The Journal of Experimental Biology 208, 3003-3012
Published by The Company of Biologists 2005
doi:10.1242/jeb.01747

Ontogenetic effects on aerobic and anaerobic metabolism during jumping in the American locust, *Schistocerca americana*

Scott D. Kirkton*, Jared A. Niska and Jon F. Harrison

Section of Organismal, Integrative, and Systems Biology, School of Life Sciences, Arizona State University, PO Box 874601, Tempe, AZ 85287-4501, USA

*Author for correspondence at present address: Division of Physiology, Department of Medicine, School of Medicine, University of California, San Diego, 9500 Gilman Drive, 0623A, La Jolla, CA 92093-0623, USA (e-mail: skirkton@ucsd.edu)

Accepted 14 June 2005

Summary

Developing vertebrates increase both their locomotory power output and endurance due to ontogenetic improvements in anaerobic and aerobic metabolic capacities. Do similar patterns hold for insect locomotion, or do longer tracheal lengths create problems for oxygen delivery in older animals? We forced developing American locust grasshoppers (*Schistocerca americana*) to jump repeatedly and examined the effect of development on power output, endurance, lactate concentration, oxygen consumption and the oxygen sensitivity of jump performance. As previously shown, power outputs, relative leg lengths and leg cuticular content increased with age. A key finding of this study is that both lactate concentration and aerobic metabolic rate of the jumping muscle increase with age, explaining how the increased leg cuticular stiffness can result in increased power output. After two minutes of jumping, grasshoppers rely completely on aerobic ATP production. The rise in mass-specific, active aerobic metabolic rates with age indicates that problems with longer tracheae can be overcome; however, the reduced endurance, higher lactate concentrations and increased oxygen sensitivity of locomotory performance in older animals indicate that larger/older grasshoppers have smaller safety margins for oxygen delivery during hopping.

Key words: development, endurance, power output, lactate, oxygen consumption, grasshopper, *Schistocerca americana*.

Introduction

In vertebrate locomotion, older animals have both increased power outputs (lizards – Garland, 1984; carp – Wakeling et al., 1999; humans – Doré et al., 2000) and greater endurance (lizards – Garland, 1984; Garland and Else, 1987; snakes and anurans – Pough, 1977, 1978; Pough and Kamel, 1984). These vertebrate locomotory performance improvements with age appear to be facilitated by increased aerobic (squirrels – Chappell and Bachman, 1995; sparrows – Chappell et al., 1999) and anaerobic metabolic rates (fish – Somero and Childress, 1980; humans – Taylor et al., 1997). For insects, some authors have suggested that larger sizes may cause increased problems with oxygen delivery due to slower diffusion rates through longer tracheae (Graham et al., 1995). This raises the question, do insects show improvements in locomotory performance and metabolic rates with age, as seen in vertebrates, or do gas exchange constraints produce a different pattern in tracheated organisms?

Among invertebrates, developmental effects on locomotory performance have been best studied in jumping grasshoppers. During ontogeny, the power output during single, maximal jumps increased sixfold in *Schistocerca gregaria* grasshoppers (Gabriel, 1985a; Queathem, 1991; Katz and Gosline, 1993). This increase in single jump power output was first attributed to a greater proportion of jumping muscle mass and improved energy storage in the exoskeleton of the femur (Gabriel, 1985a,b). However, a second study showed that adults have stiffer cuticular springs but may have the same proportion of muscle as juveniles, leaving the mechanism of increased power output of maximal jumps during development unresolved (Katz and Gosline, 1993). No studies to date have examined the effect of ontogeny on sustained locomotory performance in grasshoppers or other insects.

Ontogenic effects on aerobic and anaerobic metabolism of active insects are also not known. Adult grasshoppers produce lactate at relatively high rates during jumping (Zebe and McShan, 1957; Gade, 1984; Harrison et al., 1991). If juveniles do not produce lactate during jumping, this could at least partially explain the increase in single-jump power output in grasshoppers, and such a pattern would be consistent with the suggestion that larger insects experience more problems with oxygen delivery (Graham et al., 1995). Resting mass-specific metabolic rates fall with age in grasshoppers, but the safety margin for oxygen delivery increases, suggesting an improved oxygen delivery system in larger/older grasshoppers (Greenlee and Harrison, 2004). Morphological measures indicate that mitochondrial content and tracheal oxygen delivery capacities

within the jumping leg of grasshoppers increase strongly with age (Hartung et al., 2004). Thus, the data to date suggest that larger/older grasshoppers do not experience increased problems with oxygen delivery that might reduce locomotory performance. In the present study, we used a wide range of developmental stages (2nd, 4th, 6th instars and adults), representing a 30-fold increase in body mass, to determine how ontogeny affected power output, endurance, lactate concentrations, oxygen consumption and carbon dioxide production during repeated jumping in *S. americana*.

If older/larger grasshoppers experience more problems with oxygen delivery due to longer tracheae, as suggested by Graham et al. (1995), then their locomotion may be more oxygen sensitive than juveniles. For example, if older/larger grasshoppers experience oxygen limitations during jumping, we would predict that their jump performance would be stimulated by hyperoxia, and strongly inhibited by hypoxia; if smaller/younger grasshoppers have excessive oxygen delivery capacities, their locomotory performance should be relatively oxygen insensitive. As noted above, evidence from resting grasshoppers suggests that older *S. americana* grasshoppers have improved oxygen delivery relative to younger animals, probably due to improved convective ventilation (Greenlee and Harrison, 2004). However, metabolic rates and critical oxygen partial pressure (P_{O_2}) values for insects during flight are much higher than measured for insects at rest (Chadwick and Gilmour, 1940; Davis and Fraenkel, 1940; Joos et al., 1997; Harrison and Lighton, 1998; Greenlee and Harrison, 2004). If body size imposes limits on tracheal oxygen delivery, these are most likely to be evident during locomotion, when gas exchange requirements are high.

Materials and methods

Determination of grasshopper age

S. americana Drury were reared from eggs in culture at Arizona State University as previously described (Harrison and Kennedy, 1994). To determine a grasshopper's age in days within an instar, the thorax of newly molted individuals was marked with a unique color of Testors acrylic paint (Rockford, IL, USA). The paint was shed with the exoskeleton during molting, so it was necessary to re-paint the grasshoppers at each instar. We used animals from the latter two-thirds to three-quarters of an instar because maximal jump performance was reduced during the initial days after molting (Queathem and Full, 1995). Sex could not be distinguished in the 2nd and 4th instars, but in both 6th instars and adults only the larger female grasshoppers were used to maximize the body size contrast across instars. We utilized female adults up to day 21, because they had not yet laid egg pods that dramatically alter body mass (S.D.K., K. J. Greenlee and J.F.H., unpublished).

Morphological changes during development

We measured body mass and femur mass (±0.1 mg) using a Mettler Analytical AE 240 Dual Range Balance (Hightstown, NJ, USA). Femur length was measured to the nearest 0.01 mm with a Mitutoyo Digimatic CD-6 digital micrometer (Kawasaki, Japan). We then sliced the femur longitudinally in half and placed it into 0.35 mol l^{-1} NaOH for approximately 24 h for tissue digestion (Marden, 1988). After the tissue was removed, the femoral exoskeleton was washed in distilled water, blotted dry and re-weighed. The femoral exoskeletons of 6th instars and adults were weighed on the analytical balance and those of the 2nd and 4th instars were weighed on a Cahn C-33 microbalance (±0.01 mg; Cerritos, CA, USA). Wet tissue mass was calculated as the difference between the exoskeleton mass and wet femur mass. We calculated the extensor tibia muscle mass to be 66% of the measured wet tissue mass (Hartung et al., 2004).

