

Distance and force production during jumping in wild-type and mutant *Drosophila melanogaster*

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Summary

In many insects renowned for their jumping ability, elastic storage is used so that high forces can be developed prior to jumping. We have combined physiological, behavioural and genetic approaches to test whether elastic energy storage makes a major contribution to jumping in *Drosophila*.

We describe a sensitive strain gauge setup, which measures the forces produced by tethered flies through their mesothoracic legs. The peak force produced by the main jumping muscle of female flies from a wild-type (Canton-S) strain is $101 \pm 4.4 \mu\text{N}$ [and this is indistinguishable from a second wild-type (Texas) strain]. The force takes 8.2 ms to reach its peak. The peak force is not affected significantly by altering the leg angle (femur–tibia joint angle) in the range of $75\text{--}120^\circ$, but the peak force declines as the leg is extended further.

Measurements of jumping ability (distance jumped) showed that female *Drosophila* (with their wings removed) of two wild-type strains, Canton-S and Texas, produced jumps of 28.6 ± 0.7 and 30.2 ± 1.0 mm (mean \pm S.E.M.). For a female wild-type *Drosophila*, a jump of 30 mm corresponds to a kinetic energy of 200 nJ on take-off (allowing 20% of the energy to overcome air resistance). We develop equations of motion for a linear force–time model of take-off and calculate that the time to take-off is 5.0 ms and the peak force should be $274 \mu\text{N}$ ($137 \mu\text{N leg}^{-1}$).

We predicted, from the role of octopamine in enhancing muscle tension in several locust muscles, that if stored elastic energy plays no part in force development, then genetic manipulation of the octopaminergic system would directly affect force production and jumping in *Drosophila*. Using two mutants deficient in the octopaminergic system, *Tbh^{nM18}* (*M18*) and *TyrR^{hono}* (*hono*), we found significantly reduced jumping distances (20.7 ± 0.7 and 20.7 ± 0.4 mm, respectively) and force production (52% and 55%, respectively) compared with wild type.

From the reduced distance and force production in *M18*, a mutant deficient in octopamine synthesis, and in *hono*, a tyramine/octopamine receptor mutant, we conclude that in *Drosophila*, as in locusts, octopamine modulates escape jumping. We conclude that the fly does not need to store large quantities of elastic energy in order to make its jump because (1) the measured and calculated forces agree to within 40% and (2) the reduction in distances jumped by the mutants correlates well with their reduction in measured peak force.

Key words: *Drosophila*, jumping, tyramine, octopamine, tergal depressor of trochanter, tergotrochanteral muscle, *M18*, *hono*, biomechanics.

Introduction

In many groups of insects, jumping is part of the escape behaviour, with survival dependent on rapid take-off. In high-performance jumpers, for example Orthoptera (including locusts and bush crickets), froghoppers and fleas, the biomechanics of jumping has been extensively analysed (Bennet-Clark and Lucey, 1967; Bennet-Clark, 1975; Burrows, 2003; Burrows and Morris, 2003). In all these groups of insects, elastic energy storage makes an important contribution to the distance they can jump. Locusts and a stick insect, *Prosarthria*, store energy in the leg by co-contracting the extensors and flexors (Heitler, 1974; Bennet-Clark, 1975;

Burrows and Wolf, 2002), while energy is stored in a thoracic resilin pad in fleas (Bennet-Clark and Lucey, 1967); a similar thoracic store has been proposed for froghoppers (Burrows, 2003). Rapid release of energy is achieved through release of a catch (locust, froghopper) or through an increase in the mechanical advantage of the extensor muscle (bush crickets; Burrows and Morris, 2003), and it is the sudden release of stored energy that allows the insect to travel a long way.

However, the fruit fly *Drosophila melanogaster*, with a mass of 1 mg and a body size between that of locusts and fleas, is not noted for its jumping performance. In *Drosophila*, jumping

is normally the prelude to flight and serves merely for the fly to clear the substrate and initiate contractions of the indirect flight muscles; so, the distance jumped is more modest. From this, we hypothesise that *Drosophila* do not need to store large quantities of elastic energy in order to jump.

