EVOLUTIONARY ADAPTATION OF CONTRACTILE PERFORMANCE IN MUSCLE OF ECTOTHERMIC WINTER-FLYING MOTHS

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Summary

The temperature-sensitivity of muscle performance in a winter-flying ecotothermic moth (*Operophtera bruceata*) was examined and compared with that of a summer-flying endothermic hawkmoth (*Manduca sexta*). *O. bruceata* muscle contracted over a temperature range of 1–28 °C, whereas *M. sexta* muscle contracted at temperatures of 13–42.5 °C. Maximum (unloaded) contraction velocity (V_{max}) was greater in *O. bruceata* over most of the range of temperatures where muscle from both species was excitable (3–4 lengths s^{-1} *versus* 0.6–3.6 lengths s^{-1} at 13–28 °C), but *M. sexta* muscle achieved a much higher V_{max} at the temperature that this species maintains during flight (10 lengths s^{-1} at 40–42.5 °C). The capacity of *O. bruceata* muscle to generate tension was approximately twice that of *M. sexta* muscle (peak tetanic tension of 13.9 *versus* 7.0 N cm^{-2}). This greater force-generating capacity in *O. bruceata* largely offset its lower shortening velocity, such that maximum instantaneous power output was equivalent in both species at temperatures below 35 °C (approximately 100–120 W kg^{-1}). *M. sexta* muscle achieved instantaneous power outputs of up to 202 W kg^{-1} at temperatures of 40–42.5 °C. Muscle activation and deactivation (measured by times to peak tension and to half-relaxation during isometric twitches) were most rapid for *O. bruceata* at temperatures of 15–30 °C and for *M. sexta* at temperatures of 30–40 °C.

Data for power output of flight muscle from these moths are combined with estimates of induced power required for flight in order to show how adaptations for thermal sensitivity of muscle power output interact with morphology (low wing-loading, high flight muscle ratio) to allow *O. bruceata* moths to fly at extremely low body temperatures, and to construct a model showing how the fecundity of flightless *O. bruceata* females would decline if they were to regain the ability to fly. Marginal flight over a narrow range of temperatures for *O. bruceata* females would require a 17 % reduction in fecundity; to fly over as large a range of temperatures as do males would require an 82 % reduction in fecundity.

Introduction

The ability of large flying insects to attain and regulate high body temperatures has dominated recent research on the thermal physiology of insects (Heinrich, 1993); however, the 'average' insect is small, ectothermic and flies over a broad range of body temperatures (Josephson, 1981). The metabolic biochemistry (reviewed in Hoffmann, 1985) and neurophysiology (Esch, 1988; Goller and Esch, 1990) of ectothermic flying insects have received some attention, yet there remain large gaps in our understanding of the physiological adaptations of ectothermic insects. In particular, no studies have examined the thermal physiology of muscle contractile performance of insects that differ in active body temperatures, such as has been done for polar *versus* tropical fish (Johnston and Brill, 1984; Johnston and Altringham, 1985; Johnston and Harrison, 1985; Johnson and Johnston, 1991).

Small winter-flying geometrid moths (Lepidoptera: Geometridae) are spectacular examples of insects that fly with a broad range of muscle temperatures. These moths are thermoconformers, able to fly at environmental and tissue temperatures from −3 °C to approximately 25 °C (Heinrich and Mommsen, 1985). The *in vitro* thermal sensitivity of the metabolic enzymes citrate synthase and pyruvate kinase does not differ between winter-flying geometrid moths and either winter- or summer-flying endothermic moths (noctuids and sphingids; Heinrich and Mommsen, 1985). These authors concluded that the ability of winter-flying geometrid moths to fly with low muscle temperature is largely attributable to morphological adaptation. *O. bruceata* possesses the lowest wing-loading of any moth yet measured, which reduces wingbeat frequency and lowers the energetic cost of flight.

The study presented here examines the thermal sensitivity of contractile performance of flight muscle from a winter-flying

geometrid moth, *Operophtera bruceata*, in comparison with the warm-adapted endotherm *Manduca sexta* (Lepidoptera: Sphingidae; Heinrich, 1971). The results of this study are used to test the null hypothesis, that these moths do not differ in muscle contractile performance and thermal sensitivity of muscle power output, and to construct a quantitative model that predicts how fecundity and thermal breadth for flight should vary as a function of body design in female ectothermic moths.

Materials and methods

Operophtera bruceata Hulst adults were collected in the field near Hanover, New Hampshire, USA, during November 1993, and were maintained for up to 2 weeks in a laboratory refrigerator at 4 °C. Although the possibility of thermal acclimation was not explicitly examined, there were no systematic differences in data obtained from freshly caught *versus* stored individuals. *Manduca sexta* L. pupae were obtained commercially (Carolina Biological Supply) and adults of both sexes were used 2–5 days after emergence.

