

Body size-independent safety margins for gas exchange across grasshopper species

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Summary

Why is maximal insect body size relatively small compared to that of vertebrates? Possibly insect body size is limited by the capacity of the tracheal respiratory system to delivery oxygen down longer and longer tracheae to the tissues. If so, one possible outcome would be that larger insect species would have a smaller safety margin for oxygen delivery (higher critical P_{O_2} , P_c). We tested this idea by exposing inactive adult grasshoppers of a range of species and body sizes (0.07–6.4 g) to progressively lower oxygen atmospheres and measuring their ventilation frequency and their ability to maintain metabolic rate (indexed by CO_2 emission rate). We analyzed effects of body size on these parameters by simple linear regressions, as well as methods to control for

phylogenetic relatedness among species. We found interspecific variation in P_c , but P_c did not significantly correlate with body mass (average P_c across all species = 4 kPa). Maximal tracheal system conductance scaled approximately with $mass^{0.7}$, and estimated ventilation in hypoxia (ventilatory frequency \times tidal volume) scaled directly with mass, suggesting that convection is the major mechanism of gas exchange in all these species. These comparative data strengthen the growing body of evidence that body size does not affect the safety margin for oxygen delivery in insects.

Key words: oxygen delivery, scaling, insect, gigantism.

Introduction

Why are the largest insects relatively small compared to vertebrates? Many explanations have been proposed. Some have argued that the exoskeleton would collapse if insects grew much larger; the larger size of aquatic than terrestrial invertebrates supports this hypothesis (Currey, 1967; Price, 1997). Possibly the vulnerability of insects during molting, combined with the enhanced benefit of larger prey to predators leads to size-dependent natural selection that reduces insect size (McGavin, 2001). Perhaps the open circulatory system of insects cannot generate sufficient pressures to support fluid flow in very large insects; in support of this hypothesis, insects and spiders reach similar maximal sizes (about 100 g). Multiple authors have suggested that it is possible that the tracheal respiratory system cannot supply sufficient oxygen to sustain very large insects (Miller, 1966; Rutten, 1966; Tappan, 1974). In support of the latter idea, the appearance of gigantic insects in the fossil record coincides with the occurrence of high atmospheric P_{O_2} levels (35%) (Berner and Landis, 1988; Graham et al., 1995; Dudley, 1998). Researchers have proposed that diffusive gas exchange was enhanced by these higher levels of O_2 , allowing insects to evolve larger body sizes (Miller, 1966; Graham et al., 1995; Dudley, 1998). One possible prediction of this hypothesis is that the safety margin

for gas exchange should decrease with size across extant insect species, perhaps reaching zero in the largest species currently alive.

Safety margins for gas exchange can be measured by exposing insects to decreasing levels of O_2 and recording the level at which metabolism can no longer be sustained (the critical P_{O_2} , P_c). Insects typically tolerate very low levels of O_2 (<5%) (Greenlee and Harrison, 2004a), not only maintaining normal levels of O_2 consumption and CO_2 emission, but also being able to ventilate (Greenlee and Harrison, 2004a), eat (Greenlee and Harrison, 2005), jump (Kirkton et al., 2005), and even fly (Chadwick and Williams, 1949; Joos et al., 1997) during substantial hypoxic exposures. However, to date it is unclear how the ability to function in hypoxia scales with body mass.

In insects, as with most other organisms, metabolic rates (and O_2 consumption needs) change with body size, resulting from changes in tissue oxygen needs. Generally, as body size increases, absolute metabolic rate increases, while mass-specific metabolic rate decreases (Schmidt-Nielsen, 1984). Metabolic rate usually scales approximately with body mass to the power 0.75, with reported values ranging from 0.47 to 1.02 (Peters, 1983). As insects increase in body size during development across instars, the safety margin for O_2 delivery

increases (grasshoppers) (Greenlee and Harrison, 2004a) or remains the same (caterpillars) (Greenlee and Harrison, 2005). However, it is possible that ontogenetic patterns in the safety margin for O₂ delivery may be due to developmental changes such as the ability to respond to hypoxia rather than changes in body size *per se* (Greenlee and Harrison, 2004a). To address this question, in this study we examined adults of grasshopper species differing in body size, challenging the respiratory system of each species with hypoxia to determine the P_c .

