RESEARCH ARTICLE

Maximum metabolic rate, relative lift, wingbeat frequency and stroke amplitude during tethered flight in the adult locust *Locusta migratoria*

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SUMMARY

Flying insects achieve the highest mass-specific aerobic metabolic rates of all animals. However, few studies attempt to maximise the metabolic cost of flight and so many estimates could be sub-maximal, especially where insects have been tethered. To address this issue, oxygen consumption was measured during tethered flight in adult locusts *Locusta migratoria*, some of which had a weight attached to each wing (totalling 30–45% of body mass). Mass-specific metabolic rate increased from $28\pm2\,\mu mol$ $O_2\,g^{-1}\,h^{-1}$ at rest to $896\pm101\,\mu mol\,O_2\,g^{-1}\,h^{-1}$ during flight in weighted locusts, and to $1032\pm69\,\mu mol\,O_2\,g^{-1}\,h^{-1}$ in unweighted locusts. Maximum metabolic rate of locusts during tethered flight ($\dot{M}_{M_{O_2}}$; $\mu mol\,O_2\,h^{-1}$) increased with body mass (M_b; g) according to the allometric equation $\dot{M}_{M_{O_2}}=994M_b^{0.75\pm0.19}$, whereas published metabolic rates of moths and orchid bees during hovering free flight ($\dot{M}_{H_{O_2}}$) are approximately 2.8-fold higher, $\dot{M}_{H_{O_2}}=2767M_b^{0.72\pm0.08}$. The modest flight metabolic rate of locusts is unlikely to be an artefact of individuals failing to exert themselves, because mean maximum lift was not significantly different from that required to support body mass (95±8%), mean wingbeat frequency was 23.7±0.6 Hz, and mean stroke amplitude was 105±5 deg in the forewing and 96±5 deg in the hindwing – all of which are close to free-flight values. Instead, the low cost of flight could reflect the relatively small size and relatively modest anatomical power density of the locust flight motor, which is a likely evolutionary trade-off between flight muscle maintenance costs and aerial performance.

Key words: insect, lift, locust, maximum metabolic rate, respirometry, tethered flight, weights, stroke amplitude, wingbeat frequency.

Received 28 December 2011; Accepted 1 June 2012

INTRODUCTION

Flying insects achieve the highest mass-specific oxygen consumption rates of all animals (Sacktor, 1976; Suarez, 2000). This has been demonstrated across a range of insect groups, including bees, dragonflies, locusts and moths (Bartholomew and Casey, 1978; Casey et al., 1985; Harrison and Lighton, 1998; Kammer and Heinrich, 1974; Weis-Fogh, 1967). However, many metabolic flight estimates could be sub-maximal given few studies take steps to maximise the work rate of the flight motor. The potential to underestimate flight metabolic capacity has been demonstrated in carpenter bees *Xylocopa varipuncta* where hovering metabolic rates increase by up to 50% in low-density heliox atmospheres compared with normal air (Roberts et al., 2004). Other studies have revealed hidden metabolic potential by adding either natural or artificial weights to free-flying insects, which effectively increases their wing loading. However, the results of these experiments are conflicting – while some studies have found no 'clear-cut' dependence of metabolic rate on the mass of lead weight or crop load carried by foraging honeybees *Apis mellifera* (Balderrama et al., 1992; Moffatt, 2000), another study found that pollen and nectar loads increase honeybee energy requirements by 6% (Feuerbacher et al., 2003), while an earlier study found that nectar loads increase the cost of honeybee flight by up to 40% (Wolf et al., 1989). This last study is in line with results from bumblebees *Bombus edwardsii* in which energy expenditure during flight is correlated with body mass, as affected by stomach contents (Heinrich, 1975).

Given that the energetics of insect flight can increase when additional demands are placed on the system, it is reasonable to suspect that sub-maximal metabolic rates could occur when no effort is made by researchers to increase the cost of flight. Studies involving locusts should come under particular scrutiny because this insect appears incapable of hovering in a metabolic flight chamber and so needs to be tethered (Armstrong and Mordue, 1985; Rascón and Harrison, 2005; Weis-Fogh, 1967). The problem with tethering is that it interrupts sensory feedback loops, which can lead to unnatural flight behaviour and sub-maximal flight efforts (Dudley and Ellington, 1990; Kutsch et al., 1999; Preiss and Gewecke, 1991; Zarnack and Wortmann, 1989). Some studies have found that tethered locusts have lower wingbeat frequencies than free-flying locusts (Baker et al., 1981; Kutsch and Stevenson, 1981; Kutsch et al., 1999), while others have reported relative lift values that are less than that required to support body mass (Gee and Robertson, 1998; Gewecke, 1975; Kutsch and Gewecke, 1979; Robertson and Johnson, 1993; Wolf, 1993). In other insects, such as the fruit fly *Drosophila melanogaster*, tethering can lead to underestimation of peak flight performance by around 20% (Lehmann and Dickinson, 1997), and the rate of oxygen uptake by tethered sphinx moths *Manduca sexta* is one-half that of free-flying moths (Heinrich, 1971). Similarly, for honeybees, energy expenditure and lift production during tethered flight are likely to be less than in free flight (Esch, 1976; Harrison and Fewell, 2002; Heinrich, 1993; Rothe and Nachtigall, 1989).