In *Drosophila*, the jump is produced by extension of the mesothoracic legs, as a result of contraction of the tergal depressor of trochanter muscle (TDT; M66 of Miller, 1950), also known as the tergotrochanteral muscle (Bacon and Strausfeld, 1986). This substantial, triangular, pennate muscle runs between the dorsal surface of the thorax and the proximal end of the trochanter, and its contraction extends the femur because the trochanter and femur are fused (Trimarchi and Schneiderman, 1993). A second muscle, the tibia-levator muscle (TLM), extends the femur-tibia joint during take-off (Trimarchi and Schneiderman, 1993), prolonging the time for which force is applied to the substrate. The TDT is activated by the descending giant fibre from the brain through a mixed electrical-chemical synapse (Blagburn et al., 1999). Many workers have exploited the restrained preparation developed by Tanouye and Wyman (1980), in which the giant fibre is stimulated visually or electrically by electrodes implanted in the eyes or neck, so the physiology of the giant fibre-TDT system is well known. Among these are Trimarchi and Schneiderman (1995a), who showed that excitatory junction potential (EJP) in the TDT is activated with constant latency and amplitude.

In locusts, the contraction of the metathoracic slow extensor tibia muscle (SETi) is modulated both pre- and post-synaptically by octopamine. The overall effect of octopamine is to make the twitch of the isolated muscle stronger, increasing the peak force and narrowing the tension transient (Evans and O'Shea, 1977). During the locust jump, the octopaminergic midline neuron, DUM5A (originally called DUMETI) is activated (Duch et al., 1999), so that octopamine is delivered in time for the contraction to be enhanced. Nonetheless, in locusts, the extension of the leg is not derived directly from muscle contraction but occurs when Heitler's catch is suddenly relaxed to permit rapid release of stored elastic energy (Heitler, 1974). If, in flies, muscle contraction during jumping is directly coupled to leg extension, we predict that interference in the octopaminergic system would be expected to reduce distance jumped as well as force production.

Among mutants known in *Drosophila* to affect the octopaminergic system are *Tbh^{nM18}* (M18; Monastirioti et al., 1996) and *TyrR^{hono}* (*hono*; Kutsukake et al., 2000). The M18 mutation is in the gene encoding tyramine β hydroxylase, which converts tyramine to octopamine. It is a hypomorph with <0.2% of the normal level of octopamine but with elevated ($\times 10$) levels of tyramine (Monastirioti et al., 1996). The *hono* mutation is in the gene for the tyr/oct receptor (also known as the tyramine receptor). This receptor binds tyramine 33 times better than octopamine in cos-7 cells (Saudou et al., 1990). However, when expressed in *Xenopus* oocytes, this receptor couples to different second messenger pathways depending on whether tyramine or octopamine was applied (Robb et al., 1994).

The main aim of the work presented here was to investigate

whether *Drosophila* store energy elastically for jumping. We have tested this by measuring both the distance jumped and the forces produced by the jump muscle (TDT). The distance jumped by unrestrained flies from which the wings had been removed provides a behavioural estimate of the energy used in jumping, which we have used to calculate the force exerted during take-off. The forces produced by the TDT were obtained by an extension of the physiological preparation of Trimarchi and Schneiderman (1995a). Our calculations show good agreement between behavioural and physiological estimates of force in the wild-type flies, from which we conclude that *Drosophila* do not store energy elastically for jumping. We predict that in the absence of stored elastic energy, defects of the octopaminergic system would lead to direct effects on force production and jumping.

We therefore compared our wild-type data with the distances jumped and forces produced in two *Drosophila* mutants of the octopaminergic system: *Tbh^{nM18}* (M18) and *TyrR^{hono}* (*hono*). Both mutants jump less well than the wild type and generate less force. Our data are consistent with the hypothesis that elastic energy storage is not a major factor in fly jumping.

Materials and methods

Flies

Drosophila melanogaster Meigen were reared at constant temperature (25°C) on a yeast-sugar-agar medium (Carpenter, 1950). Canton-S (CS) and Texas (TX) wild-type flies were taken from laboratory culture. *Tbh^{nM18}* (M18) and *TyrR^{hono}* (*hono*) mutations were the kind gifts of Maria Monastirioti and Mayako Kutsukake.

Jumping behaviour

Newly hatched flies were isolated and left for two days. They were then lightly anaesthetised using carbon dioxide and their wings removed. The flies were weighed in batches of 5–8, and their mean masses calculated. After a recovery period of 30 min, each fly was placed on lined paper. Flies were stimulated to jump by moving a fine paintbrush towards them from the rear. The flies were not touched but jumped in response to the visual and air movement stimuli. The distances of the first 5–8 full jumps were recorded; we ignored the push-ups or small tumbling responses (Kaplan and Trout, 1974) that were sometimes elicited by these stimuli.

Mutants were always tested at the same times as wild-type controls: either CS or TX of the same gender. Comparisons between fly strains were assessed using *t*-tests, and significance was $P < 0.05$. Unless otherwise stated, results are means \pm S.E.M.

