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RESEARCH ARTICLE

Scaling of resting and maximum hopping metabolic rate throughout the life cycle of the locust *Locusta migratoria*

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SUMMARY

The hemimetabolous migratory locust *Locusta migratoria* progresses through five instars to the adult, increasing in size from 0.02 to 0.95 g, a 45-fold change. Hopping locomotion occurs at all life stages and is supported by aerobic metabolism and provision of oxygen through the tracheal system. This allometric study investigates the effect of body mass (M_b) on oxygen consumption rate ($\dot{M}_{O_2}$, μmol h^{-1}) to establish resting metabolic rate ($\dot{M}_{RO_2}$), maximum metabolic rate during hopping ($\dot{M}_{MO_2}$) and maximum metabolic rate of the hopping muscles ($\dot{M}_{MO_2,hop}$) in first instar, third instar, fifth instar and adult locusts. Oxygen consumption rates increased throughout development according to the allometric equations $\dot{M}_{RO_2}=30.1M_b^{0.83\pm0.02}$, $\dot{M}_{MO_2}=155M_b^{1.01\pm0.02}$, $\dot{M}_{MO_2,hop}=120M_b^{1.07\pm0.02}$ and, if adults are excluded, $\dot{M}_{MO_2,juv}=136M_b^{0.97\pm0.02}$ and $\dot{M}_{MO_2,juv,hop}=103M_b^{1.02\pm0.02}$. Increasing body mass by 20–45% with attached weights did not increase mass-specific $\dot{M}_{MO_2}$ significantly at any life stage, although mean mass-specific hopping $\dot{M}_{O_2}$ was slightly higher (ca. 8%) when juvenile data were pooled. The allometric exponents for all measures of metabolic rate are much greater than 0.75, and therefore do not support West, Brown and Enquist's optimised fractal network model, which predicts that metabolism scales with a ¾-power exponent owing to limitations in the rate at which resources can be transported within the body.

Key words: allometry, fractal network model, hopping, insect, locust, maximum metabolic rate, respirometry, weight.

INTRODUCTION

Metabolic rate generally follows an allometric relationship with body mass, such that small animals consume more energy per unit body mass than large animals over a given period of time (Savage et al., 2004; White et al., 2006). In recent years, a range of mechanistic theories have been put forward to explain the allometric scaling of metabolism (Banavar et al., 2002; Banavar et al., 2010; Barbosa et al., 2006; Darveau et al., 2002; Kozlowski and Konarzewski, 2004; Price et al., 2007; West et al., 1997). The fractal network model proposed by West, Brown and Enquist has received the most attention because it purports to explain the metabolic rate of all living organisms (West et al., 1997; West et al., 1999; West et al., 2003). The model is based on the theory that metabolism is matched to the rate at which resources are most efficiently transported within fractal distribution networks of the body. Their original optimised model predicts that metabolic rate scales allometrically with an exponent of 0.75 (West et al., 1997). Three key assumptions are central to the model: the delivery network is a space-filling fractal-like branching pattern that supplies all cells with oxygen, the final branch of the network is a size-invariant unit, and the energy required to distribute resources is minimised (West et al., 1997). These assumptions are met by the fractal-like branches of the insect tracheal system, where terminal tracheoles represent the final size-invariant unit of the network (West et al., 1997). Therefore, the model predicts that the standard metabolic rate (SMR) of insects should scale with a ¾-power exponent. However, there is little consensus as to whether this actually occurs. On the one hand, the results of a global meta-analysis show that insect SMR scales with an exponent close to 0.75 (Addo-Bediako et al., 2002). On the other hand, a number of studies report significant variation in the derived exponents for insect SMR, particularly at lower taxonomic levels where allometric slopes range from 0.67 to 1 (Chown et al., 2007; Strauss and Reinhold, 2010; Terblanche et al., 2004).

In theory, variation in the metabolic scaling exponents can be accommodated in the network model, and recent presentations that relax some assumptions of the model by incorporating a range of branching patterns predict allometric slopes from 0.5 to 1 (e.g. Banavar et al., 2010; Price et al., 2007). Nonetheless, the ¾-power exponent predicted by the model of West et al. (West et al., 1997) remains a valid prediction against which the scaling of insect metabolic rate can be compared, for two reasons. Firstly, West, Brown and Enquist explicitly state that their original model "...predicts structural and functional properties of... insect tracheal tubes" [(West et al., 1997) p. 122], although they later state that West et al. (West et al., 1997) "...do not present a model for insect tracheal systems", but suggest that if insect metabolic rate does scale with an exponent of ¾, then the principles of fractal-like design should apply to the structure and function of the tracheal system, and that this represents a testable hypothesis [(Brown et al., 2005) p. 737]. Thus, there is some confusion in the literature about the extent to which the optimised fractal model applies to insects. Secondly, a recent comparative analysis concludes that insect metabolic rate does scale with a ¾-power exponent (Riveros and Enquist, 2011), and the authors use this as evidence to provide broad

support for the core predictions of the West et al. (West et al., 1997) model, but suggest that more work is required.