The shortening velocity during isotonic contraction of the large dorsal longitudinal flight muscle (DLFM) was measured from *in situ* preparations of *O. bruceata* and *M. sexta* muscles. The head, wings, legs and abdomen of moths were removed and the thorax was denuded of scales. For *M. sexta* preparations, a small incision was made in the ventral thorax and the underlying thoracic ganglion was removed in order to prevent spontaneous muscle contraction. Spontaneous muscle activity occurred at low levels in *O. bruceata* and did not interfere with measurements; in these preparations, the thoracic ganglion was left intact.

Thoraces were oriented with their anterior end downwards and glued with quick-setting epoxy resin to the floor of a water-jacketed chamber. The shaft of an insect pin, suspended from the lever arm of an ergometer (Cambridge Technology 300b dual-mode lever system), was attached with cyanoacrylate glue (gel form) to the phragmatum of the metathorax, the posterior origin of the DLFM (Snodgrass, 1935). In this arrangement, the DLFM was held in a vertical orientation at its resting length. Incisions were made around the periphery of the metathoracic phragmatum in order to free the posterior end of the DLFM mechanically and to allow it to shorten against loads set by the ergometer.

The analog output from the lever system was sampled at 4 kHz with a MacLab8 A/D converter and displayed using Scope software on a Macintosh Quadra 700 computer. Fine-gauge platinum electrodes inserted into the lateral thorax were used to stimulate tetanic contractions of the DLFM. A Grass 44 stimulator and SIU5 stimulus isolation unit delivered 300 ms duration trains of 125 % supramaximal voltage, 1 ms duration, 100 Hz square waves. Analog voltage output from the MacLab unit was used to trigger the stimulator and to switch the lever system from isometric to isotonic mode during the plateau of tetanic contractions. Switching between stable isometric and isotonic contraction occurred in less than 5 ms, with only minor force and length transients (Fig. 1).

Water was pumped from a temperature-controlled bath through the water jacket of the test chamber in order to maintain muscle temperature at desired levels. A fine-gauge copper–constantan thermocouple connected to a Physitemp Bat-12 thermocouple thermometer was implanted in the approximate center of the thorax (but away from the DLFM) in order to monitor muscle temperature. Four *O. bruceata* preparations were tested at 4–7 temperatures each, from 1 to 28 °C. Temperatures below 25 °C were tested in random order; temperatures above 25 °C were always tested last because tetanic tension and shortening velocity of *O. bruceata* preparations declined quickly at these temperatures. Six *M. sexta* preparations were tested at 1–6 temperatures each, from 13 to 42.5 °C (the order of temperatures was determined randomly; the number of temperatures tested depended on how long the preparation maintained maximum isometric tension, P_0, within 10 % of original P_0 at 25 °C). At the conclusion of experiments, cuticle and tissue surrounding the DLFM were dissected away, and the resting length of the DLFM was measured to the nearest 0.1 mm with vernier calipers. The mass of the DLFM was measured to the nearest 0.1 mg with a Mettler balance. The cross-sectional area of the DLFM was estimated by dividing its mass by its length.

At each experimental temperature, the shortening velocity was measured from the first 1 ms of length change following the establishment of a stable tension during isotonic contraction (Fig. 1). Maximum (unloaded) shortening velocity (V_{max}) was determined from a curve fitted to velocity as a
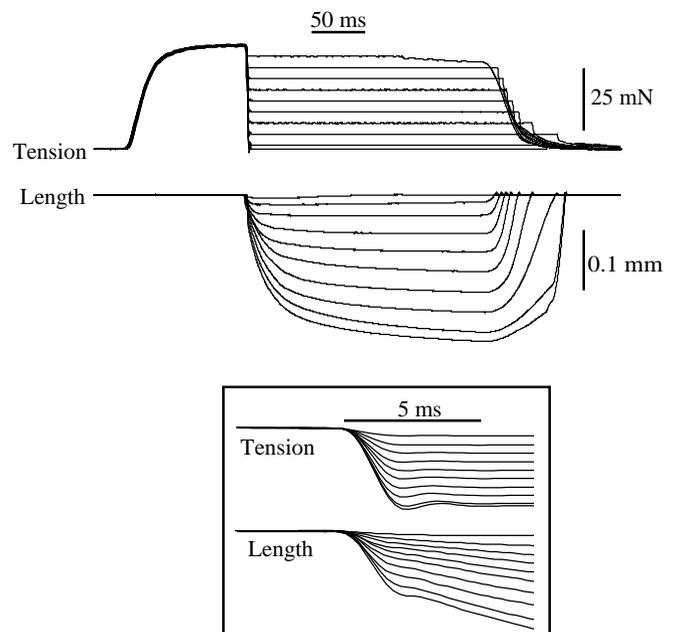