Muscle force determination

Two-day-old flies were lightly anaesthetised with carbon dioxide and the dorsal surface of the thorax was attached to a fine tungsten needle using rubber solution. All legs, except the right mesothoracic leg, were glued either to the tungsten needle or to the fly's abdomen. The flies were then allowed to recover for ~30 min.

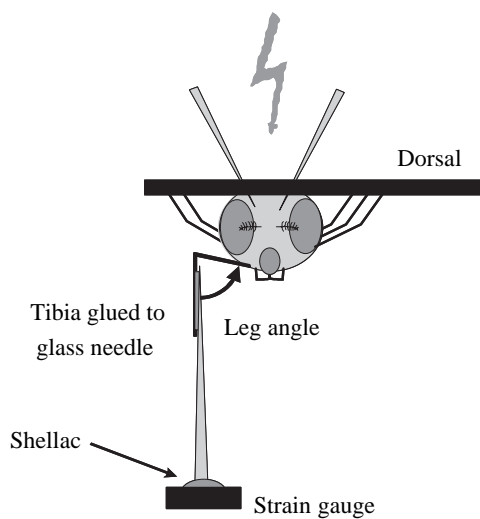


Fig. 1. Preparation for recording the force produced by the tergal depressor of trochanter (TDT) muscle. A glass needle was bonded to a strain gauge using shellac. Each fly was mounted on a tungsten needle mounted on a micromanipulator. Its tibia was set in line with the glass needle to which it was glued. Electrical stimuli were delivered to the giant fibre system by two tungsten needle electrodes in the neck or head. The contraction of the TDT causes the fused trochanter-coxa to press downwards against the strain gauge.

The tip of a glass capillary, drawn to a point, was mounted perpendicularly using shellac on a sensitive strain gauge, AE801 [MEMSCAP (formerly Capto AS), Skoppum, Norway]. The output was connected in a Wheatstone bridge circuit, dc amplified and recorded on a Gould recording oscilloscope (Model 1604) or PC using a National Instruments (Austin, TX, USA) PCI-6052E analog-digital card and DasyLab software (Bedford, NH, USA), sampling at 100 kHz.

The tungsten needle holding the fly and the strain gauge were mounted on micromanipulators arranged so that the tibia was parallel to the glass needle on the strain gauge. Cellulose nitrate glue was used to stick the tibia of the mesothoracic leg to the glass needle, leaving the femur-tibia joint free of any glue (Fig. 1).

Finally, the fly was impaled with two sharpened tungsten needles in the neck (or less often, in the eyes). These were connected to a stimulator, which generated variable amplitude pulses of 25, 50 or 100 μ s, separated by 500–1500 ms. When stimulated above threshold, the giant fibre was activated, causing the TDT muscle to contract. The force produced by the muscle was transmitted to the femur and thence to the tibia. As the tibia was stuck to the glass needle, the strain gauge was activated.

The output of the strain gauge was calibrated by pressing on it with glass capillaries, drawn to different lengths. The deflection of glass capillary was recorded under a travelling microscope, along with the voltage from the dc amplifier. The force needed to deflect the capillary was determined by hanging short coils of copper wire, of known mass, on their tips and recording the deflection of the glass.