Measurements of jumping performance

Grasshoppers were removed from the colony on the day of the experiment and kept in a group with food at 35°C. Approximately 1 h before a jumping trial, we weighed the individual to be jumped and removed the distal third of the adult wings to prevent flight. Grasshoppers were encouraged to jump by physical prodding for up to 5 min or until fatigued, defined as a 30-s pause between subsequent jumps with continued prodding. The method of physical stimulation was varied throughout the trial to motivate the grasshopper. Grasshoppers were jumped on a cotton bed-sheet (310×80 cm) that was divided into a 10 cm (adult) or 5 cm (juvenile) numbered grid system within a 35°C temperature controlled room. As grasshoppers jumped between squares during the trial, the grid number was called out and recorded onto an audiotape. Distances were measured from the center of each square.

Jump frequency, total distance jumped, and mean distance per jump were measured during each minute of the trial. The jump energy (E) was calculated from:

$$E = (m_b \, \boldsymbol{g} \, d)/(2 \sin 2\theta) , \quad (1)$$

where m_b is the body mass, $\boldsymbol{g}$ is the acceleration due to gravity, d is the distance jumped and θ is the take-off angle of the jump (Bennet-Clark, 1975). Across all developmental stages in *S. americana*, the average take-off angle is 45° (Katz and Gosline, 1993; Queathem and Full, 1995). We calculated the mass-specific power output (P) as the ability of grasshoppers to generate jumping power over a time interval spanning numerous jumps:

$$P = E_{sum}/(t \, m_b) , \quad (2)$$

where E_{sum} is the sum of all jump energy within a time period, and t is the time over which the power output was calculated.

Lactate concentration during jumping

Grasshoppers similar in age to the ones used in the jumping performance experiment were jumped in a 100 liter Plexiglas gloved box at 35°C for 0, 15, 30, 60, 120, 300 or 600 s (N=8–10 at each time). After jumping, animals were frozen in liquid nitrogen and stored at –20°C. Later, the body mass, femur mass, femur length and lactate concentrations were measured.

Frozen femurs were diluted 9× with chilled 0.6 mol l^{-1} perchloric acid and pulverized with a ground-glass-tissue homogenizer immersed in ice-water. After centrifugation (Beckman Microfuge E, Palo Alto, CA, USA) for 5 min at 15 850 ***g***, the supernatant was removed and diluted 9× with a 25 mmol l^{-1} 2-amino-2-methyl-1-propanol buffer. Lactate was measured in the supernatant using a fluorometric assay (Passonneau and Lowry, 1993) with a Standard Curve Filter Fluorometer (Optical Technology Devices Inc., Elmsford, NY, USA). Each sample was run six times [three with lactate dehydrogenase (LDH) and three without LDH to account for background noise].

O_2 consumption and CO_2 production during jumping and recovery

Gas exchange for jumping *S. americana* was measured using Plexiglas chambers for all ages except the 2nd instars, which were jumped in a portion of a 60 cm^3 syringe. Each age group (N=7 per group) was tested using a different-sized chamber and flow rates so that the 95% equilibrium time was approximately 45 s in each case (Lasiewski et al., 1966): 2nd instars, 10 ml chamber, 35.0 ml min^{-1} flow rate; 4th instars, 39.5 ml chamber, 138.4 ml min^{-1}; 6th instars, 116.4 ml chamber, 377.8 ml min^{-1}; adults, 246.3 ml chamber, 563.1 ml min^{-1}. Each jumping chamber was air tight except for incurrent and excurrent air-flow ports. The smooth bottom surface of the chamber was covered with coarse sand paper to facilitate jumping. Grasshoppers were encouraged to jump using the end of a small paint brush attached to a wire that was inserted through a rubber septum.

To reduce fluctuations in the background oxygen level, dry, CO_2-free air (Balston purge-gas generator; Havervill, MA, USA) was generated and stored in a gas cylinder, which was warmed with heating pads to improve mixing. The purge gas generator was then disconnected, and air flow from the cylinder to the respirometry system was controlled by a Brooks 5878 mass flow controller and three 5850i Brooks mass flow meters (Brooks Instruments, Hatfield, PA, USA). One meter controlled air flow to the respirometry chamber and the grasshopper, the second meter was used to flush the respirometry chamber between trials, and the third meter controlled a line that flowed to the reference channel of the gas analyzers (Fig. 1). Using three-way valves, we could switch between recording either the baseline O_2 and CO_2 levels (while the animal chamber was being flushed) or the grasshopper's gas exchange.

Both the reference stream and the excurrent stream from the jumping chamber were dried using $MgClO_4$ (except for the 2nd instars, for whom this step was omitted to improve temporal resolution). Next, the air in each line emptied into a syringe open to the atmosphere, from which air was sub-sampled (at 10 ml min^{-1} for 2nd instars and 50 ml min^{-1} for the other ages) using a Li-Cor pump (Li-Cor, Lincoln, NE, USA) and pulled through a Li-6252 Li-Cor CO_2 analyzer and then an Oxilla O_2 analyzer (Sable Systems, Las Vegas, NV, USA). Both gas analyzers were used in the differential mode to improve resolution. CO_2 and water were removed from each stream between the analyzers using Ascarite® and Drierite®, respectively. The flow rates through the analyzers were controlled by FMA-A2405 Omega mass flow controllers (0–500 ml min^{-1}; Stamford, CT, USA) located between the O_2 analyzer and the Li-Cor pump. The output of both gas analyzers was digitized and recorded (Sable Systems).

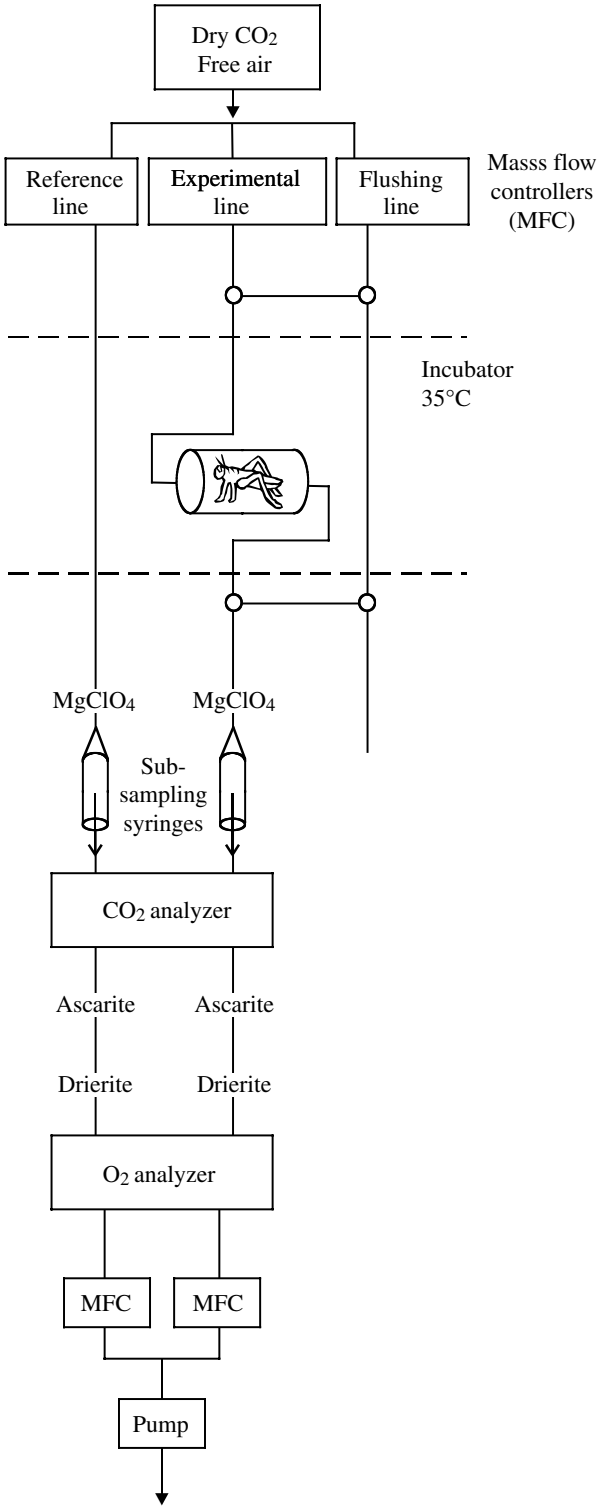

Fig. 1. Schematic of the respirometry set-up.