If tethering does result in sub-maximal flight efforts, then it might explain why locust flight metabolic rates appear to be lower than those of other insects. For example, energy expenditure during tethered flight in the American locust *Schistocerca americana* and desert locust *S. gregaria* is within the range of 450–1300 μmol $O_2 g^{-1} h^{-1}$ (Armstrong and Mordue, 1985; Krogh and Weis-Fogh, 1951; Weis-Fogh, 1952; Weis-Fogh, 1967) [see also Rascon and Harrison (Rascon and Harrison, 2005), calculated from CO_2 production assuming RQ=0.83], whereas oxygen uptake in similarly sized bees, beetles and moths is generally around 2000–4000 μmol $O_2 g^{-1} h^{-1}$ (Auerswald et al., 1998; Bartholomew and Casey, 1978; Casey et al., 1985; Suarez et al., 2005).

Because tethering can elicit sub-maximal flight efforts, and given that some insects reveal hidden metabolic reserves when extra demands are placed on the flight system, we hypothesise that the apparently low flight oxygen consumption rate of tethered locusts may not reflect their true metabolic potential. Therefore, the aim of this study was to clarify whether previous estimates of energy expenditure during locust flight are representative of maximum metabolic values, and whether flight oxygen consumption rates increase with the attachment of weights to the wings. In doing so, we attempted to increase the metabolic work of the flight muscles in an insect that is intractable to measurements of untethered flight. Accurate measurements of maximum oxygen consumption rate during locust flight are also necessary to evaluate the structure and function of the tracheal oxygen delivery system and mitochondria of the flight motor. This analysis is the subject of the companion paper (Snelling et al., 2012).

MATERIALS AND METHODS
Animals

Gregarious-phase locusts *Locusta migratoria* (Linnaeus 1758) were reared at 33±2°C, under a 12h:12h light–dark cycle, with *ad libitum* access to seedling wheatgrass and wheat germ, as previously described (Snelling et al., 2011). Respirometry during rest and flight, and measurements of lift, wingbeat frequency and stroke amplitude were made on adult male locusts, 2–3 weeks post-final moult, by which time the flight muscles are fully developed (Mizisin and Ready, 1986). All measurements were carried out at 35±2°C, the temperature at which locust flight muscles operate at peak power (Neville and Weis-Fogh, 1963). All locusts were fasted 6–10h prior to commencement of experiments. Immediately following experiments, locusts were weighed to 0.1mg on an analytical balance (AE163, Mettler, Greifensee, Switzerland). No insects were reused.

Respirometry system

Oxygen consumption was measured in an initial cohort of adult male locusts using a flow-through respirometry system described previously (Snelling et al., 2011). Briefly, dry CO_2-free air was pushed through an experimental line and a reference line connected to mass flow controllers (810C 0–1000 ml min^{-1}, Mass-Trak, Sierra Instruments, Monterey, CA, USA) that matched their flow rates. Both lines then entered a temperature cabinet, where the experimental line was connected to a metabolic chamber that contained the locust. Upon exiting the temperature cabinet, the experimental line was scrubbed of H_2O vapour and CO_2, then both the reference and experimental lines were directed into an oxygen analyser (FC-2 Sable Systems, Las Vegas, NV, USA; calibrated with dry, CO_2-free outside air) operating in differential mode. The analog outputs from the oxygen analyser and both mass flow controllers were recorded at 1s intervals to a computer with a PowerLab data acquisition system and LabChart software

(ADInstruments, Bella Vista, NSW, Australia). These outputs were then used to calculate oxygen consumption rate (μmol h^{-1}) before an instantaneous correction was applied to account for chamber washout, as previously detailed (Snelling et al., 2011).

Instantaneous oxygen consumption rates ($\dot{M}_{O_2}$) were then used for all analyses. Resting oxygen consumption rates ($\dot{M}_{R_{O_2}}$) were calculated for each individual either by averaging the lowest oxygen consumption rate over 2min during an initial period of rest, or by averaging at least two consecutive gas exchange cycles when oxygen uptake was intermittent. Maximum oxygen consumption rates during flight ($\dot{M}_{M_{O_2}}$) were calculated for each individual by averaging the highest 30s period of oxygen uptake. Finally, maximum oxygen consumption rate of the flight muscle ($\dot{M}_{M_{O_2,\text{flight muscle}}}$) was calculated by subtracting $\dot{M}_{R_{O_2}}$ from $\dot{M}_{M_{O_2}}$ in locusts from which data for both metabolic states were available. This ignores the metabolic contribution made by the flight muscle to overall $\dot{M}_{R_{O_2}}$; however, even if oxygen uptake by flight muscle equalled that of the whole body at rest, the error would still be less than 3% owing to the 37-fold metabolic scope.