To add further complexity, some researchers believe that the fractal network model is in fact more relevant to the scaling of maximum metabolic rate (MMR) than it is to SMR. They argue that because the model is based on the theory that metabolic rate arises due to scaling of resource delivery, it is under conditions of maximum activity that this is most likely to apply (Suarez and Darveau, 2005). Very few studies have scaled MMR with body mass in insects. A recent study on Mormon crickets, *Anabrus simplex*, reports that overall MMR at ambient temperatures spanning 10–40°C scales with an exponent of 0.62, but only over a fourfold range in adult body mass (Chappell et al., 2009). Interspecific insect flight studies spanning a 100-fold range in mass, however, suggest that the scaling exponent for flight metabolic rate could trend towards 0.82–0.87 (Bartholomew and Casey, 1978; Niven and Scharlemann, 2005), which is similar to the exponent of MMR for birds and mammals (Bishop, 1999; Savage et al., 2004; Weibel et al., 2004; Weibel and Hoppeler, 2005; White and Seymour, 2005). Clearly, the lack of insect MMR scaling data precludes researchers from assessing whether metabolic scaling theories, such as the fractal network model, are supported empirically.

The lack of research undertaken on the aerobic capacity of insects could be partly due to the difficulty in obtaining maximum aerobic activity levels from these animals. Insects appear so adept at meeting the challenges of oxygen delivery that even the most energetically demanding of tasks, such as flight, are thought to be almost entirely aerobic (Beenakkers et al., 1984; Komai, 1998; Worm and Beenakkers, 1980). This makes the accurate determination of maximum aerobic metabolic rate particularly difficult. One option available to researchers is to force insects to exercise while carrying a load. A number of studies have already calculated the metabolic cost of ants carrying pupae (Bartholomew et al., 1988) and the transport costs of ants and beetles carrying artificial weights (Kram, 1996; Lighton et al., 1987; Lighton et al., 1993). This technique could be adapted to insects that are then forced to undertake strenuous exercise. Potentially, the energetic burden of carrying a load during heavy exercise could increase aerobic metabolism and reveal an untapped metabolic reserve that would have otherwise gone undetected.

The aim of this study was to determine the resting and maximum hopping metabolic rate of the migratory locust *Locusta migratoria* throughout ontogeny, during which body mass increases 45-fold. Weights were attached to exercising insects in an attempt to increase oxygen consumption rates during terrestrial locomotion. The allometric exponents derived for resting and maximum metabolic rate were then used to assess whether there is empirical support for the hypothesis that insect metabolism conforms to the ¾-power exponent predicted by the optimised fractal network model.

MATERIALS AND METHODS

Animals

Gregarious-phase locusts *Locusta migratoria* (Linnaeus 1758) were sourced from a breeding colony at the University of Sydney, Australia, and then reared under crowded conditions at the University of Adelaide, Australia. They were kept in a large breeding container at 33±1°C, at a relative humidity of ~30%, under a 12 h:12 h light:dark cycle, and had *ad libitum* access to seedling wheatgrass and wheat germ.

The developmental stage of each locust was determined based on instar-specific differences in wing morphology. Newly moulted individuals were transferred into separate plastic terraria with other locusts of the same age. Measurements of resting and hopping metabolic rate were conducted on first, third and fifth instar locusts, as well as adults. Insects were measured three to four days post-moult to provide sufficient time for the exoskeleton to stiffen while minimising compression of the tracheal system due to growth (Greenlee and Harrison, 2004b; Queathem, 1991). All insects were fasted for 6–10 h prior to experiments to minimise elevation in oxygen consumption due to the heat increment of feeding (Gouveia et al., 2000; Nespolo et al., 2005) while limiting the possibility that over-fasting might reduce jump performance.

Respirometry system

Flow-through respirometry was carried out using a dual-channel oxygen analyser (FC-2 Sable Systems, Las Vegas, NV, USA). First, outside air was pumped into an air pressure buffer cylinder using an air compressor (Sparmax, AT-250A, Taipei, Taiwan) before being scrubbed of H_2O vapour and CO_2 using a series of Drierite (W. A. Hammond Drierite Co. Ltd, Xenia, OH, USA), soda lime and Drierite columns. This dry, CO_2-free air was then split into an experimental line and a reference line, both of which were directed through mass flow controllers (Model 810C, Mass-Trak, Sierra Instruments, Monterey, CA, USA; 0–100 ml min^{-1} and 0–1000 ml min^{-1} used depending on insect chamber volume; calibrated with a bubble flow meter, Gilibrator, Sensidyne, Clearwater, FL, USA) where their flow rates were matched. Both lines then entered a temperature cabinet set to 35±2°C [consistent with gas exchange measurements in other locust studies (Greenlee and Harrison, 2004a; Harrison et al., 2005; Harrison et al., 1991; Kirkton et al., 2005)], where the experimental line was connected to a metabolic chamber that contained the insect. Upon exiting the temperature cabinet, the reference line was directed straight into the oxygen analyser, whereas the experimental line was first scrubbed of H_2O vapour and CO_2 using a small Drierite, Ascarite (A. H. Thomas Co., Philadelphia, PA, USA) and Drierite column. The experimental line also had a bypass around the metabolic chamber that was controlled with two three-way valves and allowed for baseline measurements of oxygen concentration before and after measurements with insects.