Table 1. *Scaling of metathoracic femur mass, extensor tibia muscle mass, femoral exoskeleton mass, and metathoracic leg length with body mass across instars of* S. americana *(N=196)*

Log variable	Slope (S.E.M.)	Upper 95% C.L.	Lower 95% C.L.	Intercept (S.E.M.)	r^2
Femur mass (mg)	1.046 (0.007)	1.059	1.032	–1.117 (0.019)	0.99
Extensor tibia muscle mass (mg)	1.006 (0.007)	1.020	0.992	–1.06 (0.019)	0.99
Femoral exoskeleton mass (mg)	1.470 (0.013)	1.496	1.444	–3.305 (0.037)	0.98
Leg length (mm)	0.375 (0.006)	0.387	0.363	0.156 (0.016)	0.96

All equations are in the form: log variable = intercept + slope (log body mass).

After recording a starting baseline CO_2 and O_2 level for 5 min, the three-way valves were switched to record the animal's resting CO_2 production and O_2 consumption for 10 min. Next, the jumping metabolic rate was measured for 5 min by turning on a light in the incubator and stimulating the grasshopper to jump with the brush. After 5 min, the light was turned off and the grasshopper's whole-body CO_2 production and O_2 consumption during recovery were measured for approximately 10 min before switching back to the bypass and recording baseline values for the last 5 min of the trial. In control tests, there was no evidence of leaks when switching between the different experimental streams nor when manipulating the brush in the chamber. The signal-to-noise resolution of the respirometry system ranged from 12 (resting 2nd instars) to 95 (jumping adults) for O_2, and from 337 (resting 2nd instars) to 1554 (jumping adults) for CO_2. Immediately following the trial, the animal was weighed on the analytical balance (±0.1 mg).

To quantify how the aerobic ATP production of the jumping muscles may change during ontogeny, we calculated the locomotory rate of oxygen uptake ($\dot{V}_{O_2}$) of the jumping muscle by comparing the $\dot{V}_{O_2}$ values for animals stimulated to locomote before and after autotomizing their jumping legs. Each grasshopper was encouraged to autotomatize its hind femurs by gently rubbing the femoral joints with warmed scissors. Autotomy caused minimal damage to the animal because there are no direct muscles in the autotomical plane, and after the leg is detached a membrane closes the wound, minimizing haemolymph bleeding (Arbas and Weidner, 1991). Also, the thoracic muscles responsible for moving the femur do not show denervation for at least 3 days (Personius and Arbas, 1998) or muscular atrophy for 7 days after the autotomy (Arbas and Weidner, 1991). The hindleg-less grasshopper was returned to a 35°C container with food and water. The metathoracic hind femurs were weighed on an analytical balance, and extensor tibia muscle mass was calculated as previously described. O_2 consumption and CO_2 emission were re-measured exactly as described above for juveniles on the following day and for adults after 2 days.

Injections of room air were used for each chamber and flow rate to correct for lag time from the chamber to the analyzers (approximately 1 min) and between analyzers. The rates of whole-body O_2 consumption and CO_2 emission ($\dot{M}_{O_2,wb}$ and $\dot{M}_{CO_2,wb}$, respectively; μmol g^{-1} h^{-1}) were calculated similarly to Greenlee and Harrison (2004) and converted from ml g^{-1} min^{-1} to μmol g^{-1} h^{-1}. There were no statistically significant differences in jump frequencies between the grasshoppers forced to jump in the respiratory chamber and those jumped in the temperature-controlled room, so these values are not reported.

Oxygen sensitivity during jumping

Oxygen sensitivity during jumping was measured in a 100 liter Plexiglas gloved box at 35°C. The gloved box was perfused at 6 l min^{-1} with artificial oxygen atmospheres (5 kPa, 12 kPa, 21 kPa or 45 kPa – balance nitrogen) created by mixing oxygen and nitrogen supplied from compressed tanks *via* needle valves with mixtures confirmed using an S-3A/I AEI Technologies Oxygen Analyzer (Pittsburgh, PA, USA). Within the gloved box, a fan promoted air circulation, a thermocouple recorded air temperature, and the bottom was covered with a soft paper to provide traction for the jumping grasshoppers. $\dot{P}_{O_2}$ did not vary from the reported mix in the empty gloved box.

Individual grasshoppers were placed in the adjoining airlock for 30 s to equilibrate to the oxygen tension before being released into the chamber. Grasshoppers were forced to jump for up to 10 min or until fatigued (defined as when 30 s passed between jumps). Each animal was tested in a single oxygen atmosphere (N=5–8 per oxygen tension).

Statistical analysis

The effects of age and time during the trial on jump performance data were analyzed using repeated-measures analysis of variance (ANOVA) with Systat 10.2 (Systat Software Inc., Richmond, CA, USA). We utilized ANOVA with Bonferroni-corrected *post-hoc* comparisons to compare the lactate concentrations among different aged grasshoppers at specific minutes during the trial (Sokal and Rohlf, 1995). The $\dot{M}_{O_2}$, $\dot{M}_{CO_2}$ and oxygen sensitivity data were analyzed using ANOVA with Bonferroni-corrected *post-hoc* comparisons. In all cases, our level of significance was 0.05. Unless otherwise noted, all reported values are the means ± standard errors.

Results

Morphological changes

During development in *S. americana*, femur mass and muscle mass grew isometrically relative to body mass (Table 1). In contrast to muscle development, femurs from

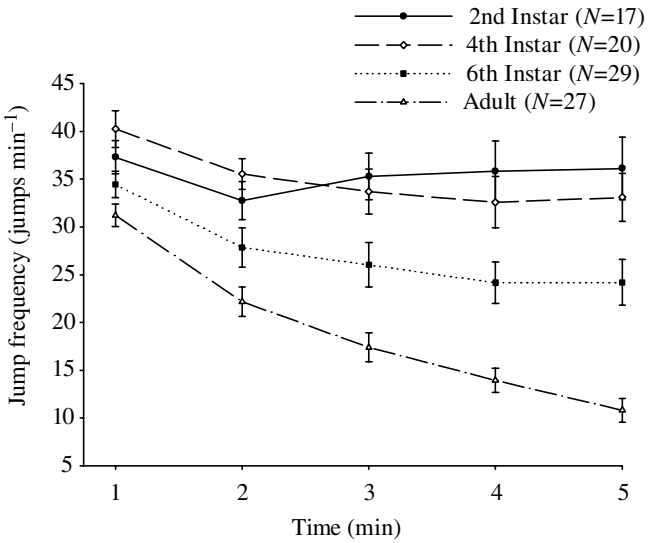

Fig. 2. Endurance of jump frequency was negatively correlated with age across instars. Values are means ± S.E.M.

larger animals were proportionally longer and had a greater amount of exoskeleton (Table 1). For the grasshoppers used in the jumping experiment, body mass increased ~30-fold as animals developed from the 2nd instars to adults (2nd instars, 60±1.6 mg; 4th instars, 262±6.5 mg; 6th instars, 1219±37.3 mg; adults, 1782±62.0 mg).