Respirometry during rest and flight

Oxygen consumption in resting locusts was measured in a 50ml polymethyl methacrylate metabolic chamber, which was sealed at the top and bottom with rubber stoppers, where incurrent and excurrent air-flow ports were located, through which air was pushed at a rate of 200ml min^{-1} standard temperature and pressure, dry (STPD). Each locust was given 30min to acclimate to the conditions of the chamber and then resting oxygen consumption rates were recorded over a 10min period or up to 30min if cyclic gas exchange occurred. During this time, locusts were monitored for movement although most individuals settled within minutes of the initial acclimation period.

Locusts were then transferred to a cylindrical 500ml polymethyl methacrylate metabolic chamber for flight oxygen consumption measurements. The flight chamber was orientated horizontally and was sealed at both ends with rubber stoppers, where incurrent and excurrent air-flow ports were located, through which air was pushed at a rate of 1000ml min^{-1} STPD. Locusts were tethered to a metal rod descending from the roof of the chamber, which was attached to the pronotum of the insect with warm depilatory wax (Klorane, Boulogne, France). Careful attention was paid to ensure the wax was not excessively hot upon application. Each locust was given 10min to acclimate to the conditions of the chamber, before oxygen consumption was measured over a 5min period while locusts performed tethered-flight exercise. Flight was induced by invoking the 'tarsal reflex' (Weis-Fogh, 1956b), which involved the rapid removal of a metal perch from beneath the locust using a rare-earth magnet located outside the chamber. In addition, flight was encouraged by positioning the incurrent air stream directly in front of the locust's head, which provided stimulation of the aerodynamic sense organ also known to elicit a flight response (Weis-Fogh, 1949). Flight was generally maintained over the 5min exercise period; however, in some individuals it was necessary to reinitiate flight by re-perching the locust and invoking the tarsal reflex.

Respirometry during flight with weights attached to the wings

In an attempt to increase maximum oxygen consumption rate from the exercising flight muscles, a second cohort of adult male locusts was exercised exactly as described above, but with a steel plate attached with putty (Bostik, Regency Park, SA, Australia) to the mid-proximal part of each of the four wings. The combined mass of the four steel plates and putty ranged between 30 and 45% of

body mass for all locusts, with each weight equivalent to ~10% of body mass. While the addition of weights to the wings is not particularly reflective of natural flight, it does provide a means to augment the power required to accelerate and decelerate the wings during flapping, and if all else is equal, this should increase metabolic requirements. The weights were not positioned too far from the axis of rotation, for if they were, the consequent increase in wing moment of inertia became excessive and normal wing-stroke was reduced. Maximum oxygen consumption rates from individuals with weights attached to their wings ($\dot{M}_{MO_2,weight}$) were calculated exactly as described for individuals without weights.

Lift, wingbeat frequency and stroke amplitude during flight

Lift, wingbeat frequency and stroke amplitude during flight were measured in a third cohort of adult male locusts. Individuals were tethered to a vertical force-displacement transducer (FT.03, Grass Instrument Co., Quincy, MA, USA) coupled to a low-level DC pre-amplifier (7P1E, Grass Instrument Co.) and driver amplifier (7DAF, Grass Instrument Co.) with analog output recorded every 0.001 s to a computer with the PowerLab data acquisition system and LabChart software.

Warm depilatory wax was used to attach a metal rod descending from the force transducer to the pronotum of the insect. Locusts were tethered ~10–20 deg to the horizontal, head-up, consistent with the body angle of locusts in level free flight (Baker et al., 1981; Fischer and Kutsch, 1999). A 4×4 cm variable speed fan (XC5054, Sirocco, Taipei, Taiwan) was positioned 3 cm in front of the locust to replicate the flow of air that would occur during free forward flight (Cloupeau et al., 1979). Using a calibrated hot wire anemometer (AM-4204HA, Lutron Electronic Enterprise Co., Taipei, Taiwan) the velocity of air flowing over the locust was maintained between 4 and 5 m s^{-1}, similar to the forward velocity of locusts in free flight (Baker et al., 1981; Fischer and Kutsch, 1999). Potential heterogeneity in air velocities moving over the wings and body were not measured for this study.