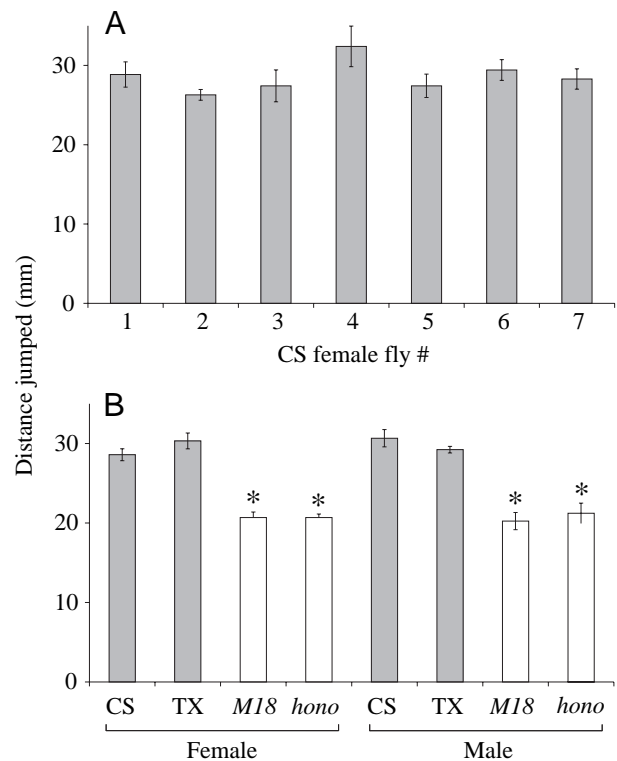


Fig. 2. The distance jumped by *Drosophila* from which the wings had been removed. (A) Repeated jumps from seven individual Canton-S (CS) females. (B) Jumps from two wild-type strains [Canton-S (CS) and Texas (TX)] and two mutants: *M18*, which synthesises no octopamine, and *hono*, a null mutant of the tyr/oct receptor. Both mutants jump significantly less far (* $P<0.001$). Values are means \pm S.E.M.

The spring constant of the strain gauge is 2 kN m^{-1} (Capto data sheet); for the peak force we measured, 300 μ N, the deflection will be 150 nm, so that the measurements are effectively isometric.

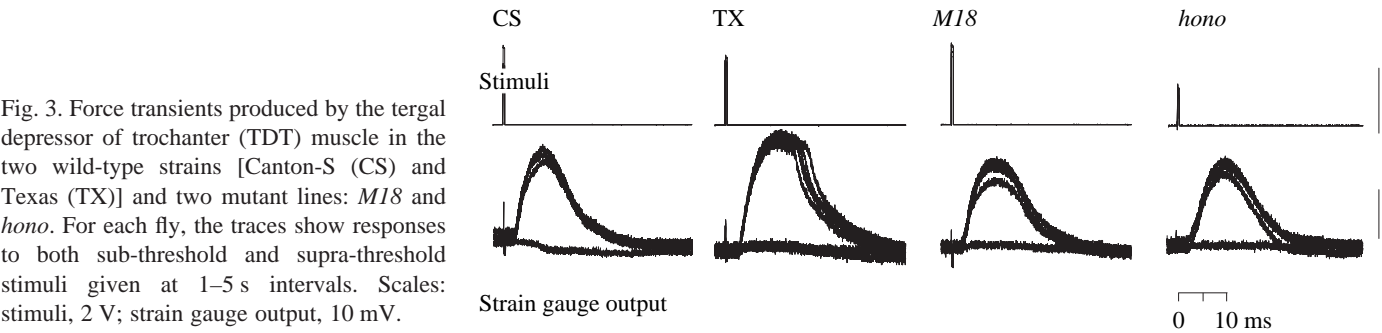
Results

Jumping

Individual female CS flies from which the wings had been removed produced repeated jumps consistently in the range of 28–32 mm, and the S.E.M. averaged 1.5 mm (Fig. 2A). Overall, the mean distance jumped by female CS flies was 28.6 ± 0.7 mm, while TX females jumped 30.2 ± 1.0 mm, which is not significantly different from the CS flies (Fig. 2B).

We found no evidence for any differences in jumping ability between males and females, even though females are significantly heavier than males (CS ♀, 1.13 ± 0.03 mg; ♂, 0.81 ± 0.03 mg).

For flies from both the mutant lines we tested, the distance jumped is reduced compared with the wild type; female *M18* and *hono* mutants jumped 20.7 ± 0.7 and 20.7 ± 0.4 mm, respectively. This is two-thirds of the distance jumped by the wild-type flies, (Fig. 2B) and is statistically significant (*t*-test:



CS vs *M18*, $t_{12d.f.}=7.8$, $P<0.001$; CS vs *hono*, $t_{10d.f.}=8.15$, $P<0.001$). For the mutants, there is no difference in distances jumped between male and female flies, even though the females are again heavier (*hono* ♀, 1.14 ± 0.03 mg; ♂, 0.85 ± 0.04 mg; *M18* ♀, 1.10 ± 0.03 mg; ♂, 0.81 ± 0.04 mg).

Force measurement

Representative force–time traces for the forces generated by the TDT muscle by female wild-type, *M18* and *hono* flies are shown in Fig. 3, where sub- and supra-threshold stimuli were applied to the stimulating electrodes. The stimulus–response latency is 2.3 ± 0.2 ms ($N=8$ CS flies), probably mostly due to the neural conduction delay between the head and the neuromuscular junction. The force occurs in an all-or-nothing fashion. It does not seem to habituate quickly when repetitive 0.1–10 Hz stimuli are applied. In the typical response, the force rises rapidly, reaches $88\pm2\%$ of its maximum after 5 ms and peaks after 8.2 ± 0.5 ms. It declines more slowly, reaching 50% of peak force by 14.5 ± 0.6 ms, and becomes indistinguishable from the baseline by 25.0 ± 1.4 ms.