Jump performance

Jump performance was significantly affected by age. The mean number of jumps per minute declined more rapidly in older animals (Fig. 2; repeated-measures ANOVA, instar × time, $F_{12,227}$=3.7, P<0.001). The body mass-specific power output decreased during the trial regardless of age; however, power output decreased more rapidly in older animals (Fig. 3; repeated-measures ANOVA, instar × time, $F_{12,227}$=13.2, P<0.001).

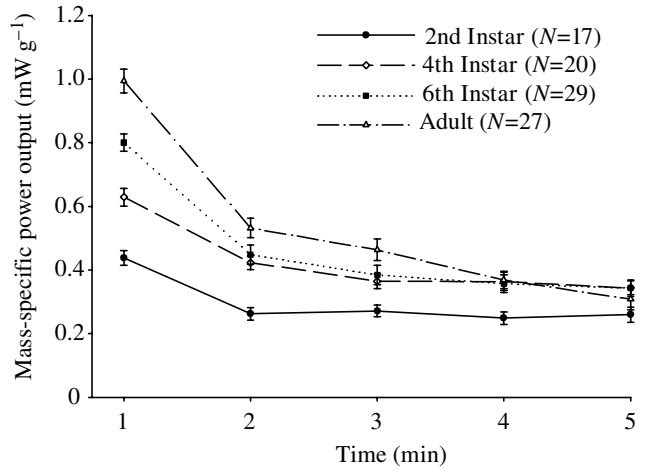

Fig. 3. Older/larger grasshoppers showed greater body mass-specific power outputs and increased fatigue during the jumping trial. Values are means ± S.E.M.

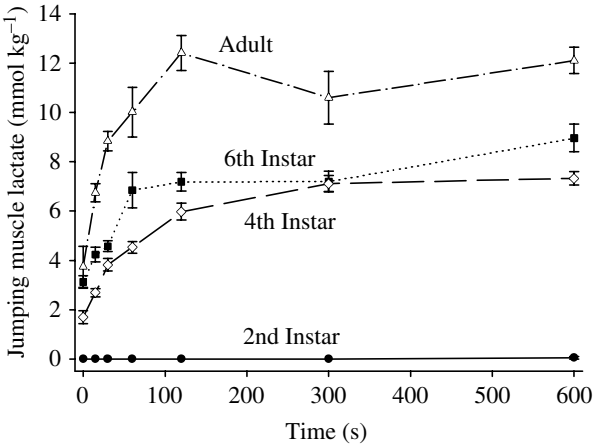

Fig. 4. Lactate levels increased with age and time spent jumping in the first 2 min (N=8–10 at each point). Values are means ± S.E.M.

Anaerobic capacity and lactate concentration

Lactate concentrations in the extensor tibia muscle increased across developmental stages and with time spent jumping (Fig. 4). There was no detectable lactate in the muscles of 2nd instars during the trial (assay detection limit was 0.3 mmol kg^{-1}). Muscle-specific lactate levels measured at time 0 increased with age, rising from 1.7±0.3 mmol kg^{-1} in 4th instars to 3.1±0.2 mmol kg^{-1} in 6th instars and 3.7±0.8 mmol kg^{-1} in adults (Fig. 4; ANOVA, $F_{3,28}$=13.4, P<0.001). During 2 min of jumping, muscle lactate increased more rapidly in older animals (Fig. 4; ANOVA, $F_{3,33}$=157.7, P<0.001). After 2 min of jumping, lactate levels reached a peak for each age group (6.0±0.3 mmol kg^{-1} in 4th instars, 7.2±0.4 mmol kg^{-1} in 6th instars and 12.4±0.7 mmol kg^{-1} in adults) and did not increase during the rest of the trial. No lactate was detected in the haemolymph.

O_2 consumption and CO_2 production during rest, jumping and recovery

During jumping, maximal (30 s period) $\dot{M}_{O_2,wb}$ and $\dot{M}_{CO_2,wb}$ increased significantly with age (O_2: Fig. 5A; ANOVA, $F_{3,24}$=38.2, P<0.001; CO_2: Fig. 5B; ANOVA, $F_{3,24}$=33.1, P<0.001). $\dot{M}_{O_2,wb}$ and $\dot{M}_{CO_2,wb}$ of jumping adults were double that of the 2nd instars (Fig. 5). The scope in $\dot{M}_{O_2,wb}$ (jumping relative to resting values) increased with age (2nd instars, 2.0; 4th instars, 2.4; 6th instars, 3.4; adults, 4.2). After 2 min of recovering, there was no effect of age on $\dot{M}_{O_2,wb}$ (Fig. 6A), but $\dot{M}_{CO_2,wb}$ was inversely related to age (Fig. 6B; ANOVA, $F_{3,24}$=13.8, P<0.001). Similarly, during jumping, age had a significant effect on the amount of O_2 consumed by the jumping muscle (Fig. 7A; ANOVA, $F_{3,24}$=6.6, P<0.01) and CO_2 produced (Fig. 7B; ANOVA, $F_{3,24}$=4.6, P<0.05), with both increasing with ontogeny.

Regardless of activity state (resting, jumping or recovery), grasshopper age did not significantly affect the respiratory exchange ratio (RER, the ratio of CO_2 produced to O_2 consumed), so RER values were combined across ages. RER did not change between rest and thirty seconds of jumping

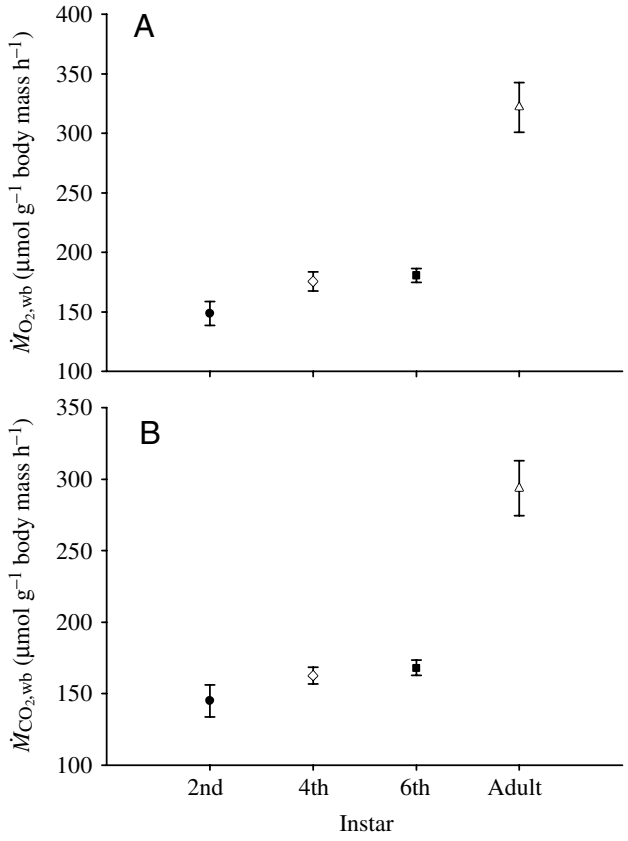

Fig. 5. Whole body oxygen consumption (A) and carbon dioxide emission (B) during jumping across different instars of *S. americana* (N=7 at each point). Values are means ± S.E.M.