The differential mode of the oxygen analyser was used to measure the difference in oxygen level between the reference and experimental lines at 1 s intervals. The analog outputs from the oxygen analyser and both mass flow controllers were recorded to a computer with a PowerLab data acquisition system and LabChart software (ADInstruments, Bella Vista, NSW, Australia). Baseline measurements of ambient oxygen concentration taken before and after insect measurements were used to correct experimental data for drift. Oxygen consumption rates were then calculated as:

$$\dot{M}_{O_2,\text{non-inst.}} = \dot{M}_I(F_{I_{O_2}} - F_{E_{O_2}}) / (1 - F_{E_{O_2}}), \quad (1)$$

where $\dot{M}_{O_2,\text{non-inst.}}$ is the oxygen consumption rate of the locust (μmol O_2 h^{-1}) prior to instantaneous correction, $\dot{M}_I$ is the flow rate of the dry CO_2-free air prior to entering the metabolic chamber (μmol h^{-1}), $F_{I_{O_2}}$ is the fractional O_2 concentration of the dry CO_2-free air prior to entering the metabolic chamber (i.e. 0.2095) and $F_{E_{O_2}}$ is the fractional O_2 concentration in air that has exited the metabolic chamber following the removal of CO_2 and H_2O vapour. This calculation for the rate of oxygen consumption is appropriate for respirometry setups where both H_2O vapour and CO_2 are removed prior to measurement of $\dot{M}_I$ and $F_{E_{O_2}}$ (Withers, 2001). Oxygen consumption rates were then instantaneously corrected as:

$$\dot{M}_{O_2} = [\dot{M}_{O_2,\text{non-inst.1}} - \dot{M}_{O_2,\text{non-inst.2}} e^{k(t2-t1)}] / [1 - e^{k(t2-t1)}], \quad (2)$$

where $\dot{M}_{O_2}$ is the instantaneous oxygen consumption rate of the locust, $\dot{M}_{O_2,\text{non-inst.1}}$ and $\dot{M}_{O_2,\text{non-inst.2}}$ are the oxygen consumption

rates of the locust at times t_1 and t_2, respectively, and k is the washout constant determined for each chamber at the appropriate flow rate by injecting a bolus of nitrogen immediately upstream of the chamber and analysing the washout curve (Seymour et al., 1998).

$\dot{M}_{O_2}$ values were then used for all analyses. Measurements of oxygen consumption provide a comparable indication of metabolic rate between developmental stages if there are no significant changes in respiratory substrate during ontogeny. Resting oxygen consumption rates ($\dot{M}_{R_{O_2}}$) were calculated for each individual by averaging the lowest oxygen consumption rate over 2 min during an initial period of rest. In a number of experiments, individuals exhibited cyclic gas exchange; in such cases, $\dot{M}_{R_{O_2}}$ was calculated as the mean over two or more successive ventilation cycles. Maximum oxygen consumption rates during hopping exercise ($\dot{M}_{M_{O_2}}$) were calculated for each individual by averaging the highest oxygen consumption rate over a 30 s period [consistent with Kirkton et al. (Kirkton et al., 2005)]. The maximum metabolic rate of the hopping muscle ($\dot{M}_{M_{O_2,hop}}$) was calculated by subtracting $\dot{M}_{R_{O_2}}$ from $\dot{M}_{M_{O_2}}$ in each locust. This ignores the metabolic contribution made by the hopping muscle to overall $\dot{M}_{R_{O_2}}$; however, the error in this approach is likely to be small given that the metathoracic hopping femurs represent just 5–10% of locust body mass (Kirkton et al., 2005; Snelling et al., 2011).

Respirometry during resting and hopping

Resting and hopping oxygen consumption rates were measured in locusts using metabolic hopping chambers constructed specifically for each developmental stage. Briefly, cylindrical hopping chambers were fashioned from either polymethyl methacrylate or polyethylene tube and sealed at the top and bottom with rubber stoppers. Incurrent and excurrent airflow ports were located in the top and bottom stoppers, respectively, and wire mesh was fastened over the inner surface of the bottom stopper to provide insects with a coarse horizontal platform on which to jump (Fig. 1A).

Chamber volumes and flow rates varied to account for the large size range of insects tested. For first instars (N=37 individuals), the chamber volume was 8 ml, through which dry CO_2-free air was pushed at a rate of 20 ml min^{-1} standard temperature and pressure, dry (STPD). Third instar (N=32), fifth instar (N=21) and adult (N=12) hopping chambers had volumes of 50, 120 and 500 ml, with flow rates of 90, 150 and 410 ml min^{-1} STPD, respectively.

Each insect was given 30 min to acclimate to the hopping chamber, during which time a starting baseline oxygen level was recorded. Using the three-way valves, incurrent air was then directed from the bypass line into the chamber so that resting oxygen consumption rate could be recorded for 10 min (up to 30 min if cyclic gas exchange was evident). During this time, locusts were monitored for movement, although most individuals settled within minutes of the initial acclimation period.

Next, the metabolic chamber was illuminated with a fibre optic cold light source (Microlight 150, Fibreoptic Lightguides, Hornsby, NSW, Australia) and hopping was induced for 5 min by persistently harassing the insect with five plastic beads located in the chamber while oxygen consumption was recorded (Fig. 1A). The external diameter of the beads ranged from 3 mm for first instars, 5 mm for third instars, 10 mm for fifth instars and 12 mm for adults. This technique elicited high and continual hopping activity from locusts. After the hopping period, the fibre optic light was removed and oxygen consumption during recovery was recorded for a further 8 min, after which time the chamber was bypassed and baseline values were measured for the final 10 min of the trial. Immediately following the experiment, each locust was weighed to 0.1 mg on an analytical balance (AE163, Mettler, Greifensee, Switzerland). No animals were reused within a given life stage; however, it is possible that some individuals were reused at a later life stage.

