A new approach to animal flight mechanics. *J. exp. Biol.* **80**, 17–54.

STEVENSON, R. D. AND JOSEPHSON, R. K. (1990). Effects of operating frequency and temperature on mechanical power output from moth flight muscle. *J. exp. Biol.* **149**, 61–78.

WEIS-FOGH, T. (1973). Quick estimates of flight fitness in hovering animals, including novel mechanisms for lift production. *J. exp. Biol.* **59**, 169–230.

WEIS-FOGH, T. AND ALEXANDER, R. McN. (1977). The sustained power output from striated muscle. In *Scale Effects in Animal Locomotion* (ed. T. J. Pedley), pp. 511–525. London: Academic Press.