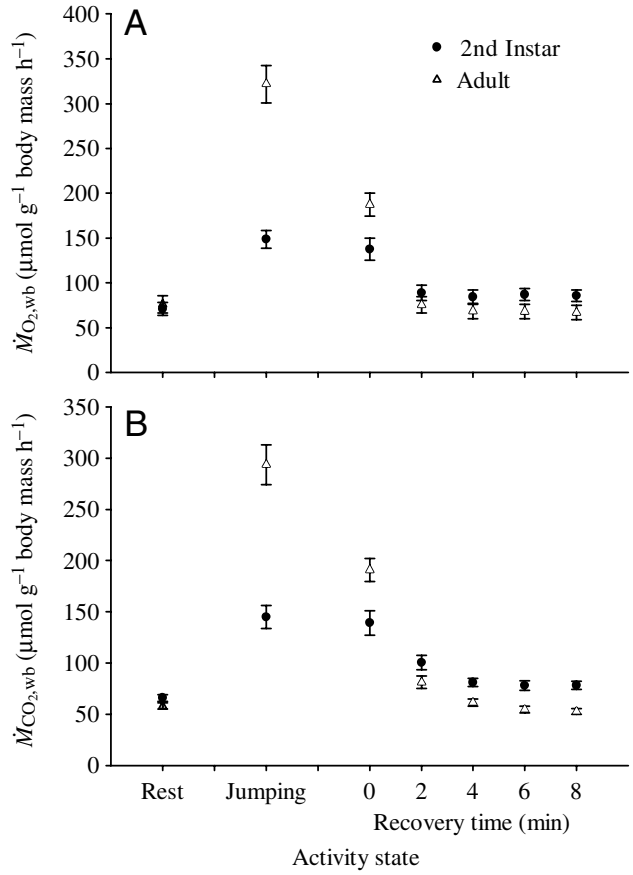

Fig. 6. Whole-body oxygen consumption (A) and carbon dioxide emission (B) at rest, jumping and recovery for 2nd instars and adult *S. americana* grasshoppers. The values of 4th and 6th instars were intermediate and not shown for sake of clarity (N=7 at each point). Values are means ± S.E.M.

(0.94) but significantly increased to 1.0 when jumping ended (Fig. 8; repeated-measures ANOVA, $F_{6,19}$=10.3, P<0.001). The RER was greatest after 2 min of recovery (1.2) and then decreased back to values observed at rest.

Oxygen sensitivity during jumping

Within an age group, hypoxia affected first-minute jump rates. Extreme hypoxia (5 kPa O_2) significantly reduced first-minute jump rate for grasshoppers of all ages, while moderate hypoxia (12 kPa O_2) significantly reduced first-minute jump rate only in the 2nd instars (Fig. 9; ANOVA, $F_{3,20}$=4.8, P<0.05) and adults (Fig. 9; ANOVA, $F_{3,30}$=28.6, P<0.001). When compared with normoxia (21 kPa), there was no effect of hyperoxia (45 kPa) on first-minute jump rate for 2nd and 4th instar grasshoppers of any age; however, hyperoxia increased the first-minute jump rate in 6th instars (Fig. 9; ANOVA, $F_{3,21}$=20.3, P<0.001) and adults (Fig. 9; ANOVA, $F_{3,30}$=28.6, P<0.001).

Age did not significantly affect endurance in the gloved box (Fig. 10), perhaps due to overall lower jump rates in the glove box. Moderate hypoxia (12 kPa O_2) reduced endurance in older grasshoppers (6th instars – ANOVA, $F_{3,21}$=70.0, P<0.001; adults – ANOVA, $F_{3,30}$=22.5, P<0.001) but not the younger animals (2nd and 4th instars; Fig. 10). Endurance was strongly reduced in extreme hypoxia (5 kPa) for all ages. Decreasing the oxygen level reduced jumping endurance for all juveniles, with hypoxic effects increasing with age in juveniles (Fig. 10). Endurance of the largest juveniles (6th instars) was significantly affected by moderate hypoxia while the endurance of 2nd instars was only significantly affected at extreme hypoxia (Fig. 10). The jumping endurance of adults was significantly affected by moderate hypoxia but proportionally less so than that of 6th instars (Fig. 10).

Discussion

Our data indicate that, as generally shown for vertebrates, most measures of locomotory and metabolic performance increase with age in jumping grasshoppers. Mass-specific power output, aerobic metabolic rate, and lactate concentrations during jumping all increase dramatically and steadily throughout ontogeny. The increase in mass-specific aerobic metabolism during development seems inconsistent with the hypothesis that larger size and longer tracheae necessarily lead to increased problems with oxygen delivery in insects. However, the rise in lactate concentrations, the decrease in endurance and the increase in the oxygen

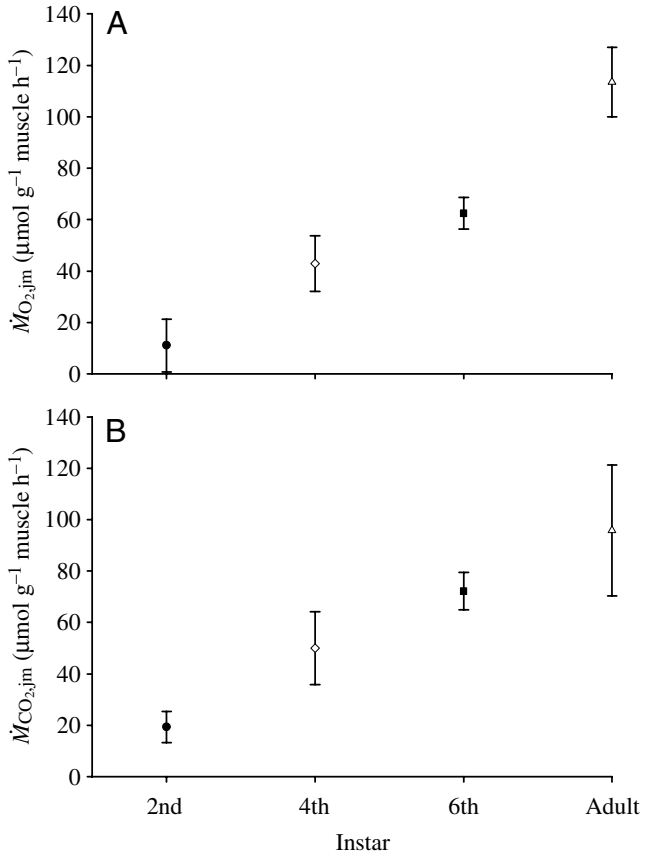

Fig. 7. Muscle-specific oxygen consumption (A) and carbon dioxide emission (B) increases with instar during jumping (N=7 at each point). Values are means ± S.E.M.

sensitivity of locomotion do indicate that the safety margin for oxygen delivery is reduced in older jumping grasshoppers. However, these safety margins decrease with age due to increases in mass-specific power outputs (and ATP

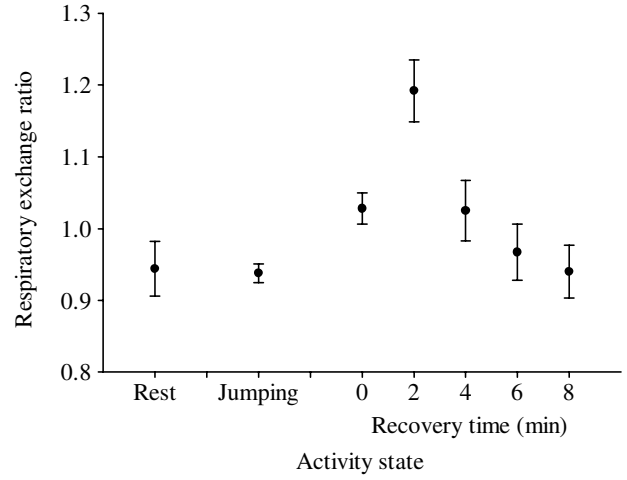

Fig. 8. The changes in respiratory exchange ratio during rest, jumping and recovery, with all ages of grasshoppers pooled together (N=7 at each point). Values are means ± S.E.M.