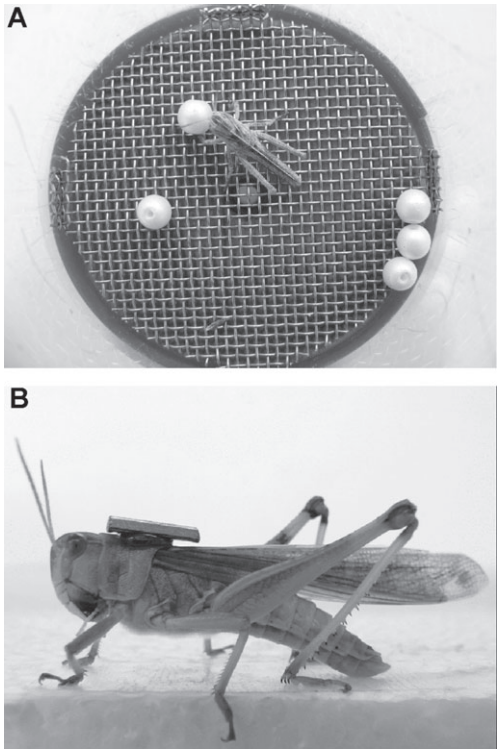

Fig. 1. (A) Third instar locust inside a metabolic hopping chamber with five plastic exercising beads. (B) Adult locust with a steel plate weight attached with depilatory wax to the pronotum.

Respirometry with weight attachments

In an attempt to increase maximum oxygen consumption rate, a second cohort of locusts were exercised exactly as previously described but with a weight attached to their body (Fig. 1B). For first instars (N=18 individuals), the weight was a bead of depilatory wax (Klorane, Boulogne, France) that was attached while warm to the pronotum of the insect and allowed to set. Careful attention was paid to ensure that the wax was not excessively hot upon application. For larger third instars (N=21), fifth instars (N=16) and adults (N=11), the same procedure was employed except that a small steel plate was affixed to the wax before it set. The mass of the attached weight varied depending on the insect's developmental stage, ranging between 20 and 45% (including wax mass) of body mass for all animals. Insects at all life stages behaved normally following the attachment of the weight. Oxygen consumption rates from individuals with a weight attached to their body, $\dot{M}_{O_2,weight}$, $\dot{M}_{R_{O_2,weight}}$ and $\dot{M}_{M_{O_2,weight}}$, were calculated exactly as described for individuals without a weight attached.

All mean values and allometric exponents include ±95% confidence intervals (CI), unless otherwise stated. Statistical significance between means was tested using paired and unpaired t-tests for equal or unequal variance, as appropriate. When three or more means were compared, an ANOVA was performed followed by a Tukey's or Dunnett's *post hoc* test, as appropriate. Allometric data were $\log_{10}$-transformed before statistical analysis using ordinary least-squares regressions. Analysis of covariance (ANCOVA) comparisons of regressions (Zar, 1998), ANOVAs and t-tests were

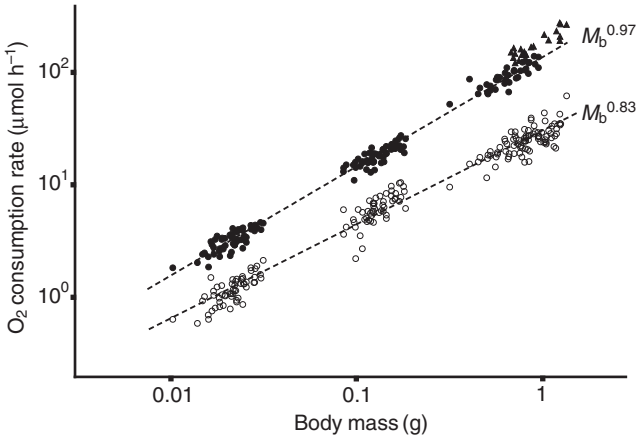

Fig. 2. Relationship between body mass (M_b) and resting metabolic rate ($\dot{M}_{RO_2}$) in first instar (N=55), third instar (N=53), fifth instar (N=37) and adult locusts (N=49) (unfilled circles). Also shown is the relationship between body mass and juvenile maximum metabolic rate during hopping ($\dot{M}_{MO_2,juv}$) in first (N=55), third (N=53) and fifth instar locusts (N=37) (filled circles). The $\dot{M}_{MO_2}$ of adult locusts is also presented (N=23) (filled triangles), but was excluded from the regression due to the disproportionately high $\dot{M}_{MO_2}$ values.

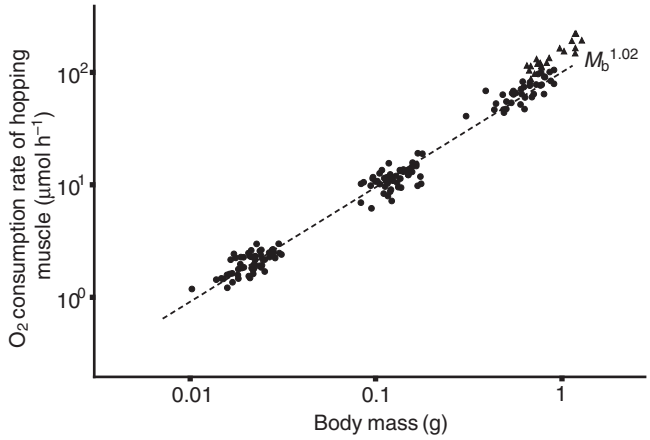

Fig. 3. Relationship between body mass (M_b) and the maximum metabolic rate of juvenile hopping muscle ($\dot{M}_{MO_2,juv,hop}$) in first (N=55), third (N=53) and fifth instar locusts (N=37) (filled circles). The $\dot{M}_{MO_2,hop}$ of adult locusts is also presented (N=23) (filled triangles), but was excluded from the regression due to the disproportionately high $\dot{M}_{MO_2,hop}$ values.

carried out with GraphPad Prism 5 statistical software (GraphPad Software, La Jolla, CA, USA).