Immediately after each locust was tethered to the force transducer, it was provided with a metal perch and given a few minutes to settle. The perch was then removed very slowly from beneath the locust so as to prevent flight by tarsal reflex. With the locust suspended in still air, but not making any wing movements, the baseline vertical force voltage of the locust's body mass was zeroed and recorded for 60 s. A precision calibration weight (2.5 g) was then carefully attached to the tether above the locust and another 60 s of voltage was recorded. The increase in voltage produced by the calibration weight was used to calculate a voltage–gram equivalent, and so it was imperative no wing movements occurred during these initial baseline measurements. The calibration weight was then removed from the tether and flight was initiated by engaging the variable speed fan and, when necessary, eliciting the tarsal reflex. Lift (g) generated during tethered flight was recorded over a 5 min exercise bout, consistent with the duration of flight respirometry. A sub-set of these locusts also had a high-speed digital video camera (Xacti VPC-FH1, Sanyo Electric Co., Osaka, Japan) positioned lateral to the tethered insect, which recorded right forewing and hindwing stroke movements during flight at 600 frames s^{-1}. Tracking wing movements through the stroke plane was facilitated by a small black ink dot applied to the wing tips prior to flight. The base-to-tip length of the right forewing and hindwing was also measured in these locusts using a digital micrometer.

Mean maximum lift relative to body mass (L_r) was calculated for each locust by averaging the 30 s period in which lift was greatest, consistent with flight $\dot{M}_{MO_2}$ calculation. The cyclic nature of wing flapping meant that wingbeat frequency was also recorded in the cyclic plot of lift production over time (Cloupeau et al., 1979; Robertson and Johnson, 1993). Mean wingbeat frequency (f) was calculated over the entire 30 s period of maximum lift using the sine-shape frequency analysis in the LabChart software. Individual frames from the high-speed video footage capturing upper and lower reversal points of five random complete down-strokes performed during the 30 s period of maximum lift were identified for both the forewing and hindwing, and then imported into a computer graphics program for analysis (CorelDRAW 11, Corel Corp., Ottawa, ON, Canada). The images of the upper and lower reversal points for individual down-strokes were superimposed, and the distance between the wing tips was measured and corrected to scale. Mean stroke amplitude of the forewing (Ψ_{fw}) and hindwing (Ψ_{hw}) was calculated using these distance measurements as a cosine function of wing length (Fischer and Kutsch, 1999; Fischer et al., 2002).

All mean values and allometric exponents include 95% confidence intervals (CI). Resting oxygen consumption rates obtained from the literature were, when necessary, corrected to 35°C assuming $Q_{10}=1.83$ (Harrison et al., 1991), while CO_2 production rates were converted to oxygen consumption assuming RQ=0.83 (Hadley and Quinlan, 1993). Statistical significance between means was tested using paired or unpaired t-tests for equal or unequal variance, as appropriate. Allometric data were log$_{10}$ transformed before statistical analysis using ordinary least squares regressions. ANCOVA comparisons of regressions (Zar, 1998) and t-tests were carried out with GraphPad Prism 5 statistical software (GraphPad Software, La Jolla, CA, USA).

RESULTS
Respirometry

During rest, locusts regularly exhibited cyclic or discontinuous gas exchange, which is indicative of inactivity (Chown et al., 2006). Mean mass-specific $\dot{M}_{RO_2}$ was 28±2 μmol O_2 g^{-1} h^{-1} (N=28; Fig. 1). During tethered flight, oxygen consumption rate increased 37-fold, with mean mass-specific $\dot{M}_{MO_2}$ reaching 1032±69 μmol O_2 g^{-1} h^{-1} (N=23), which is significantly higher than resting levels (ANOVA with Tukey's *post hoc* test, $P<0.05$).

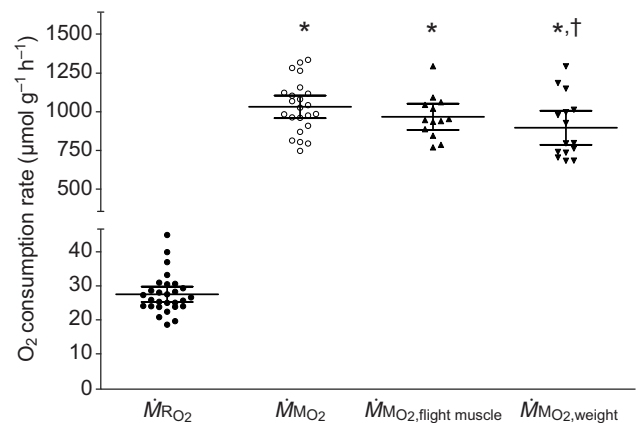Fig. 1. Body-mass-specific resting metabolic rate ($\dot{M}_{RO_2}$; N=28), maximum metabolic rate during tethered flight ($\dot{M}_{MO_2}$; N=23), maximum metabolic rate of the flight muscles ($\dot{M}_{MO_2,flight muscle}$; N=13) and maximum metabolic rate during tethered flight with weights attached to each wing ($\dot{M}_{MO_2,weight}$; N=15). Each data point represents the mean of an individual adult locust. The grand mean ±95% confidence interval (CI) is also presented. *Statistically different from $\dot{M}_{RO_2}$. †Statistically different from $\dot{M}_{MO_2}$ (ANOVA with Tukey's *post hoc* test, $P<0.05$).
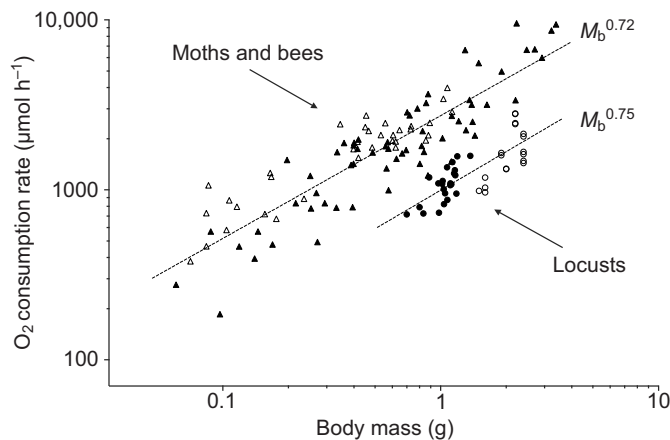
Fig. 2. Relationship between body mass (M_b) and metabolic rate during tethered flight in locusts measured in the present study (filled circles; N=23) and that in *Schistocerca gregaria* (open circles; N=18) measured by Krogh and Weis-Fogh (Krogh and Weis-Fogh, 1951). Also shown is the relationship between body mass and metabolic rate in 31 moth species (filled triangles; N=63) and nine orchid bee species (open triangles; N=35) during hovering free flight measured by Bartholomew and colleagues (Bartholomew and Casey, 1978; Casey et al., 1985).