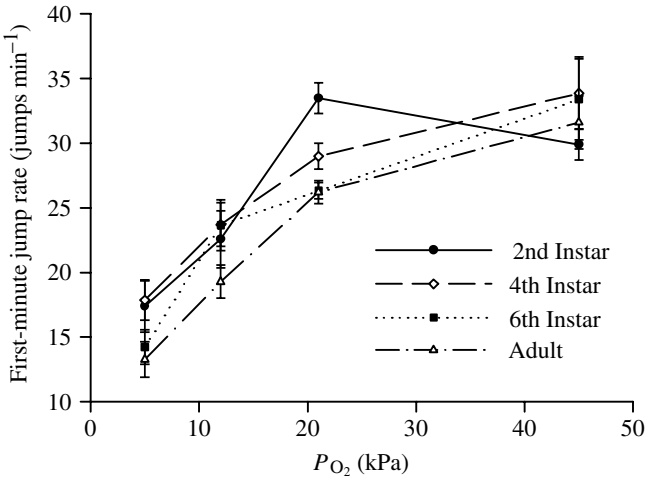

Fig. 9. The effects of atmospheric oxygen content on first-minute jump rates for each age (N=5–8 at each point). Values are means ± S.E.M.

requirements) rather than because mass-specific oxygen delivery capacities fall.

Similar to other developmental studies of locomotory $\dot{V}_{O_2}$ (Full, 1987; Chappell and Bachman, 1995; Chappell et al., 1999), the factorial aerobic scope increased with ontogeny. The whole-body aerobic scope increased from 2.1 in 2nd instars up to 4.2 in adults. Compared with other types of insect locomotion, the aerobic scope calculated during jumping is low (running ectothermic insects, 8; running endothermic insects, 26; flying insects, 129; Full, 1997). While the aerobic scopes are low, the amount of oxygen consumed during jumping in *S. americana* adults (7.2 ml g^{-1} h^{-1}) was greater than the mean oxygen consumption of running insects (2.4–4.5 ml g^{-1} h^{-1}; Harrison and Roberts, 2000), suggesting that the relatively low scopes are due to elevated resting rates in this study. Our measured values for resting $\dot{M}_{O_2}$ were approximately double

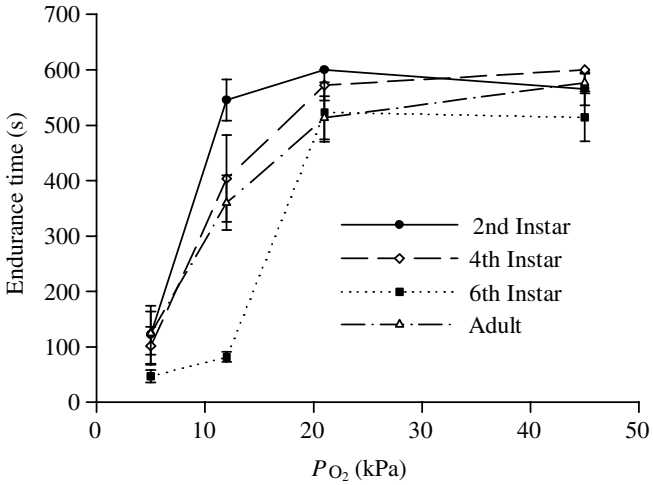

Fig. 10. The effects of atmospheric oxygen content on jump endurance for each age (N=5–8 at each point). Values are means ± S.E.M.

Table 2. *Relative contributions of jumping muscle-specific aerobic and anaerobic ATP production during the first 2 min of jumping in* S. americana *grasshoppers*

Age	$\dot{M}_{O_2,jm}$ (μmol g^{-1} min^{-1})	Aerobic ATP production* (μmol g^{-1} min^{-1})	$Lactate_{jm}$ (μmol g^{-1} min^{-1})	Anaerobic ATP production (μmol g^{-1} min^{-1})	% of ATP from O_2
2nd	0.17	0.96	0	0	100
4th	0.60	3.39	2.15	2.15	61
6th	0.69	3.90	2.05	2.05	66
Adult	1.12	6.33	4.35	4.35	59

*1 μmole of O_2 = 5.65 ATP (Hochachka and Somero, 1984).

those reported by Greenlee and Harrison (2004), perhaps because the animals could be much more active in the larger chamber we used (we did not monitor activity during the prejump, resting $\dot{M}_{O_2}$ measurements). If the resting $\dot{M}_{O_2}$ from Greenlee and Harrison (2004) is used, aerobic scopes increase to approximately 10 in adults, a value well within the range for an ectothermic running insect.

The high oxygen consumption of jumping *S. americana* compared with insect runners is consistent with the high mitochondrial contents of the muscle (Hartung et al., 2004). The only other reported $\dot{V}_{O_2max}$ for grasshopper jumping (*Melanoplus bivittatus*; 1.3 ml g^{-1} h^{-1}, 58 μmol g^{-1} h^{-1}; Harrison et al., 1991) was ~5-fold lower than measured in this study. *M. bivittatus* may not have such a well-developed respiratory system as *S. americana* since peak gas exchange rates occurred after, rather than during, jumping in *M. bivittatus*, and oxygen consumption rates returned to resting rates much more slowly (Harrison et al., 1991). Strong support for the validity of this surprising interspecific difference is provided by the observation that traveling speeds during jumping are 4-fold higher in *S. americana* than in *M. bivittatus* (Harrison et al., 1991).

What explains the reduced endurance of older grasshoppers?

Unlike the situation for most vertebrates, older animals had reduced endurance in *S. americana* grasshoppers (Figs 2, 3). These trade-offs in performance variables may relate to life history. While juvenile grasshoppers utilize jumping as their primary mechanism for escape from predators and migration (Ellis, 1951), the powerful single jumps of adults are thought to be necessary to achieve the take-off velocity required for flight (Katz and Gosline, 1993).

The stimulation method used during this study seems unlikely to be the cause of the decreased endurance with development. Based on data from running vertebrates (Heglund et al., 1974) and invertebrates (reviewed in Full, 1997), one might expect the jump frequency of grasshoppers to scale with body mass raised to the –0.14 to –0.25. Within the first minute of our experiment, when grasshoppers needed no prodding, the scaling of jump frequency with body mass was –0.06, suggesting that larger grasshoppers jumped more than expected based on vertebrate data. During the second minute of repeated jumping, the scaling coefficient was –0.12. However, by the third minute, the scaling coefficient (–0.21) was similar to values reported for running animals. This suggests that the increased jump frequency of older grasshoppers in the first minute was due to increased reliance on aerobic metabolism in larger animals, rather than the method of stimulation. After two minutes of repeated jumping, when aerobic ATP production dominates, the scaling of jump performance matches running animals.