RESULTS

Body mass

Body mass increased 45-fold from hatching to early adulthood. First instars had a mean body mass of 0.022±0.001 g, which increased to 0.131±0.007 g by the third instar stage, and 0.666±0.052 g by the fifth instar stage. Male and female adults 3–4 days post-moult had a mean mass of 0.945±0.096 g.

Allometry

$\dot{M}_{RO_2}$ and $\dot{M}_{RO_2,weight}$ values were pooled because they had statistically indistinguishable allometric slopes (ANCOVA, $F_{1,190}$=0.07, P=0.79) and elevations (ANCOVA, $F_{1,191}$=0.07, P=0.79). The pooled data show that throughout ontogeny, resting metabolic rate increases with body mass according to the allometric equation $\dot{M}_{RO_2}=30.1M_b^{0.83\pm0.02}$ μmol h^{-1} at 35°C (r^2=0.97, N=194; Fig. 2). If adults are excluded from the regression, the resting metabolic rate of first, third and fifth instar juveniles scales as $\dot{M}_{RO_2,juv}=32.3M_b^{0.86\pm0.02}$ μmol h^{-1} at 35°C (r^2=0.97, N=145). The exponents for $\dot{M}_{RO_2}$ and $\dot{M}_{RO_2,juv}$ are statistically similar (ANCOVA, $F_{1,335}$=2.1, P=0.15).

There was no significant difference in the slope (ANCOVA, $F_{1,163}$=0.07, P=0.79) or elevation (ANCOVA, $F_{1,164}$=3.26, P=0.07) between $\dot{M}_{MO_2}$ and $\dot{M}_{MO_2,weight}$. Pooled hopping data show that maximum hopping metabolic rate increases with body mass according to $\dot{M}_{MO_2}=155M_b^{1.01\pm0.02}$ μmol h^{-1} at 35°C (r^2=0.99, N=168). However, adults have a disproportionately high $\dot{M}_{MO_2}$ and if they are excluded from the regression, juvenile maximum oxygen consumption rate scales as $\dot{M}_{MO_2,juv}=136M_b^{0.97\pm0.02}$ μmol h^{-1} at 35°C (r^2=0.99, N=145; Fig. 2). The exponent for $\dot{M}_{MO_2,juv}$ is significantly lower than when adults are included in the analysis (ANCOVA, $F_{1,308}$=10.9, P<0.01). There is also a significant difference between the exponents derived for $\dot{M}_{RO_2}$ and $\dot{M}_{MO_2}$ (ANCOVA, $F_{1,357}$=182, P<0.0001) and $\dot{M}_{RO_2,juv}$ and $\dot{M}_{MO_2,juv}$ (ANCOVA, $F_{1,286}$=66, P<0.0001).

The maximum oxygen consumption rate of the hopping muscle increases with body mass according to the allometric equation $\dot{M}_{MO_2,hop}=120M_b^{1.07\pm0.02}$ μmol h^{-1} at 35°C (r^2=0.98, N=168). If adults are excluded, the juvenile maximum oxygen consumption rate of the hopping muscle scales as $\dot{M}_{MO_2,juv,hop}=103M_b^{1.02\pm0.02}$ μmol h^{-1} at 35°C (r^2=0.98, N=145; Fig. 3). The exponent for $\dot{M}_{MO_2,juv,hop}$ is significantly lower than when adults are included in the analysis (ANCOVA, $F_{1,307}$=8.1, P<0.01).

Resting oxygen consumption

During the initial rest period, locusts at all developmental stages regularly exhibited cyclic gas exchange, which is indicative of inactivity (Chown et al., 2006). $\dot{M}_{RO_2}$ increased nearly 25-fold throughout development from 1.2±0.1 μmol h^{-1} in first instars to approximately 28±3 μmol h^{-1} in adults. However, mass-specific $\dot{M}_{RO_2}$ was highest in first instars at approximately 55±3 μmol g^{-1} h^{-1}, and declined steadily with age to 48±3 μmol g^{-1} h^{-1} in third instars, 33±2 μmol g^{-1} h^{-1} in fifth instars and 29±2 μmol g^{-1} h^{-1} in adults (ANOVA, P<0.0001; Fig. 4). *Post hoc* analysis revealed a significant difference in mass-specific $\dot{M}_{RO_2}$ between all life stages (Tukey's, P<0.05) except between fifth instars and adults (Tukey's, P>0.05).

The attachment of weights had no effect on mass-specific $\dot{M}_{RO_2}$ at any developmental stage (first instars, Mann–Whitney U-test, P=0.99; third instars, t-test, P=0.96; fifth instars, t-test, P=0.51; adults, t-test, P=0.48; Fig. 4).

Maximum oxygen consumption

$\dot{M}_{MO_2}$ during hopping exercise increased nearly 55-fold throughout development from 3.3±0.2 μmol h^{-1} in first instars to 176±24 μmol h^{-1} in adults. However, mass-specific $\dot{M}_{MO_2}$ was similar amongst the juvenile instars in that first, third and fifth instars consumed a maximum of 150±6, 139±6 and 141±10 μmol g^{-1} h^{-1}, respectively (Fig. 4). Adults had a higher mass-specific $\dot{M}_{MO_2}$ of 195±13 μmol g^{-1} h^{-1}. Statistical analysis confirms that the mean value of adults is significantly greater than each of the juvenile stages (ANOVA, P<0.0001; Tukey's *post hoc*, P<0.05), but that juvenile values are all similar to one another (Tukey's *post hoc*, P>0.05).