When the position of the micromanipulator holding the strain gauge was adjusted so that the peak force was maximised for each individual, it became clear that mutant flies produced less peak force than the wild type (Fig. 3). This is confirmed by the summarised data (Fig. 4), where the means for *M18* and *hono* are 52% and 55%, respectively, of the CS wild type. These reductions are both significant at the 0.1% level (female CS vs *M18*, $t_{15d.f.}=5.6$; CS vs *hono*, $t_{16d.f.}=5.6$). We have found no significant differences in the latency, time to maximum force development or half-width of the force transient when the *M18* and *hono* mutants are compared with wild-type flies. No significant difference was found between the CS and TX wild types in any parameter.

The leg angle is critical for force production; as the TDT muscle contracts, the leg straightens and the angle of the femur–tibial joint increases towards 180° (see Fig. 1). Over the range of $75\text{--}120^\circ$, the averaged peak force is constant at 101 ± 4.4 μN (Fig. 5). As the leg is straightened by adjusting the micromanipulators, the isometric force declines, until zero force is produced at $\sim 160^\circ$.

Analysis

We have determined the distance jumped by unrestrained

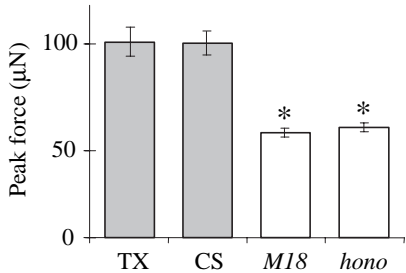


Fig. 4. Mean peak force produced by the tergal depressor of trochanter (TDT) muscle in female flies. The peak force is significantly less in the *M18* and *hono* mutants than in the Canton-S (CS) and Texas (TX) wild-type strains (* $P<0.001$). Values are means \pm S.E.M.

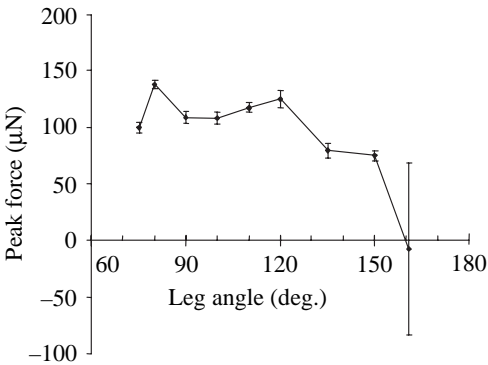


Fig. 5. Mean peak force from 42 measurements from six Canton-S (CS) flies as the angle between coxa and tibia was adjusted. The peak isometric force is constant at ~ 100 μN from $75\text{--}120^\circ$ and then declines to zero force at 160° . At this angle, it was hard to adjust the micromanipulators holding the fly without distortion of the joints, which accounts for the large standard error.

flies from which the wings had been removed. From this distance, we now calculate the work done during take-off and use this to estimate the minimum force required to propel the fly. This force will then be compared with our direct TDT measurements.

Our analysis of biomechanics of jumping follows the outline in the recent review by Alexander (2003). Neglecting air resistance, the distance (d) jumped by a fly is determined solely by its velocity on take-off (v) and the angle of take-off (α):

$$d = \frac{v^2}{g} \cdot \sin(2\alpha), \quad (1)$$

where g is the acceleration due to gravity.

At $\alpha=45^\circ$, $\sin(2\alpha)$ is the maximum, 1, and so the distance jumped for a given velocity is also a maximum. At 45° :

$$d = \frac{v^2}{g} \Leftrightarrow v^2 = g \cdot d \Leftrightarrow v = \sqrt{g \cdot d}, \quad (2)$$

and the work done during the jump [kinetic energy (KE)] can be calculated from the velocity and mass (m) of the fly as:

$$KE = \frac{1}{2} m \cdot v^2 = \frac{1}{2} m \cdot g \cdot d. \quad (3)$$

In small insects, air resistance is an important energy loss, so that the actual KE at take-off is larger than equation 3 predicts. In order to estimate the actual KE at take-off, we note that Bennet-Clark and Alder (1979) projected *Drosophila* (from which the wings had been removed) vertically upwards in air and *in vacuo*. For flies projected upwards 100 mm, 20% of the energy was lost to air resistance. If we assume that the same loss occurs in our experiments, the KE at take-off, allowing for air resistance (KE_{air}), will be 1.25 times as much. This would require the take-off velocity to be increased by $\sqrt{1.25}$ ($=1.1$):