Fig. 1. Representative traces of tension and length from the dorsal longitudinal flight muscle of moths during force–velocity experiments. The inset shows details of the interval during the switch from isometric to isotonic tension. Shortening velocity was measured during the final 1 ms of the time reference bar in the inset figure. These data were collected at a temperature of 18 °C from an *O. bruceata* muscle that weighed 0.7 mg and was 0.23 cm in length.

function of relative tension (Fig. 2) using a hyperbolic–linear equation (Marsh and Bennett, 1986, performed iteratively with an Igor software routine supplied by R. L. Marsh). Estimates of shortening velocity ($m\,s^{-1}$) and tension (N) at the tension yielding maximum power output (as determined by the hyperbolic–linear equation) were multiplied together and
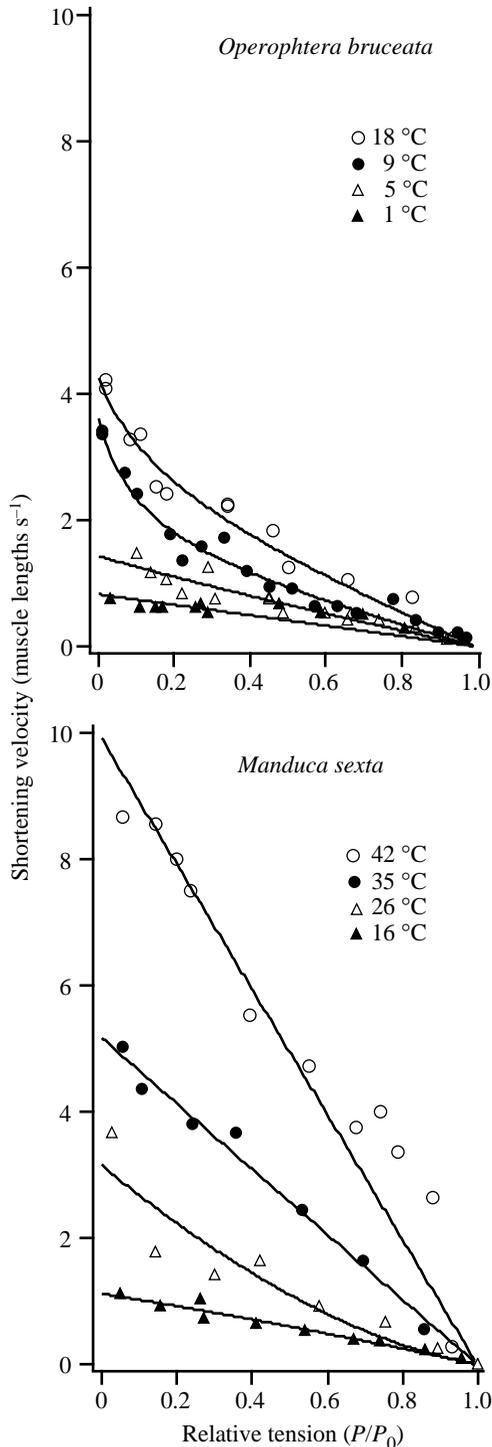

Fig. 2. Representative force–velocity data used to calculate maximal shortening velocity (V_{max}). Curves were fitted using a hyperbolic–linear equation (Marsh and Bennett, 1986).

divided by muscle mass (kg) in order to derive maximum instantaneous power output ($W\,kg^{-1}$).

A separate sample of moths (three from each species) was used to measure the temperature-sensitivity of activation (time to peak tension) and deactivation (time to half-relaxation) of the DLFM. The experimental arrangement was identical to that described above, except that stimulation was accomplished with single pulses (0.5 ms) and the muscles were held at constant length. Twitches were elicited at 0.5 °C intervals over the entire range of temperatures where noticeable force development occurred.

Results

The flight muscle of winter-flying *O. bruceata* moths was excitable from 1 °C (the lowest temperature that could be regulated in the test chamber) to 28 °C. Temperatures above 25 °C caused a rapid and irreversible loss of contractility in all *O. bruceata* preparations. Muscle from *M. sexta* could not be stimulated to contract at temperatures below 13 °C, and some preparations remained stable at temperatures up to 42.5 °C. Thus, these two species differ greatly in the range of temperatures over which their flight muscles can function.