$\dot{M}_{M_{O_2},\text{flight muscle}}$ was calculated by subtracting $\dot{M}_{R_{O_2}}$ from $\dot{M}_{M_{O_2}}$ measurements. Mean mass-specific $\dot{M}_{M_{O_2},\text{flight muscle}}$ was $967\pm76\,\mu\text{mol O}_2\,\text{g}^{-1}\,\text{h}^{-1}$ (N=13; Fig. 1), which is statistically indistinguishable from $\dot{M}_{M_{O_2}}$ (Tukey's *post hoc* test, P>0.05).

In an effort to increase the work rate of the flight motor, a second cohort of locusts was flown with weights attached to their wings. Mean mass-specific $\dot{M}_{M_{O_2},\text{weight}}$ in these locusts was $896\pm101\,\mu\text{mol O}_2\,\text{g}^{-1}\,\text{h}^{-1}$ (N=15; Fig. 1), which is approximately 13% lower than the mean $\dot{M}_{M_{O_2}}$ of unweighted individuals (Tukey's *post hoc* test, P<0.05).

Allometry

When the tethered-flight $\dot{M}_{M_{O_2}}$ data of locusts in the present study are combined with published tethered-flight metabolic data from larger desert locusts *S. gregaria* (Krogh and Weis-Fogh, 1951), it is possible to generate the allometric equation $\dot{M}_{M_{O_2}}=994M_b^{0.75\pm0.19\,(95\%\,\text{CI})}\,\mu\text{mol O}_2\,\text{h}^{-1}$ (r^2=0.61, N=41; Fig. 2). In comparison, published aerobic metabolic rates from 31 moth species (Bartholomew and Casey, 1978) and nine orchid bee species (Casey et al., 1985) in hovering free flight ($\dot{M}_{H_{O_2}}$) scale with body mass according to the equation $\dot{M}_{H_{O_2}}=2767M_b^{0.72\pm0.08}\,\mu\text{mol O}_2\,\text{h}^{-1}$ (r^2=0.79, N=98). While the slopes of the two allometric regressions

are similar (ANCOVA, $F_{1,135}$=0.04, P=0.83), the metabolic cost of flight in moths and bees is approximately 2.8-fold higher than in locusts (ANCOVA, $F_{1,136}$=217, P<0.05).

Lift, wingbeat frequency and stroke amplitude

Mean maximum lift during tethered flight was, on average, sufficient to support 95±8% of locust body mass (N=30; Fig. 3). This is not significantly different from 100%, which equates to level flight. However, there was variability between individuals, with 18 of the 30 locusts recording lift that exceeded body mass, while the remaining locusts recorded relative lift values between 41 and 89% of body mass.

Wingbeat frequency (f) during this period of maximum lift averaged 23.7±0.6 Hz, and only varied between 20.3 and 26.1 Hz (N=30; Fig. 3). Over this narrow range of wingbeat frequencies, there was no clear-cut relationship with percentage lift, L_r=3.7f+7.8% (r^2=0.07, N=30).

Mean stroke amplitude of the forewings during this period of maximum lift was 105±5 deg, with individual means ranging from 89 to 122 deg (N=15; Fig. 3). The absolute maximum forewing stroke amplitude reached 129 deg (N=75 wing-strokes). However, there was no clear-cut relationship between mean forewing stroke amplitude (Ψ_{fw}) and percentage lift, L_r=–0.45Ψ_{fw}+148% (r^2=0.06, N=15).