$$KE_{\text{air}} = \frac{1.25}{2} m \cdot g \cdot d \quad (4)$$

and

$$v_{\text{air}} = \sqrt{1.25 \cdot g \cdot d}. \quad (5)$$

For female flies travelling a distance of 30 mm, allowing for air-resistance, with a mass of 1.1 mg and taking gravity as 10 m s^{-2} , the take-off velocity is 0.61 m s^{-1} and the KE is 206 nJ. For the mutant flies, travelling only 20 mm, take-off velocity is 0.50 m s^{-1} and the KE is 137 nJ.

For a fly jumping off the ground, accelerating from rest, force can only be applied while the leg is touching the ground. On the assumption of a constant force (CF) being applied, the KE is the product of the extension of the leg (s) and the force (F), so that:

$$F = \frac{KE_{\text{air}}}{s}. \quad (6)$$

Equation 6 can be rewritten in terms of measured variables, using equation 4:

$$F = \frac{KE_{\text{air}}}{s} = \frac{1.25 \cdot m \cdot g \cdot d}{2s}. \quad (7)$$

With constant force, and therefore constant acceleration, the time (t) to extend the leg is:

$$t = \frac{2 \cdot s}{v_{\text{air}}}. \quad (8)$$

The combined length of the femur tibia and tarsus is

1.36 mm (Miller, 1950). Assuming that, from rest to take-off, the mesothoracic legs extend by 1 mm, the force required to take off is 206 mN and each leg would have to contribute $\sim 103 \text{ mN}$. The duration of the force will be 3.3 ms. If the flies extend their legs less than 1 mm before they leave the ground, the force will be higher and take-off time will be reduced. Mutant flies will need two-thirds of the force ($135 \mu\text{N}$) and will take off in 4.0 ms.

However, our force measurements show that the force produced by the TDT is not constant. Over the 5 ms that it takes for the fly to leave the ground (Trimarchi and Schneiderman, 1995b), the TDT force increases, approximately linearly with time. After 5 ms, the force starts to fall below the linear relationship (Fig. 3), but by then the fly will have left the ground. Appendix 1 derives the equations of motion for a linear relationship between force and time. Equations A6 and A7 give the force and time at the take-off point as 274 mN at 5.0 ms for the wild-type fly (137 mN per leg) and 183 mN at 6.1 ms for the mutants.

Discussion

Whereas elastic energy storage is important for long-distance jumpers, we have examined jumping in an insect that is not noted for the distance it can jump. Normally, *Drosophila* start to fly as soon as they are airborne, as shown in high-speed filming (Trimarchi and Schneiderman, 1995b). However, we have found that two wild-type strains of *Drosophila* females (from which the wings have been removed) jump 30 mm (~ 5 – 6 body lengths). The lighter males jump an equal distance, as would be expected if their muscle mass/body mass ratio is the same as that of the females (Alexander, 2003). We calculate that the minimum kinetic energy (KE) used to jump this far is $\sim 200 \text{ nJ}$, assuming a take-off angle of 45° and taking air resistance into account. If the fly takes off at a sub-optimal angle, the KE required to travel 30 mm is increased. For example, at an angle of 25° (or 65°), the KE would be increased by a factor of 1.3, i.e. 260 nJ. High-speed film shows flies taking off at 45° (Trimarchi and Schneiderman, 1995b) and 51° (Kaplan and Trout, 1974). Our data give a standard error of $\sim 1 \text{ mm}$, suggesting a consistency in take-off velocities. The calculated take-off velocity, 0.61 m s^{-1} , corresponds well with the filmed speeds of 0.6 – 0.7 m s^{-1} (Trimarchi and Schneiderman, 1995b). The other factor in our KE calculation is air resistance. When Bennet-Clark and Alder (1979) catapulted wingless flies vertically upwards, the loss of KE to air resistance increased with take-off velocity. At 100 mm, their lowest height, 20% of energy was lost. We assumed the same loss for our flies that travelled 30 mm horizontally. The smaller distance may mean that we have over-estimated the loss due to air resistance. If only 10% of the energy is lost to air resistance, the KE at take-off would be 180 nJ rather than 200 nJ.