The results for thermal sensitivity of shortening velocity, tetanic tension and instantaneous power output are summarized in Fig. 3. Maximum shortening velocity (V_{max}) for *O. bruceata* increased from $0.5\,lengths\,s^{-1}$ at 1 °C to $4\,lengths\,s^{-1}$ at 15–20 °C, then declined at higher temperatures (Fig. 3A). *M. sexta* V_{max} increased in a linear fashion from $0.6\,lengths\,s^{-1}$ at 13 °C to a maximum of nearly $10\,lengths\,s^{-1}$ at 42.5 °C (Fig. 3A). *O. bruceata* V_{max} showed a Q_{10} of 2.96 from 1 to 20 °C in comparison with the Q_{10} for V_{max} of *M. sexta* of 2.40 from 13 to 42 °C. V_{max} for *O. bruceata* at optimal temperatures (15–20 °C) was significantly higher than V_{max} for *M. sexta* at the same temperatures ($P=0.006$), but was lower than V_{max} achieved by *M. sexta* at temperatures above 30 °C ($P<0.0001$).

Maximum tetanic tension (Fig. 3B) was twice as high in *O. bruceata* ($13.9\pm1.0\,N\,cm^{-2}$, mean ± S.D., at temperatures from 15 to 20 °C) as in *M. sexta* ($7.0\pm0.8\,N\,cm^{-2}$ at temperatures from 20 to 42 °C; the difference between means is significant at $P<0.0001$).

These data show that *O. bruceata* muscle contracts more slowly but generates more force than does *M. sexta* muscle. As a result of these nearly symmetrical differences in contractile speed and tension, the maximum instantaneous power output of *O. bruceata* at 15–20 °C was as high as that of *M. sexta* at temperatures up to about 35 °C (Fig. 3C). At temperatures above 35 °C, the best *M. sexta* preparations attained $160–202\,W\,kg^{-1}$; the latter figure is nearly twice the highest power output attained by *O. bruceata*. Because maximum instantaneous power derived from force–velocity data is about twice the maximum power output achieved during normal cyclical contraction (Josephson, 1993), a peak instantaneous performance of $160–202\,W\,kg^{-1}$ for *M. sexta* agrees closely with the $90\,W\,kg^{-1}$ continuous power output that Stevenson and Josephson (1990) measured for *M. sexta* flight muscle during sinusoidal contraction at 40 °C.
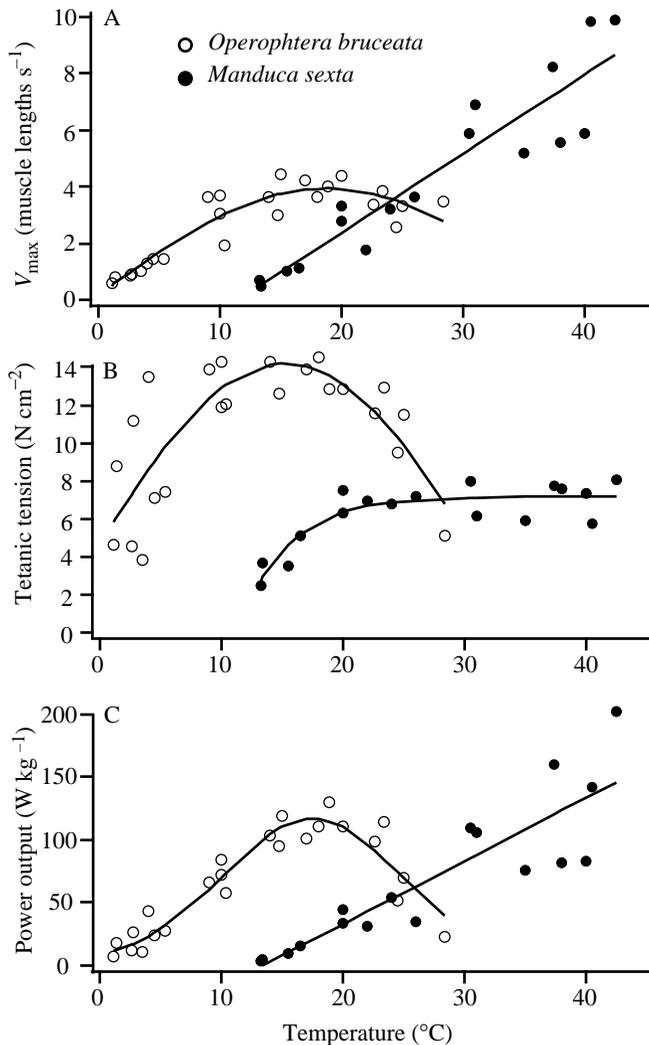
Fig. 3. Maximum shortening velocity V_{max} (A), tetanic tension (B) and instantaneous power output (C) as a function of temperature in flight muscle of *O. bruceata* and *M. sexta* moths.