One possibility is that larger animals compensate for their increased body size (and increased gas exchange needs) by changing ventilatory parameters such as breathing frequency or tidal volume. In mammals and birds, breathing frequency scales with body mass to the -0.25 power, and tidal volume scales directly with mass, providing a scaling of pulmonary ventilation that matches the scaling of metabolic rate (Peters, 1983). The scaling of these ventilatory parameters has never been measured for insects. In addition, animals commonly respond to hypoxia with compensatory breathing (increasing ventilation frequency, tidal volume or both). In developing grasshoppers, ventilatory compensation for hypoxia (increasing frequency and tidal volume) increased with size across instars (Greenlee and Harrison, 2004a). Again, it is possible that the observed pattern was merely due to development of the respiratory system as opposed to a compensatory mechanism in response to body size *per se*. To determine how ventilatory parameters scale with body size and to begin to investigate how the response to hypoxia varies with size in insects, we measured metabolic rates, tidal volumes and ventilatory frequencies in adults of grasshopper species across an order of magnitude range of body sizes while individuals were exposed to decreasing levels of atmospheric oxygen.

Materials and methods

Scaling and determination of P_c

Twenty-three species of grasshopper were collected throughout Arizona, USA. Animals were collected in 2001–2003 from the vicinities of Prescott Valley (112°25'W: 34°58'N), from Arivaca (111°33'W: 31°58'N) and from Yuma (114°67'W: 33°25'N) (Table 1). We also include data from laboratory-reared *Schistocerca americana* Drury for comparison. We transported animals back to the laboratory in screen cages with grass and forbs from their collection site. At the laboratory, up until the time of their experimental use, animals were kept at room temperature (25°C) with a light source provided for warmth during the day and given *ad libitum* access to green leaf lettuce and kale, in addition to their native plants. Animals were tested within 3 days of arrival at the laboratory. All animals were weighed to the nearest 0.001 g on a Mettler (Hightstown, NJ, USA) analytical balance prior to testing.

We measured CO₂ emission at 25°C as previously described (Greenlee and Harrison, 2004a). Briefly, grasshoppers were placed in a respirometry chamber small enough to restrict

movement and allowed to acclimate for 20 min before recording began. All animals were measured in normoxia for 3 min to determine normoxic scaling coefficients for CO₂ emission. A subset of these species (identified by a dagger in Table 1) was then exposed for 3 min to 10 different levels of P_{O_2} in decreasing order (16, 13, 9, 7, 5, 3, 2, 1, 0.5 and 0 kPa O₂) to determine the P_c value for each species (Table 1). We identified the P_c for each grasshopper by comparing confidence intervals for CO₂ emission at each P_{O_2} (Greenlee and Harrison, 2004a). A few animals exhibited discontinuous gas exchange in response to hypoxia and were not included in the computation of P_c . To determine whether our short-term exposures produced P_c values representative of steady-state values, we also measured P_c using 1 h exposures to each P_{O_2} for one relatively large (*Melanoplus differentialis*) and one relatively small (*Melanoplus sanguinipes*) species.