The take-off time can be calculated from the KE : on the basis of our constant force (CF) model, take-off occurs after 3.3 ms, implying a power of $60 \mu\text{W}$. Our force data show, however,

that the muscle force rises with time, and on the more realistic linear force–time (LFT) model, the duration to take-off is calculated to be 5.0 ms and the mean power reduced to 40 μ W. High-speed photography of *Drosophila* taking off shows that there is 4.9 ± 1.6 ms between the start of the jump and the legs losing contact with the ground (Trimarchi and Schneiderman, 1995b). This agrees very well with our LFT estimate of 5.0 ms derived from the *KE* and from the length of the mesothoracic femur and tibia.

We estimate that the TDT is $\sim 900 \mu\text{m}$ long and, at its insertion on the dorsal cuticle, is $\sim 300 \mu\text{m}$ wide and 150 μm thick. Treating this as a pyramid, its volume will be $13 \times 10^6 \mu\text{m}^3$ and its mass will be 13 μg . The two TDT muscles would then be just over 2% of the mass of the fly. The power output, 40 μW , corresponds to a specific power output of 1.5 W g^{-1} during the period of take-off. Taken over the full time to contract and relax (25 ms), the specific power output of the TDT is lower, but still an impressive 300 mW g^{-1} . This is much higher than the continuous power output of *Drosophila* flight muscle, 80 mW g^{-1} , measured by respirometry (Lehmann and Dickinson, 1997) and is higher than the power output calculated from work loops of the flight muscle of the beetle *Cotinus* (200 mW g^{-1} ; Josephson et al., 2000). The *Drosophila* TDT power output is also larger than the specific power output of locust muscle during jumping, which averages 200 mW g^{-1} but peaks at 450 mW g^{-1} during the contraction (Bennet-Clark, 1975). All of this indicates that jumping is energetically demanding.

The force exerted by the leg of female wild-type flies in the present study was measured to peak at 101 μN at 8.2 ms. This force was with the leg held at an angle of 90° , i.e. with the femur horizontal and the tibia vertical. The peak force produced was not significantly affected by adjustments of the leg angle over a range of $75\text{--}120^\circ$, but as the leg was extended further the force dropped as its mechanical advantage declined. As the mean mass of female flies is 1.1 mg, their weight will be 11 μN . Thus, the peak force exerted by the two legs corresponds approximately to the weight of 20 flies, and the net force is upwards.

If there is no need for a substantial energy storage mechanism, the measured force (101 μN) should agree with the force estimated from the distance data. We calculated above that the *KE* was in the range of 180–260 nJ and that the value of 200 nJ corresponded well with data from high-speed video. On the assumption of a *CF*, this *KE* gives a force of 100 μN for each leg, with a take-off time of 3.3 ms. If force increases linearly with time (LFT model), this *KE* gives a peak force of 137 $\mu\text{N leg}^{-1}$ and take-off in 5.0 ms. In both models, force is proportional to *KE* and so the range of *KE* corresponds to force in the region of 90–180 μN . The measured force peaks at 100 μN , at the lower end of the range. While the agreement here is good, we need to consider four factors affecting the measured force. (1) The fly produces a force that acts downwards and forwards, while we measured the downwards component only. If we had measured in the direction of take-

off, optimally 45° , the force would be larger by a factor of $\sqrt{2}$ ($=1.414$), increasing the force by 40 μN . (2) The fly takes off in 4.9 ms and the force at 5 ms was 88% of the peak, i.e. a reduction of $\sim 10 \mu\text{N}$. (3) As the fly extends its leg, the mechanical advantage decreases so that, as the leg angle reaches 150° , the force has been reduced by 25 μN . (4) Our measurements were done isometrically, and this is likely to produce a maximal estimate of muscle force, as during jumping the muscle contracts and so will produce less force as its thin filaments slide together (Gordon et al., 1966). Since the TDT inserts on the trochanter at the thoracic end (Miller, 1950; Trimarchi and Schneiderman, 1993; Peckham et al., 1990), it is unlikely to contract more than 5%. Assuming that the TDT, like other muscles, starts at the optimal filament position, a 5% change will not substantially change the sarcomeric thick–thin filament overlap and therefore the force will remain similar to that measured at the isometric level. On balance, these factors indicate that the measured force has a scope of 60–140 μN , which agrees well with the range (90–180 μN) estimated from jumping. We therefore conclude that no substantial energy storage is needed to account for the distance jumped by *Drosophila*.

This conclusion is supported by analysis of the *M18* and *hono* mutant flies, where the measured force is reduced to 52 and 55%, respectively. Our calculations show that distance travelled in a jump is proportional to the force produced (equations 7 or A4), so we expect that the mutant flies should travel 52 and 55% of the wild-type distance. This is consistent with the measured jump distance reductions to 66% for both mutants. If an elastic storage mechanism dominated the jump, we would not have expected the force to be proportionately reduced in these mutations of the aminergic systems.