Differences in tetanic force between *O. bruceata* and *M. sexta* muscle are not a result of differences in the proportional composition of myofibrils. Electron micrographs of cross sections of the DLFM (J. H. Marden, unpublished data; see also Fig. 1.28 in Heinrich, 1993) show that myofibrils constitute a similar proportional area in both species.

The shape of the force–velocity relationship, as measured by the power ratio (maximum power estimated from the force–velocity relationship divided by V_{max}; Marsh and Bennett, 1986), was quite linear in both species (Figs 2, 4). Concavity (indicated by a power ratio less than 0.25) increased significantly with increasing muscle temperature in *O. bruceata* (slope significantly different from zero, $P=0.017$), but not in *M. sexta* ($P=0.75$; Fig. 4). These results differ from the observation of less curved force–velocity relationships in cold-adapted fish (Johnston and Altringham, 1985; Johnson and Johnston, 1991), where this pattern has been interpreted as one of the ways in which cold-adapted species increase their muscle power output at cold temperatures.
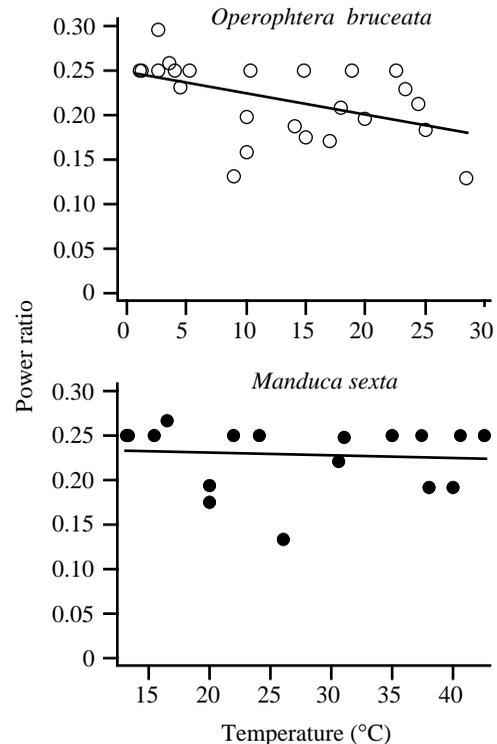

Fig. 4. Curvature of the force–velocity relationship (as characterized by the power ratio; Marsh and Bennett, 1986) as a function of muscle temperature. Concavity (power ratio<0.25) increases significantly with increasing temperature in *O. bruceata*, but not in *M. sexta*.

The temperature-sensitivity of muscle activation and deactivation was dramatically different between the two species. Time to peak tension (TTP) and time to half-relaxation (THR) during isometric twitches were minimal and fairly temperature-insensitive ($Q_{10}<1.3$) for *M. sexta* muscle at temperatures of 30–40 °C and for *O. bruceata* muscle at temperatures of 15–30 °C (Fig. 5). At temperatures below 30 °C in *M. sexta* and below 15 °C in *O. bruceata*, TTP and THR were highly temperature-sensitive ($Q_{10}>2$).

It is interesting to note how similar the values of TTP and THR (Fig. 5) are for the two species at their respective optimal temperatures for tetanic force development and shortening velocity (Fig. 3). This apparent conservation of muscle activation and deactivation rates at optimal temperatures is similar to the conservation of substrate affinity (K_m) in metabolic enzymes of fish from different thermal environments (Hochachka and Somero, 1984). In each of these cases, an evolutionary shift in temperature-sensitivity has maintained a functional characteristic at a constant value in different species operating at their different optimal temperatures.

Discussion

The winter-flying ectothermic moth *O. bruceata* has flight muscle contractile characteristics that are very different from those of the warm-adapted endothermic moth *M. sexta*. *O. bruceata* muscle appears to compensate for a relatively low

Table 1. *Morphometric data and estimates of induced power output required for flight*

Animal	N	Body mass (mg)	FMR	Wing span (cm)	P_{ind} for flight (μW)	Muscle mass-specific P_{ind} for flight (W kg^{-1})	Number of eggs
Operophtera bruceata males	6	11.7±2.9	0.41±0.04	3.24±0.18	27.9±10.1	5.7±1.0	–
Operophtera bruceata females	4	43.7±2.6	–	–	138.0*	–	143±18
Manduca sexta males	2	1473±180	0.30±0.03	9.7±0.17	12960±2139	29.5±1.9	–
Manduca sexta females	4	1772±174	0.23±0.02	10.4±0.25	16032±2448	39.1±4.0	–