The attachment of weights had no effect on mass-specific $\dot{M}_{MO_2}$ at any of the developmental stages (first instars, t-test, P=0.15; third

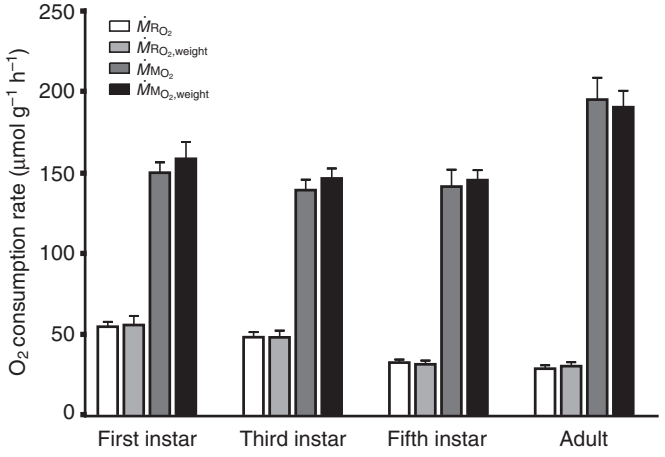

Fig. 4. Mass-specific resting metabolic rate ($\dot{M}_{R_{O_2}}$) and maximum hopping metabolic rate ($\dot{M}_{M_{O_2}}$) (mean ± 95% CI) in first instars with (N=18) and without weights (N=37), in third instars with (N=21) and without weights (N=32), and in fifth instars with (N=16) and without weights (N=21). Also shown is $\dot{M}_{R_{O_2}}$ in adults with (N=11) and without weights (N=38) and $\dot{M}_{M_{O_2}}$in adults with (N=11) and without weights (N=12).

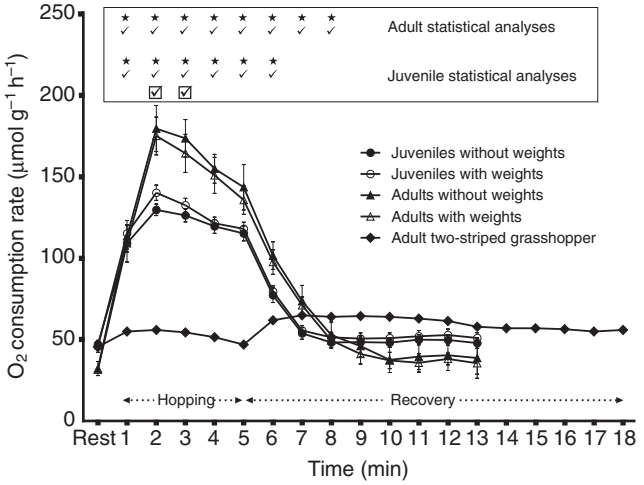

Fig. 5. Mass-specific metabolic rate ($\dot{M}_{O_2}$; mean ± 95% CI) during rest, 5 min hopping and 8 min recovery for combined juvenile data with (N=55) and without weights (N=90), and adults with (N=11) and without weights (N=12). Statistical analyses: ★, the individual mean $\dot{M}_{O_2}$ is significantly greater than the initial resting metabolic rate in locusts without a weight attached (ANOVA with Dunnett's, P<0.05); ✓, the individual mean $\dot{M}_{O_2}$ is significantly greater than the initial resting metabolic rate in locusts with a weight attached (ANOVA with Dunnett's, P<0.05); ☑, significant difference between individual mean $\dot{M}_{O_2}$ for locusts with and without a weight attached at a given time (t-tests, P<0.05). Also shown is the mean mass-specific $\dot{M}_{O_2}$ during rest, 5 min hopping and 13 min recovery in adult two-striped grasshoppers, *Melanoplus bivittatus* (N=8), measured by Harrison and colleagues (Harrison et al., 1991).

instars, t-test, P=0.14; fifth instars, t-test, P=0.56; adults, t-test, P=0.59; Fig. 4).

Difference between sexes

It was possible to determine the sex of adult locusts. Mean mass-specific pooled $\dot{M}_{R_{O_2}}$ was 31±1 μmol g^{-1} h^{-1} in males (N=14) and 32±3 μmol g^{-1} h^{-1} in females (N=9), with no significant difference between sexes (t-test, P=0.84). Mean mass-specific pooled $\dot{M}_{M_{O_2}}$ was 193±5 μmol g^{-1} h^{-1} in males (N=14) and 191±8 μmol g^{-1} h^{-1} in females (N=9), and once again there was no significant difference between sexes (t-test, P=0.84).

Oxygen consumption during rest, hopping and recovery

The pattern of change in mass-specific $\dot{M}_{O_2}$ during hopping experiments in first, third and fifth instar locusts was very similar and so combined juvenile data are presented (Fig. 5). From initial low resting levels, juvenile $\dot{M}_{O_2}$ increased significantly at the commencement of hopping with the highest rates occurring 2–3 min into exercise when mean hopping $\dot{M}_{O_2}$ ranged from 124 to 133 μmol g^{-1} h^{-1}. In a similar pattern, adult $\dot{M}_{O_2}$ also increased quickly during forced hopping, and after 1 min of exercise mean $\dot{M}_{O_2}$ was matched with that of juveniles at the same time (t-test, P=0.44; Fig. 5). However, for the remainder of the exercise period, mean mass-specific $\dot{M}_{O_2}$ in adults remained significantly above juvenile $\dot{M}_{O_2}$ (t-tests and Mann–Whitney U-test, P<0.05), reaching 180 μmol g^{-1} h^{-1} before a final decline.