Mean stroke amplitude of the hindwings was 96±5 deg, with individual means ranging from 82 to 117 deg (N=15; Fig. 3). Once again, the absolute maximum stroke amplitude reached 129 deg (N=75 wing-strokes). Similar to the forewings, there was no definitive relationship between mean hindwing stroke amplitude (Ψ_{hw}) and percentage lift, L_r=–0.22 Ψ_{hw}+122% (r^2=0.02, N=15).

The mean body mass of all adult male locusts used in the present study was 0.9630±0.0364 g (N=68).

DISCUSSION
Resting oxygen consumption

The resting oxygen consumption rate of adult migratory locusts is approximately 28 $\mu\text{mol O}_2\,\text{g}^{-1}\,\text{h}^{-1}$ (Fig. 1), which is consistent with data from adult desert locusts *S. gregaria* (1.69 g) where values around 25 $\mu\text{mol O}_2\,\text{g}^{-1}\,\text{h}^{-1}$ have been reported (corrected to 35°C assuming Q_{10}=1.83) (Armstrong and Mordue, 1985). Elsewhere in the literature there is variability; at the upper end American locusts *S. americana* (1.22 g) consume 39 $\mu\text{mol O}_2\,\text{g}^{-1}\,\text{h}^{-1}$ (calculated from CO_2 production assuming RQ=0.83) (Rascon and Harrison, 2005) and resting two-striped grasshoppers *Melanoplus bivittatus* (1.65 g) consume 46 $\mu\text{mol O}_2\,\text{g}^{-1}\,\text{h}^{-1}$ (Harrison et al., 1991). At the lower end, the large and flightless eastern lubber grasshopper *Romalea guttata* (2.92 g) has a resting rate of 16 $\mu\text{mol O}_2\,\text{g}^{-1}\,\text{h}^{-1}$ (corrected to 35°C assuming Q_{10}=1.83) (Hadley and Quinlan, 1993). The large
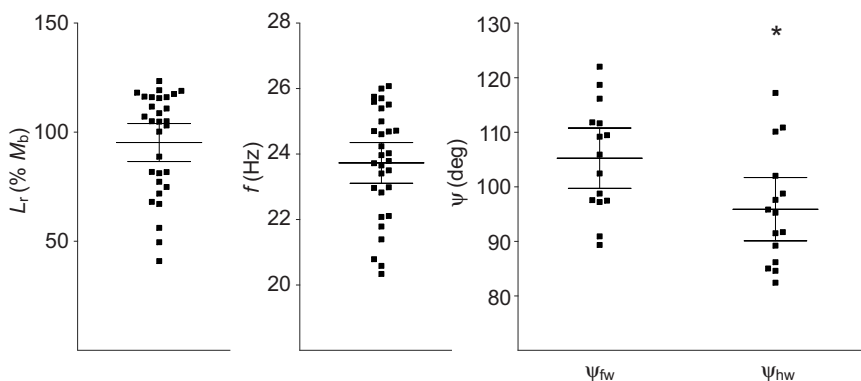

Fig. 3. Mean maximum relative lift (L_r; N=30), wingbeat frequency (f; N=30), forewing stroke amplitude (Ψ_{fw}; N=15) and hindwing stroke amplitude (Ψ_{hw}; N=15) during tethered flight. Each data point represents the mean of an individual adult locust. The grand mean ±95% CI is also presented. *Statistically different from Ψ_{fw} (t-test, P<0.05).

body mass and reduced flight muscles in *R. guttata* probably contribute to a low mass-specific resting metabolic rate in this species.

Maximum oxygen consumption

The maximum oxygen consumption rate of adult migratory locusts during tethered flight is approximately 1032 µmol $O_2\,g^{-1}\,h^{-1}$, while the flight muscles consume around 967 µmol $O_2\,g^{-1}\,h^{-1}$ (Fig. 1). Therefore, when the flight muscles are working they contribute close to 95% of the locust's whole-body energy expenditure. These oxygen consumption rates align well with those of American locusts, which consume around 1150 µmol $O_2\,g^{-1}\,h^{-1}$ during tethered flight (calculated from CO_2 production assuming RQ=0.83) (Rascon and Harrison, 2005), and are also towards the upper estimated range of 450–1300 µmol $O_2\,g^{-1}\,h^{-1}$ determined for desert locusts (Armstrong and Mordue, 1985; Krogh and Weis-Fogh, 1951; Weis-Fogh, 1952; Weis-Fogh, 1967). However, compared with other insect groups, locust flight metabolic rates appear relatively modest. On average, moths and orchid bees expend 2.8-fold more energy during hovering free flight than locusts expend during tethered flight, after allometrically correcting for the effect of body mass (Fig. 2). The apparently low cost of flight in locusts could either reflect a naturally low flight metabolic rate or arise as a consequence of tethering. It is thought that tethering can lead to sub-maximal flight efforts because it interrupts sensory feedback loops and because there is no requirement for insects to support their own weight (Dudley and Ellington, 1990; Kutsch et al., 1999; Preiss and Gewecke, 1991; Zarnack and Wortmann, 1989).