FMR is the ratio of flight muscle mass to total body mass (estimated as in Marden and Chai, 1991).
Induced power P_{ind} was calculated as in Marden (1990).
*P_{ind} for a hypothetical winged female with a 4.65 cm wing span (see text for further explanation).
Values are means ± S.D.

maximum shortening velocity by having a relatively high capacity to generate tension, such that maximal instantaneous power output at optimal temperatures in *O. bruceata* (100–120 W kg^{-1} at 15–20 °C) is equivalent to that of *M. sexta* at temperatures below 35 °C (Fig. 3). Only at the high thoracic temperatures that *M. sexta* typically maintains during flight (40–46 °C; Heinrich, 1971) does *M. sexta* muscle generate substantially more power (up to 202 W kg^{-1} instantaneously or 90 W kg^{-1} continuously; Stevenson and Josephson, 1990).

Comparisons of muscle from cold- *versus* warm-adapted fish have found quite different patterns of thermal adaptation of contractile performance. Fish muscle displays perfect evolutionary temperature compensation for force generation (i.e. species adapted to different thermal regimes show equivalent tetanic tension at their normally experienced body temperature), no temperature compensation for unloaded (maximum) contraction velocity (V_{max}), but some compensation for loaded contraction velocity (i.e. decreased curvature of the force–velocity relationship; Johnston and Brill, 1984; Johnston and Altringham, 1985; Langfeld *et al.* 1989; Johnson and Johnston, 1991). In contrast, flight muscle from cold-adapted *O. bruceata* moths generates twice the tetanic tension of muscle from warm-adapted *M. sexta* moths (Fig. 3B), has a higher unloaded contraction velocity over most of the range of temperatures where muscle from both species is excitable (Fig. 3A) and shows greater rather than lesser curvature of the force–velocity relationship (Figs 2, 5).

In addition to possessing cold-adapted muscle, morphological features also contribute greatly to the ability of *O. bruceata* moths to fly over a broad range of body temperatures, down to or even slightly below 0 °C. This can be demonstrated by combining morphological (Table 1) and muscle power output data (Fig. 3C) from *O. bruceata* and *M. sexta* moths in order to estimate the range of temperatures over which they have sufficient muscle power output for flight. Maximum instantaneous power output as measured in this study is about half of the continuous power output that a muscle achieves during normal cycles of contraction and relaxation (Josephson, 1993; R. D. Stevenson, personal communication). Furthermore, approximately half of the

mechanical power output of flying insects constitutes the induced power output (P_{ind}) necessary to accelerate air downwards at a rate sufficient to counteract body weight (Ellington, 1991). Thus, data presented in Fig. 3C for instantaneous power output can be multiplied by 0.25 (i.e. divided in half twice) to estimate roughly how available P_{ind} varies as a function of temperature. By plotting on the same axes an estimate of the temperature-independent minimum P_{ind} required for these moths to become airborne (Marden, 1990),
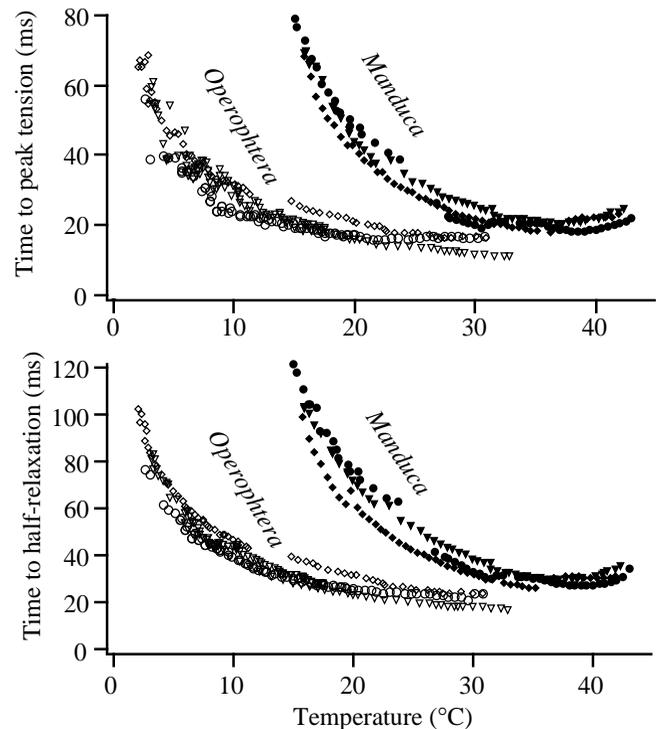