The *M18* flies synthesise no octopamine but accumulate excess tyramine, so our observations suggest that *Drosophila*, like locusts (Evans and O'Shea, 1977), enhance their muscle contraction through the action of octopamine at the leg nerve–muscle synapse. The proportional reduction in jump distance (and hence predicted force) with measured force suggests that, unlike locusts, flies do not uncouple the contraction of the muscle from leg extension through elastic energy storage. The amine could be delivered as a hormone in the blood or locally from terminals of an unpaired medial neuron. Octopamine immunoreactive fibres have been shown on the TDT of another dipteran, the blowfly *Calliphora* (Schlurmann and Hausen, 2003), and on prothoracic muscles of *Drosophila* (Rivlin et al., 2004), but the innervation of the TDT muscle in *Drosophila* is not completely known. Exogenous octopamine also increases the size of the EJP at the dorsal internal oblique muscles in the *Drosophila* larval body wall by 15%, while tyramine produced a 15% reduction (Kutsukake et al., 2000; Nagaya et al., 2002). If tyramine, rather than octopamine, were an excitatory modulator, we would not expect the *M18* flies to jump less far or generate less force, as they have higher levels of tyramine than the wild types.

Although *hono* gene expression has previously only been found in the adult central nervous system (Arakawa et al., 1990; Saudou et al., 1990; Hannan and Hall, 1996; Kutsukake et al., 2000), our data indicate a role for *hono* in the adult peripheral nervous system, specifically at the TDT neuromuscular junction. An explanation for our observations of reductions in jumping distance and force in *hono* is that octopamine action at the neuromuscular junction is blocked. Thus, the *M18* mutant fails to jump as far as the wild type because of the lack of octopamine, while the *hono* mutant does so because it cannot respond to octopamine. However, a problem with this explanation is the proposal (Kutsukake et al., 2000) that *hono* is a mutation in a pure tyramine receptor. Two lines of evidence support this. First, the receptor is much more sensitive to tyramine than to octopamine (Saudou et al., 1990). Second, in *hono*, the larval neuromuscular junction is modulated by tyramine but not octopamine (Nagaya et al., 2002). Our data favour the proposal that the *hono* mutation is in a dual tyr/oct receptor, possibly coupling to different systems (Robb et al., 1994; Reale et al., 1997). If octopamine is acting at a separate receptor to *hono*, the reductions in force and jump distance are hard to understand because the octopaminergic pathways should be the same as the wild type.

Appendix 1. Equations of motion for force increasing linearly with time

Let the force at time t be $F(t)$, producing an acceleration $a(t)$, which can be represented then as $\alpha \cdot t$, where α is a constant:

$$F(t) = m \cdot a(t) = m \cdot \alpha \cdot t. \quad (\text{A1})$$

Then, the velocity (v) at time t is:

$$v = \int a \cdot dt = \int \alpha \cdot t \cdot dt = \frac{\alpha \cdot t^2}{2}. \quad (\text{A2})$$

The distance, s , at time t is:

$$s = \int v \cdot dt = \int \frac{\alpha \cdot t^2}{2} dt = \frac{\alpha \cdot t^3}{6}. \quad (\text{A3})$$

Substituting $F(t)/m$ in equations A2 and A3:

$$v = \frac{F(t) \cdot t}{2 \cdot m} \Leftrightarrow t = \frac{2 \cdot m \cdot v}{F(t)} \Leftrightarrow t^2 = \frac{4 \cdot m^2 \cdot v^2}{F(t)^2} \quad (\text{A4})$$

and

$$s = \frac{F(t) \cdot t^2}{6 \cdot m}. \quad (\text{A5})$$

Substituting t^2 in A5 using A4:

$$s = \frac{F(t)}{6 \cdot m} \cdot \frac{4 \cdot m^2 \cdot v^2}{F(t)^2} = \frac{2}{3} \cdot \frac{m \cdot v^2}{F(t)} \Leftrightarrow F(t) = \frac{2}{3} \cdot \frac{m \cdot v^2}{s}. \quad (\text{A6})$$

Using equation A6 in A4:

$$t = \frac{2 \cdot m \cdot v}{F(t)} = \frac{2 \cdot m \cdot v}{1} \cdot \frac{3}{2} \cdot \frac{s}{m \cdot v^2} = \frac{3 \cdot s}{v}. \quad (\text{A7})$$

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