The attachment of weights to the juvenile locust body did not produce a substantial increase in oxygen uptake during hopping, although a slight but significant increase (6–9%) in mean mass-specific $\dot{M}_{O_2}$ was observed 2 and 3 min into the exercise period (t-test, P<0.05; Fig. 5). In adults, there was no detectable effect of attached weight on mean mass-specific hopping $\dot{M}_{O_2}$ (t-tests and Mann–Whitney U-test, P>0.05).

Following exercise, recovery was quick, with mean mass-specific $\dot{M}_{O_2}$ returning back to resting levels within 2 min in juveniles and 4 min in adults (Fig. 5). The attachment of weights did not have a significant effect on mean $\dot{M}_{O_2}$ during recovery in either juveniles or adults (t-tests and Mann–Whitney U-test, P>0.05).

DISCUSSION

Allometry

Resting oxygen consumption rate in locusts increased with body mass throughout development with an exponent of 0.83±0.02 (Fig. 2). This accords well with the exponents of 0.77 and 0.80 derived in two separate studies on resting American locusts, *Schistocerca americana* (Greenlee and Harrison, 2004a; Harrison et al., 2005), and the exponent of 0.84 determined for resting *Encoptolophus sordidus* grasshoppers (Bailey and Riegert, 1973). The maximum oxygen consumption rate of locusts undertaking hopping exercise increased with body mass in a near-isometric relationship, scaling with an exponent of 1.01±0.02 when adults were included in the regression, or 0.97±0.02 when adults were excluded (Fig. 2). Regardless of whether adults were included in the analysis, $\dot{M}_{M_{O_2}}$ scaled with an exponent that is significantly steeper than $\dot{M}_{R_{O_2}}$. In birds and mammals a similar pattern emerges in that MMR tends to scale with a steeper slope than basal metabolic rate (Bishop, 1999; Savage et al., 2004; Weibel et al., 2004; Weibel and Hoppeler, 2005; White and Seymour, 2005). This suggests that the factors that drive the scaling of MMR are different from those that drive basal or resting rates, and that this could be a feature of metabolic scaling in animal groups other than just birds and mammals.

Scaling exponents do not support an optimised fractal network model

The scaling exponents for $\dot{M}_{R_{O_2}}$, $\dot{M}_{M_{O_2}}$, $\dot{M}_{M_{O_2,juv}}$, $\dot{M}_{M_{O_2,hop}}$ and $\dot{M}_{M_{O_2,juv,hop}}$ are all significantly greater than 0.75 (Figs 2, 3). Therefore, the current study does not provide empirical support for the hypothesis that insect metabolism conforms to the original optimised presentation of the fractal network model (West et al., 1997). The same conclusion has emerged from a number of recent

interspecific (Chown et al., 2007) and intraspecific allometric analyses of insect metabolic rate (Chappell et al., 2009; Chown et al., 2007; Strauss and Reinhold, 2010). Perhaps a potential problem with applying the fractal network model to insects is that it assumes metabolic rate is set by the delivery of oxygen along the tracheal system. However, limited oxygen supply seems inconsistent with the fact that many insect species engage in intermittent gas exchange, during which the spiracles are occluded, sometimes for hours between breaths (Contreras and Bradley, 2009; Lighton, 1996; Quinlan and Gibbs, 2006). In fact, it has been suggested that this respiratory pattern could have evolved to prevent oxidative damage as a result of excess oxygen supply (Hetz and Bradley, 2005). Even during heavy exercise, the supply of oxygen appears to exceed, or at least match, the requirements of the locomotory muscle owing to the lack of anaerobic metabolites produced (Beenakkers et al., 1984; Kirkton et al., 2005; Worm and Beenakkers, 1980).

The lack of agreement between the metabolic scaling exponents derived in the present study and the ¾-power exponent predicted by the fractal network model might also arise if the model is not an appropriate description of oxygen transport in these animals. The apparent interspecific scaling exponent of ¾ determined for insect SMR could simply be a coincidence (e.g. Addo-Bediako et al., 2002). Certainly insects appear to violate at least one core assumption of even the most recent iterations of the model (Banavar et al., 2010): the insect tracheal system is not a network in which resources (oxygen) are distributed from a single source. Instead, atmospheric oxygen enters the tracheal system through multiple valve-like spiracles, with each spiracle typically interconnected by a longitudinal tracheal trunk, anastomosing with a series of transverse trunks that are then often interconnected with a number of medial longitudinal trunks (Harrison et al., 2005). Synchrotron X-ray imaging has also revealed that many of these large tracheae are in fact dynamic structures that undergo cyclic compressions of collapse and reinflation (Greenlee et al., 2009; Socha et al., 2010; Westneat et al., 2003). It is only at a relatively fine scale that a fractal-like branching pattern emerges, and multiple parallel branching networks serve to deliver oxygen throughout the animal. Perhaps, therefore, the near-isometric scaling of $\dot{M}_{MO_2}$ observed in the present study arises because the total maximum delivery capacity of the parallel branching system scales as M_b^{1} rather than $M_b^{3/4}$.