The reduced endurance of older grasshoppers is correlated with the increased utilization of anaerobic metabolism during the initial minutes when jump frequencies and power outputs are very high compared with younger animals (Fig. 4). During the first two minutes of jumping, the contribution of anaerobic ATP production ranged from 0% in 2nd instars to approximately 40% for older animals (Table 2). The constant lactate levels in the muscle after two minutes of jumping, and lack of appearance of lactate in the haemolymph, indicate that net lactate production is negligible after the first few minutes and suggest that prolonged hopping is completely supported by aerobic metabolism. A predominant reliance on aerobic ATP production during repeated jumping has also been shown in adults of the two-striped grasshopper (Harrison et al., 1991).

Potentially, the increased usage of anaerobic metabolism due to inadequate oxygen delivery during the first minutes of jumping may cause the reduced endurance with age. The stimulation of first-minute jump rate in older animals by hyperoxia (Fig. 9) and the increased lactate concentration in older animals suggest that the Pasteur effect is stimulating lactate production. Lactic acid has long been considered to be an agent of muscle fatigue, primarily due to effects on muscle pH (Juel, 1996). Leg $\dot{P}_{CO_2}$ also rises dramatically during jumping of adult grasshoppers (Krogh, 1913; Krolikowski and Harrison, 1996), which will further reduce muscle pH, potentially causing fatigue. However, the lack of an effect of hyperoxia on endurance argues against this hypothesis. If lactate production is caused by low muscle $\dot{P}_{O_2}$, then hyperoxia should have reduced lactate production and increased endurance in older animals. More recent studies have suggested that muscle fatigue during burst locomotion may be due to accumulation of inorganic phosphates (reviewed in Westerblad et al., 2002). Adult *Locusta migratoria* use arginine phosphate to power the initial seconds of jumping (Hitzemann, 1979; Schneider et al., 1989). Potentially, the use

of arginine phosphate increases with age, and increased accumulation of inorganic phosphate explains the reduced endurance with age.

One of the mysteries of fatigue in jumping grasshoppers is the apparent temporal dissociation between the metabolic events thought to affect fatigue and the organism-level changes in performance. Arginine phosphate utilization is completed within the first five jumps (<15 s) in adult grasshoppers (Hitzemann, 1979; Schneider et al., 1989), and lactate concentrations are similar after two minutes (Fig. 4), but power output falls steadily over many minutes (Fig. 3). These data suggest that other processes (e.g. muscle glycogen depletion, neurotransmitter depletion, changes in hormone levels, behavioral habituation) may contribute to long-term fatigue in grasshoppers.

Safety margins for oxygen delivery decrease with age

Although older *S. americana* have increased aerobic capacities and tracheal oxygen delivery capacities (Hartung et al., 2004), it does appear that there is an increasing tendency for oxygen limitation of jump performance with age. Hyperoxia improved the jump rate in the first minute (Fig. 9) only for larger/older grasshoppers, suggesting that oxygen delivery is inadequate during the first minute only in adults and 6th instars. In many tissues, inadequate oxygen concentrations can cause lactate production (Pasteur effect), and so one hypothesis is that inadequate oxygen delivery during the first minutes of jumping causes the increased lactate levels in older grasshoppers. Therefore, the usually high jump rates of 6th instar grasshoppers at 12 kPa and their rapid fatigue may be due to increased lactate production. The effect of lactate increasing initial jump frequency is supported by the finding that anaerobic and aerobic metabolism correlate with different scaling coefficients during repeated jumping. Measurements of the effect of hyperoxia on lactate production rates during jumping would test this hypothesis. Also, the increased sensitivity of endurance to hypoxia in 6th instars and adults is consistent with a reduced safety margin for oxygen delivery during ontogeny for jumping grasshoppers.

Changes in muscle metabolic potential explain the increase in power output with age

Previous research has suggested that the increased power output during jumping of older grasshoppers was attributed to increased muscle mass:body mass ratio (Gabriel, 1985a). However, our more extensive data set clearly showed that muscle mass developed isometrically with changes in body mass in *S. americana* (Table 1). In addition, when all developmental stages are included from Gabriel's study on *S. gregaria* (Gabriel, 1985a), the proportion of extensor tibia muscle to body mass appears nearly isometric (1st instars 6.1%, 2nd instars 4.7%, 3rd instars 5.0%, 4th instars 4.3%, 5th instars 5.5%, adults 6.3%; N=5 for each age). Thus, it seems likely that isometric development of jumping muscle mass occurs throughout *Schistocerca*, as in the related African migratory locust (*L. migratoria migratoriodes*; Duarte, 1938).

Originally, the increased femoral exoskeleton thickness was thought to explain the greater power output in older grasshoppers by improving cuticular energy storage (Gabriel, 1985b). However, it was later reasoned that thicker femoral walls would make it more difficult for grasshoppers with similar proportions of muscle to bend the cuticle to store energy, so a stiffer spring could not, on its own, explain improved jump performance (Katz and Gosline, 1992). These estimates of energy storage assumed constant muscle properties across instars (Gabriel, 1985a,b; Katz and Gosline, 1993). However, adult leg muscles have double the mitochondrial content and a 12-fold greater tracheal diffusing capacity than second instars (Hartung et al., 2004). Our data showed that the oxygen consumption rate of the jumping muscle increased 6-fold during development (during the first 2 min; Table 2). In addition, the muscles of older grasshoppers produced ATP anaerobically at much higher rates (Fig. 4; Table 2). The 10-fold increase in total leg-muscle specific ATP production rates exceeds the greater than 2-fold increase in body-mass specific power output, perhaps because of complexities in the mechanism of power production from a near-isometrically contracting muscle and a spring system (Bennet-Clark, 1975). Nonetheless, it is now clear that increases in the capacity of the jumping muscle to produce ATP (Table 2) allow older/larger grasshoppers to utilize their stiffer leg springs to produce greater power.

We thank Robby Roberson, Ron Rutowski, Glenn Walsberg, Wayne Willis, Brenda Rascón, Joanna Henry, Rekha Nair, Michelle Fay, Sydella Blatch and especially Kendra Greenlee for suggestions regarding this manuscript. We thank Manfred Grieshaber for providing access to K. Hitzemann's thesis. Ty C. M. Hoffman and Kendra Greenlee provided valuable insight and help with the respirometry set-up. This research was supported by the National Science Foundation through award IBN-9985857 to J.F.H. and award IBN-0104959 to S.D.K. and J.F.H.

References

Arbas, E. A. and Weidner, M. H. (1991). Transneuronal induction of muscle atrophy in grasshoppers. *J. Neurobiol.* **22**, 536-546.

Bennet-Clark, H. C. (1975). The energetics of the jump of the locust *Schistocerca gregaria*. *J. Exp. Biol.* **63**, 53-83.

Chadwick, L. E. and Gilmour, D. (1940). Respiration during flight in *Drosophilia repleta* Wollaston: oxygen consumption considered in relation to the wing-rate. *Physiol. Zool.* **13**, 398-410.

Chappell, M. A. and Bachman, G. C. (1995). Aerobic performance in Beldings ground squirrels (*Spermophilus beldingi*): variance, ontogeny, and the aerobic capacity model of endothermy. *Physiol. Zool.* **68**, 421-442.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269-2279.

Davis, R. A. and Fraenkel, G. (1940). The oxygen consumption of flies during flight. *J. Exp. Biol.* **17**, 402-407.