Fig. 5. Characteristics of isometric twitches in *O. bruceata* (open symbols) and *M. sexta* (filled symbols). Muscles from individual moths (three of each species) are represented by different symbol types. Time to peak tension measures the time from stimulation to attainment of peak tension; time to half-relaxation measures the interval between peak tension and relaxation to half of peak tension.
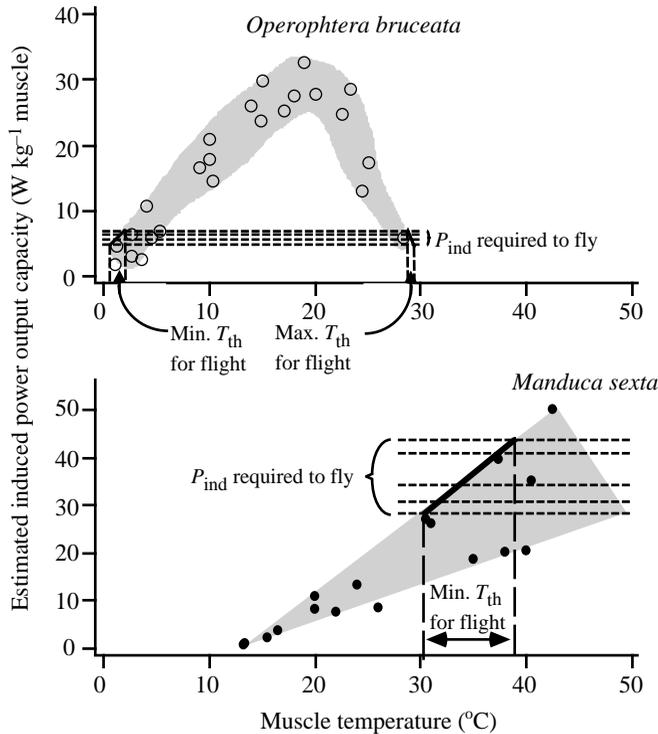
Fig. 6. Estimated capacity for induced power output (P_{ind}) as a function of flight muscle temperature in *O. bruceata* and *M. sexta* moths. Horizontal dashed lines show the temperature-independent minimum P_{ind} necessary for takeoff for a sample of individuals from each species (Table 1). *O. bruceata* males require a muscle mass-specific P_{ind} of 5–8 W kg^{-1} to offset their body mass, which the best muscle preparations achieved at temperatures of 1–29 °C. *M. sexta* moths require 28–31 W kg^{-1} (males) or 35–43 W kg^{-1} (females), which the best *M. sexta* preparations achieved at temperatures between 30 and 38 °C. T_{th}, thoracic temperature.
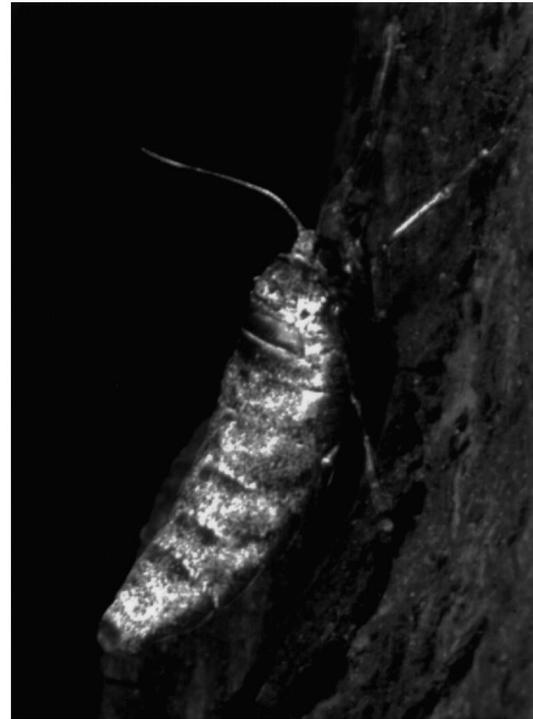

Fig. 7. *O. bruceata* (approximately 1 cm in length) female perched on a tree trunk while emitting male-attracting pheromones. All females of this and many other geometrid species are wingless and flightless; their entire body cavity is filled with eggs.

a graphical model can be constructed that shows the range of muscle temperatures over which flight is possible (Fig. 6). The relatively long wings and high flight muscle ratio (FMR; Marden, 1987) of *O. bruceata* males (Table 1) reduce their P_{ind} requirement (horizontal dashed lines in Fig. 6), thereby extending the range of temperatures over which their cold-adapted muscles can supply sufficient power for them to become airborne. The limits of temperature at which available P_{ind} from the best muscle preparations exceeds P_{ind} required for flight (Fig. 6) closely approximate both the minimum and maximum temperatures at which most *O. bruceata* males can fly (Heinrich and Mommsen, 1985; B. Heinrich, personal communication) as well as the range of minimum temperatures for which different *M. sexta* individuals can take off (Heinrich, 1971; R. D. Stevenson, personal communication).