Addition of weights

The addition of weights did not increase the $\dot{M}_{MO_2}$ or mean hopping $\dot{M}_{O_2}$ of adult locusts (Figs 4, 5). However, weights did induce a slight, but insignificant, increase in the $\dot{M}_{MO_2}$ of first, third and fifth instar locusts (Fig. 4), and when juvenile instar data were combined the mean hopping $\dot{M}_{O_2}$ of weighted juveniles was significantly higher than their unweighted counterparts at 2 and 3 min into exercise (Fig. 5). However, the increase in juvenile hopping $\dot{M}_{O_2}$ was only approximately 8%, which might be considered modest given that weighted locusts were forced to carry a load equivalent to 20–45% of their body mass. On balance, it would seem that the hopping muscles of both weighted and unweighted individuals were probably operating at or near their performance limits during exercise.

Any number of factors could set the upper functional metabolic limit of the jumping muscles, including the capacity to deliver and utilise oxygen and metabolic substrate, or even the mechanical limits of the myofibril machinery or associated nervous system. If a single factor is responsible for limiting hopping metabolic rate, then it would follow that all other components that make up the jumping system are present in excess. Alternatively, a well-known but controversial hypothesis is that all structures involved in hopping should be quantitatively matched to the functional capacity of the entire system, such that no single component limits maximum oxygen consumption rate (symmorphosis) (Snelling et al., 2011; Weibel et al., 1998; Weibel et al., 1991).

High hopping metabolic rate in adults

Another finding from this study is that adults have a 1.3-fold higher mass-specific hopping $\dot{M}_{O_2}$ and $\dot{M}_{MO_2}$ than juvenile conspecifics (Figs 4, 5). This is similar to the American locust, where mass-specific hopping metabolic rates are twofold higher in adults compared with juveniles (Kirkton et al., 2005). The high aerobic capacity of the adult hopping muscle is puzzling because it is inconsistent with the observation that its primary function is to perform single powerful jumps required to initiate flight (Katz and Gosline, 1993; Kirkton and Harrison, 2006): such short-term energy needs are more likely to be met by local ATP supplies that are temporarily maintained by arginine phosphate (Newsholme et al., 1978; Schneider et al., 1989). In light of this, it is possible that the high hopping metabolic rate of adult locusts could be due to the inadvertent stimulation of flight muscles during jumping exercise. The flight muscles only develop during adulthood (Mizisin and Ready, 1986), and although there was no evidence of obvious wing movements during adult hopping, smaller wing movements could have easily gone undetected. Potentially, the larger twofold difference between adult and juvenile hopping metabolic rates in the Kirkton et al. (Kirkton et al., 2005) study might be due to their use of more mature adults (up to 21 days post-moult), which, compared with the young adults used in the present study (3–4 days post-moult), would likely have better-developed flight muscles (Mizisin and Ready, 1986).

Comparison with the two-striped grasshopper

The hopping metabolic rate of migratory locusts in the present study differs greatly from previous measurements taken from adult two-striped grasshoppers, *Melanoplus bivittatus* (Harrison et al., 1991). In contrast to the rapid increase in oxygen consumption that occurs during hopping in migratory locusts, two-striped grasshoppers barely increase oxygen consumption rates above resting levels (Fig. 5). In fact, in *M. bivittatus*, the highest aerobic metabolic rates occur after exercise, and then they remain elevated above resting levels for the entire recovery period. Harrison and colleagues suggest that there might be an intrinsic constraint on ventilation during hopping in this insect, which limits oxygen delivery. In contrast, gas exchange during hopping in migratory locusts does not appear to be compromised and so the results of the present study probably provide a better indication of typical terrestrial locomotion energetics for the Orthoptera.

CONCLUSIONS

This study quantifies the allometric scaling of resting and maximum metabolic rate in the migratory locust throughout ontogenetic development. Similar to the pattern in birds and mammals, $\dot{M}_{MO_2}$ scales with an exponent that is significantly steeper than $\dot{M}_{RO_2}$. This study also demonstrates that $\dot{M}_{RO_2}$, $\dot{M}_{MO_2}$, $\dot{M}_{MO_2,juv}$, $\dot{M}_{MO_2,hop}$ and $\dot{M}_{MO_2,juv,hop}$ do not scale in a manner consistent with the optimised fractal network model (West et al., 1997). This might be due to the effectiveness at which oxygen is delivered along the tracheal system, or because the tracheal system is not well described by a model designed for networks that deliver resources from a single source. Finally, this study shows that the addition of weights to hopping locusts does not increase their maximum aerobic metabolic rate, implying that the functional aerobic limits of the jumping muscles were reached during exercise.

LIST OF ABBREVIATIONS

$\dot{M}_{MO_2}$	maximum oxygen consumption rate
$\dot{M}_{MO_2,hop}$	maximum oxygen consumption rate of hopping muscles
$\dot{M}_{MO_2,juv}$	maximum oxygen consumption rate of juvenile locusts only
$\dot{M}_{MO_2,juv,hop}$	maximum oxygen consumption rate of juvenile hopping muscles only
$\dot{M}_{MO_2,weight}$	maximum oxygen consumption rate with weight attached
$\dot{M}_{O_2}$	oxygen consumption rate
$\dot{M}_{O_2,weight}$	oxygen consumption rate with weight attached
$\dot{M}_{RO_2}$	resting oxygen consumption rate
$\dot{M}_{RO_2,juv}$	resting oxygen consumption rate of juvenile locusts only
$\dot{M}_{RO_2,weight}$	resting oxygen consumption rate with weight attached

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