Doré, E., Bedu, M., Franca, N. M., Diallo, O., Duche, P. and Van Praagh, E. (2000). Testing peak cycling performance: effects of braking force during growth. *Med. Sci. Sports Exerc.* **32**, 493-498.

Duarte, A. J. (1938). Problems of growth of the African migratory locust. *Bull. Entomol. Res.* **29**, 425-456.

Ellis, P. E. (1951). The marching behaviour of hoppers of the African migratory locust, *Locusta migratoria migratorioides* (R. & F.), in the laboratory. *Anti-Locust Bulletin* **7**, 1-48.

Full, R. J. (1987). Locomotion energetics of the ghost crab. I. Metabolic cost and endurance. *J. Exp. Biol.* **130**, 137-153.

Full, R. J. (1997). Invertebrate locomotor systems. In *Handbook of Physiology Section 13: Comparative Physiology*, vol. II (ed. W. H. Dantzler), pp. 853-930. New York: Oxford University Press.

Gabriel, J. M. (1985a). The development of the locust jumping mechanism. I. Allometric growth and its effects on jumping performance. *J. Exp. Biol.* **118**, 313-326.

Gabriel, J. M. (1985b). The development of the locust jumping mechanism. II. Energy storage and muscle mechanics. *J. Exp. Biol.* **118**, 327-340.

Gade, G. (1984). Anaerobic energy metabolism. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 119-136. Berlin: Springer-Verlag.

Garland, T. (1984). Physiological correlates of locomotory performance in a lizard: an allometric approach. *Am. J. Physiol.* **247**, R806-R815.

Garland, T. and Else, P. L. (1987). Seasonal, sexual, and individual variation in endurance and activity metabolism in lizards. *Am. J. Physiol.* **252**, R439-R449.

Graham, J., Dudley, R., Aguilar, N. and Gans, C. (1995). Implications of the late Palaeozoic oxygen pulse for physiology and evolution. *Nature* **375**, 117-120.

Greenlee, K. J. and Harrison, J. F. (2004). Development of respiratory function in the American locust *Schistocerca americana* I. Across-instar effects. *J. Exp. Biol.* **207**, 497-508.

Harrison, J. F. and Kennedy, M. J. (1994). *In vivo* studies of the acid-base physiology of grasshoppers: the effect of feeding state on acid-base and nitrogen excretion. *Physiol. Zool.* **67**, 120-141.

Harrison, J. F. and Lighton, J. R. B. (1998). Oxygen sensitive flight metabolism in the dragonfly *Erythemis simplicicollis*. *J. Exp. Biol.* **201**, 1739-1744.

Harrison, J. F. and Roberts, S. P. (2000). Flight respiration and energetics. *Annu. Rev. Physiol.* **62**, 179-205.

Harrison, J. F., Phillips, J. E. and Gleeson, T. T. (1991). Activity physiology of the two-striped grasshopper, *Melanoplus bivittatus*: gas exchange, hemolymph acid base status, lactate production, and the effect of temperature. *Physiol. Zool.* **64**, 451-472.

Hartung, D. K., Kirkton, S. D. and Harrison, J. F. (2004). Ontogeny of tracheal system structure: a light and electron-microscopy study of the metathoracic femur of the American locust, *Schistocerca americana*. *J. Morphol.* **262**, 800-812.

Heglund, N. C. and Taylor, C. R. (1974). Scaling stride frequency and gait to animal size: mice to horses. *Science* **186**, 1112-1113.

Hitzemann, K. (1979). Untersuchungen über den Energiestoffwechsel in der Sprungmuskulatur von *Locusta migratoria* (L.). Thesis, Zoologisches Institut Universitat Munster.

Hochachka, P. W. and Somero, G. N. (1984). *Biochemical Adaptation*. Princeton: Princeton University Press.

Joos, B., Lighton, J. R. B., Harrison, J. F., Suarez, R. K. and Roberts, S. P. (1997). Effects of ambient oxygen tension on flight performance, metabolism, and water loss of the honeybee. *Physiol. Zool.* **70**, 167-174.

Juel, C. (1996). Lactate/proton co-transport in skeletal muscle: regulation and importance for pH homeostasis. *Acta Physiol. Scand.* **156**, 369-374.

Katz, S. L. and Gosline, J. M. (1992). Ontogenetic scaling and mechanical behaviour of the tibiae of the African desert locust. *J. Exp. Biol.* **168**, 125-150.

Katz, S. L. and Gosline, J. M. (1993). Ontogenetic scaling of jump performance in the African desert locust (*Schistocerca gregaria)*. *J. Exp. Biol.* **177**, 81-111.

Krogh, A. (1913). On the composition of the air in the tracheal system of some insects. *Skank. Arch. Physiol.* **29**, 29-36.

Krolikowski, K. and Harrison, J. F. (1996). Haemolymph acid-base status, tracheal gas levels and the control of post-exercise ventilation rate in grasshoppers. *J. Exp. Biol.* **199**, 391-399.

Lasiewski, R. C., Acosta, A. L. and Bernstein, M. L. (1966). Evaporative water loss in birds. I. Characteristics of open flow method of determination and their relation to estimates of thermoregulatory ability. *Comp. Biochem. Physio.* **19**, 445-447.

Marden, J. H. (1988). Bodybuilding dragonflies: costs and benefits of maximizing flight muscle. *Physiol. Zool.* **62**, 505-521.

Personius, K. E. and Arbas, E. A. (1998). Muscle degeneration following remote nerve injury. *J. Neurobiol.* **36**, 497-508.

Pough, F. H. (1977). Ontogenetic change in blood-oxygen capacity and maximum activity in garter snakes (*Thamnophis sirtalis*). *J. Comp. Physiol.* **116**, 337-345.

Pough, F. H. (1978). Ontogenetic changes in endurance in water snakes (*Natrix sipedon*): physiological correlates and ecological consequences. *Copeia* **1978**, 69-75.

Pough, F. H. and Kamel, S. (1984). Post-metamorphic change in activity metabolism of anurans in relation to life-history. *Oecologia* **65**, 138-144.

Queathem, E. (1991). The ontogeny of grasshopper jumping performance. *J. Insect Physiol*. **37**, 129-138.

Queathem, E. J. and Full, R. J. (1995). Variation in jump force production within an instar of the grasshopper *Schistocerca americana*. *J. Zool.* **235**, 605-620.

Schneider, A., Wiesner, R. J. and Grieshaber, M. K. (1989). On the role of arginine kinase in insect flight muscle. *Insect Biochem.* **19**, 471-480.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*. New York: W. H. Freeman and Company.

Somero, G. N. and Childress, J. J. (1980). A violation of the metabolism-size scaling paradigm – activities of glycolytic-enzymes in muscle increase in larger-size fish. *Physiol. Zool.* **53**, 322-337.

Taylor, D. J., Kemp, G. J., Thompson, C. H. and Radda, G. K. (1997). Ageing: effects on oxidative function of skeletal muscle *in vivo*. *Mol. Cell. Biochem.* **174**, 321-324.

Wakeling, J. M., Kemp, K. M. and Johnston, I. A. (1999). The biomechanics of fast-starts during ontogeny in the common carp *Cyprinus carpio*. *J. Exp. Biol.* **202**, 3057-3067.

Westerblad, H., Allen, D. G. and Lannergren, J. (2002). Muscle fatigue: Lactic acid or inorganic phosphate the major cause? *News Physiol. Sci.* **17**, 17-21.

Zebe, E. C. and McShan, W. H. (1957). Lactic and a-glycerophosphate dehydrogenases in insects. *J. Gen. Physiol.* **40**, 779-790.