Attachment of weights

To address the potential problem that tethered locusts might be metabolically underperforming, a second cohort of insects was made to perform flight exercise with a small weight attached to each wing. It was reasoned that the increased work required per wing-stroke might reveal untapped metabolic potential. Instead, weighted locusts have a 13% lower maximum oxygen consumption rate during flight compared with unweighted locusts (Fig. 1). This might occur if the wing muscles are already operating close to their aerobic metabolic limits, and so little potential exists to increase oxygen uptake beyond levels attained during unweighted flight. However, the fact that maximum oxygen consumption is slightly lower in weighted locusts suggests the extra effort required to move the 'heavy' wings might have been annulled by a decrease in wingbeat frequency or stroke amplitude.

Lift, wingbeat frequency and stroke amplitude

Mean maximum lift during tethered flight is not significantly different from that required to support 100% of locust body mass (Fig. 3), which is indicative of a generally high work rate. However, there is noticeable variation between individuals, with relative lift values ranging between 41 and 123% of body mass. Nonetheless, there are several facts that indicate that the measurements are generally representative of free flight. Firstly, mean wingbeat frequency is approximately 24 Hz and is contained entirely within the narrow range of 20–26 Hz (Fig. 3), which is comparable to mean values between 23 and 24 Hz recorded from free-flying locusts (Baker et al., 1981; Fischer and Kutsch, 1999; Fischer and Kutsch, 2000; Kutsch et al., 1999). Secondly, mean stroke amplitude is approximately 100 deg in both wing-sets, with individual means occurring within the range of 89–122 for the forewings and 82–117 for the hindwings, while both wing-sets reach a maximum amplitude of approximately 130 deg (Fig. 3). These maximum values are once

again fairly close to maximum free-flight values where amplitudes of 100 and 160 deg have been reported for the forewing, and 135 and 150 deg for the hindwing (Baker and Cooter, 1979; Fischer and Kutsch, 1999). Thirdly, the relationships between wingbeat frequency, stroke amplitude and maximum relative lift are not evident, which suggests that other factors must be influencing vertical force production, such as the wings' angle of attack and stroke plane angle (Fischer and Kutsch, 2000; Sane, 2003; Walker et al., 2009). It is also noteworthy that the ascent angle of locusts in free flight is strongly and proportionately related to body angle in the range of 10–60 deg to the horizontal (Fischer and Kutsch, 1999), and so the 10–20 deg tethering angle used in the present study may have prevented locusts from achieving greater vertical force production. Fourthly, if those individuals that recorded $\dot{M}_{MO_2}$ values in the bottom four-tenths of the respirometry data are excluded from the results on the basis that 40% of tethered locusts failed to generate enough lift to support whole-body mass, the re-calculated mean mass-specific $\dot{M}_{MO_2}$ increases from 1032±69 µmol $O_2\,g^{-1}\,h^{-1}$ to 1135±63 µmol $O_2\,g^{-1}\,h^{-1}$ – a modest 10% increase. The apparent disassociation between $\dot{M}_{MO_2}$ and lift could arise if a variable fraction of the aerodynamic forces produced by the beating wings were translated along different force vectors.

Low energy expenditure of locust flight

To summarise, several lines of evidence indicate that locusts exerted themselves to near-maximum metabolic rates: (1) oxygen consumption rates are within the upper range of those recorded by other researchers, (2) the addition of weights failed to increase the energy cost of flight, (3) wingbeat frequency and stroke amplitude are largely comparable with mean free-flight values, and (4) mean maximum lift is not significantly different from that required for level flight.

If the metabolic rate of tethered locusts reflects near-maximum values, as the evidence suggests, then the energy expenditure of locust flight must be lower than that of many other insects. This might be linked to the low wing-loading and modest wingbeat frequency of locusts (Casey, 1976; Casey et al., 1985; Curtsinger and Laurie-Ahlberg, 1981; Harrison et al., 1996; May, 1981). However, it is also reasonable to suspect that the energetics of insect flight, together with wing-loading and wingbeat frequency, might also correlate with the size and anatomical power-density of the flight motor. The latter refers to the density at which mitochondria and tracheoles occur in the flight muscle, and the relative capacity to mobilise and supply fuel to the fibres.

A recent morphometric analysis of the locust flight system confirms that flight muscle represents just 17% of locust body mass (Snelling et al., 2012), which compared with other insects is at the lower limit of a range that spans 17–56% (Marden, 1987). In addition, the density of mitochondria in locust flight muscle is only 20% (Snelling et al., 2012), which is also modest compared with reports of around 40% in other insects (Casey et al., 1992; Josephson et al., 2000; Smith, 1963; Suarez et al., 2000). The study also calculated that each tracheole in the locust flight motor supplies oxygen to around 119 µm² of surrounding muscle, which cannot yet be compared against other insects because of a lack of data, but presumably species with flight motors operating at around twice the power-density of locusts should possess tracheoles that service around one-half the area of muscle. Likewise, we might also find adaptations in the fuel delivery system, such that the relative mobilisation and flux of carbohydrates (trehalose) and lipids (diacylglycerol) from the haemolymph and fat body stores (sugars from crop stores in bees)

to the sites of oxidation in the flight muscle are more rapid in high power-density flight motors than in locust.