For *O. bruceata* males, reductions in wing size or FMR would raise the muscle mass-specific P_{ind} required for flight and thereby reduce the range of temperatures over which flight is possible. Evolution of large wings and a high FMR has allowed these males to fly in search of mates during a season that is physiologically challenging (weather that is highly

variable and often cold), but ecologically beneficial, because most insectivorous birds, bats, insects and spiders have migrated, died or become inactive.

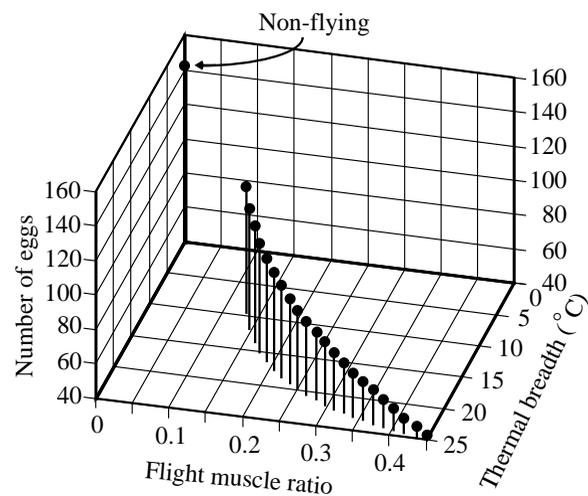*O. bruceata* females have evolved a morphology quite

Fig. 8. Three-dimensional plot of a model showing how fecundity would decline with increasing investment in flight muscle and increasing thermal breadth for flight in hypothetical flying *O. bruceata* females. Coordinates for extant flightless *O. bruceata* females are shown by the point at the top left labeled 'non-flying'.

different from that of *O. bruceata* males. *O. bruceata* females are wingless and flightless; they move about very little while perched on tree trunks, emitting pheromones that attract males (Fig. 7). These females show an extreme strategy for insect body design: from the anterior margin of their thorax to the posterior tip of their abdomen, their body cavity is completely packed with fully developed eggs; they contain no other large internal organs or tissues. *O. bruceata* females contain an average of 143 eggs that constitute 63 % of their total body mass (Table 1).

For *O. bruceata* females to re-evolve the ability to fly, not only would they need wings but they would require a substantial reduction in fecundity. On the basis of the scaling of wing length in *O. bruceata* males [length = 0.0242×body mass +1.306 (r^2=0.48, P=0.004), where length is in cm and body mass is in mg], a hypothetical winged *O. bruceata* female would have a wing length of 2.32 cm, and would require a P_{ind} of 3.2 W kg^{-1} body mass to fly. Muscle mass-specific power output required for flight would vary depending on the proportion of total body mass devoted to flight muscle. An FMR of 0.13, barely sufficient to permit flight in moths (Marden, 1987), would require a P_{ind} of approximately 24 W kg^{-1} muscle, a level of power output that *O. bruceata* muscle can achieve only between temperatures of 13 and 22 °C (Fig. 6). Assuming that flight muscle would take the place of an equal mass of eggs, such a female could carry 114 eggs, a 17 % reduction in fecundity compared with a non-flying female. An FMR of 0.45 would represent the other extreme of investment in flight muscle, and would require a P_{ind} of approximately 7 W kg^{-1} muscle, which can be achieved between temperatures of 3 and 27 °C (Fig. 6; this is equivalent to the thermal breadth for flight in males). However, such strong flight capability would allow only 40 eggs to be carried, a reduction in fecundity of 82 % compared with non-flying females. Intermediate levels of investment in flight muscle would allow intermediate levels of fecundity and thermal breadth for flight (Fig. 8).

The model presented in Fig. 8 shows that the potential thermal breadth for flight in female geometrid moths is determined by a trade-off between allocation of mass to flight muscle *versus* eggs. These data could be used to refine and extend models for the evolution of flightlessness (Roff, 1990), which previously lacked data for the temperature-sensitivity of muscle performance and flight ability of ectothermic insects. The optimal body design for a female ectothermic insect will thus depend on a complex function of weather conditions that typically occur during the adult phase, the trade-off between fecundity and thermal breadth for flight and the benefits of adult female dispersal (as well as the intensity of aerial predation; Marden and Chai, 1991). Females of geometrid species that fly in winter are predominantly flightless, whereas females of summer-flying geometrids are predominantly flight-capable (Covell, 1984; Skinner, 1984). This diversity of body design and ecology indicates that geometrid moths might provide a test of the model presented in Fig. 8 and might be an ideal system for forming and testing hypotheses about the interplay between physiology, ecology and the evolution of insect body design and life history.

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