While data for tracheole density and relative fuel flux capacity are incomplete, it is possible to calculate whether the 2.8-fold higher aerobic flight metabolic rate of bees and moths is matched by a 2.8-fold larger total mitochondrial volume. A re-examination of the data set compiled by Marden reveals that the flight muscles of both bees and moths account for 31% of whole-body mass (Marden, 1987), and we also know that mitochondria occupy approximately 43% of flight muscle fibre volume in the honeybee and orchid bee (Casey et al., 1992; Suarez et al., 2000). Thus, a crude calculation suggests that bees and moths have a 1.8-fold larger flight motor (31%/17%) and a 2.2-fold greater mitochondrial density (43%/20%) than locusts, which sum to equal a 4.0-fold larger total mitochondrial volume. Although this is somewhat higher than the 2.8-fold prediction based on the metabolic flight data presented in Fig. 2, it is possible that the estimates of energy expenditure in moths and bees are sub-maximal as both studies (Bartholomew and Casey, 1978; Casey et al., 1985) employed closed-system respirometry, which has poor temporal resolution, and neither took steps to maximise the work of the flight motor.

Evolutionary trade-offs in the design of the locust flight motor
The advantage of a flight motor that is relatively small and has a relatively low anatomical power-density is that it costs less to build and maintain, allowing resources to be directed towards other requirements, such as reproduction and migration (Marden, 2000). The significant energetic burden flight muscles pose is indirectly evident by the fact that flight motor development only begins in the lead-up to volant adulthood in locusts (Mizisin and Ready, 1986) and holometabolous insects (Fernandes et al., 1991; Skandalis et al., 2011), and by the fact that some species undergo flight muscle histolysis during periods in which flight is not required (Dingle and Winchell, 1997; Robertson, 1998). The disadvantage of a small, low power-density flight motor is reduced flight performance, and it is noteworthy that in order for insects to fly, the ratio between flight muscle mass and body mass cannot fall much below the 17% determined for locusts, and indeed, the ability to lift loads and to accelerate increases linearly from this minimum set-point (Marden, 1987; Marden, 2000). It is perhaps unsurprising then that the locust flight system, although comparatively less expensive to maintain and operate, does have some functional limitations. For instance, locusts appear unable to generate the large aerodynamic forces required to perform sustained hovering flight, and in fact, a minimum flight speed of around $3\,m\,s^{-1}$ is necessary to offset body mass (Weis-Fogh, 1956a; Zarnack and Wortmann, 1989). Likewise, the load-lifting capacity of locusts seems unlikely to exceed 30% of body mass (see Fischer and Kutsch, 2000), which is modest compared with relative loads of up to 100 and 200% carried by some bees and dragonflies, respectively (Marden, 1987). And lastly, locusts cannot initiate flight solely under the power of the wings, and instead rely on power generated from a single forceful jump to provide the initial lift and thrust for take-off flight (Katz and Gosline, 1993).

CONCLUSIONS
Measurements of oxygen consumption combined with estimates of relative lift, wingbeat frequency and stroke amplitude reveal that locusts have significantly lower flight metabolic rates compared with holometabolous bees and moths. The low cost of locust flight could reflect the relatively small size and modest anatomical power-density of the locust flight motor, which is a likely evolutionary trade-off

between flight muscle maintenance costs and aerial performance. The relatively small investment in the flight motor made by locusts is consistent with the observation that juvenile instar hoppers are highly active insects with few energy storage opportunities, whereas the juvenile larvae of bees, moths and other holometabolous insects are much less active and therefore have more opportunity to accrue the substantial energy reserves necessary to construct a high performance flight motor during the pupal lead-up to adulthood.

LIST OF ABBREVIATIONS

f	wingbeat frequency
L_r	maximum relative lift
$\dot{M}_{O_2}$	oxygen consumption rate
$\dot{M}_{H O_2}$	oxygen consumption rate of moths and orchid bees during hovering free flight
$\dot{M}_{M O_2}$	maximum oxygen consumption rate of locusts during tethered flight
$\dot{M}_{M O_2, \text{flight muscle}}$	maximum oxygen consumption rate of locust flight muscles during tethered flight
$\dot{M}_{M O_2, \text{weight}}$	maximum oxygen consumption rate of locusts during tethered flight with weights attached to each wing
$\dot{M}_{R O_2}$	resting oxygen consumption rate of locusts
Ψ_{fw}	forewing stroke amplitude
Ψ_{hw}	hindwing stroke amplitude

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