Body size effects on ventilation frequency and tidal volume index

In a separate study (different individuals and species), we quantified changes in ventilation frequency in response to hypoxia for 15 species of Arizona grasshopper (double dagger in Table 1). Animals were field-collected and maintained as described above. We measured ventilation frequency and tidal volume in 21 and 5 kPa P_{O_2} at 25°C, as we have done previously (Greenlee and Harrison, 2004a). Briefly, animals were placed into a respirometry chamber and allowed to acclimate to the chamber for 20 min while the chamber was perfused with air (21% O₂, balance N₂, flow rate = 400 ml min⁻¹). After the acclimation period, the animal's abdomen was magnified using a dissecting scope and the resulting image videotaped (Panasonic SVHS, Desktop Editor Pro-Line, Secaucus, NJ, USA) for 1–2 min with a Hitachi 3CCD camera (Hitachi, Tokyo, Japan). Magnification was adjusted so that the abdomen nearly filled the monitor, and we recorded a metric ruler for calibration. We then perfused the chamber with 5% O₂, 95% N₂ for 3 min, after which time we recorded breathing for 1–2 min. Videotapes were played back to a monitor, and we counted ventilation frequency over a 1 min period and measured changes in abdominal height from frame-by-frame analysis of the video.

During playback, we first measured the ruler on the display and used this conversion to calculate actual abdominal height changes. The height of the abdomen was measured at the third abdominal segment using a Mitutoyo digital micrometer. We recorded maximal height (inspiration) and minimal height (expiration). We calculated an index of tidal volume ($\mu\text{l breath}^{-1}$) from the abdominal height changes during inspiration and expiration. Our prior studies have shown that the grasshopper abdomen approximates an elliptical cone (Greenlee and Harrison, 1998). However, since changes in side view and top view abdominal areas during abdominal pumping were similar, and abdominal length changes are small (Greenlee and Harrison, 1998), a reasonable approximation of tidal volume can be made assuming the grasshopper abdomen approximates a cylinder of constant

Table 1. *Grasshopper species used in our experiments*

Family	Subfamily	Tribe	Genus species	Abbreviation	Collection site	Collection date (month-year)	Sample size
Romaleidae	Romaleinae	Romaleini	<i>Brachystola magna</i>	bm	Arivaca	10-01* [†]	8
			<i>Taeniopoda eques</i>	te	Arivaca	10-01* [†]	8
Acrididae	Melanoplinae	Dactyloini	<i>Dactyloptum variegatum</i>	dv	Prescott Valley	7-01*	2
			<i>Hesperotettix viridis</i>	hv	Prescott Valley	6-01* [†]	1
			<i>Hesperotettix viridis</i>	hv	Prescott Valley	7-01*	4
			<i>Poecilotettix sanguineus</i>	ps	Prescott Valley	6-01* [†]	10
		Melanoplini	<i>Melanoplus aridus</i>	ma	Arivaca	9-03 [‡]	1
			<i>Melanoplus differentialis</i>	md	Arivaca	10-01* [†]	8
			<i>Melanoplus differentialis</i>	md	Arivaca	9-03 [‡]	1
			<i>Melanoplus femurrubrum</i>	mf	Arivaca	9-03 [‡]	1
			<i>Melanoplus gladstoni</i>	mg	Arivaca	9-03 [‡]	1
			<i>Melanoplus sanguinipes</i>	ms	Prescott Valley	6-01* [†]	14
			<i>Melanoplus thomasi</i>	mt	Arivaca	9-03 [‡]	1
			<i>Melanoplus yarrowi</i>	my	Arivaca	9-03 [‡]	1
Cyrtacanthacridinae	Cyrtacanthacridini		<i>Schistocerca americana</i>	sca	Laboratory	* [†]	8
			<i>Schistocerca nitens</i>	sn	Yuma	9-03 [‡]	1
Gomphocerinae	Amblytropidiini		<i>Syrbula admirabilis</i>	sa	Arivaca	7-02* [†]	8
			<i>Syrbula montezuma</i>	sm	Arivaca	7-02* [†]	8
			<i>Boopedon nubilum</i>	bn	Arivaca	9-03 [‡]	1
	Aulocarini		<i>Ageneotettix deorum</i>	ad	Prescott Valley	7-01*	1
			<i>Aulocara ellioti</i>	ae	Prescott Valley	6-01* [†]	7
			<i>Psoloessa delicatula</i>	pd	Prescott Valley	7-01*	4
			<i>Psoloessa texana</i>	pt	Arivaca	7-02* [†]	4
	Cibolacrini		<i>Heliaula rufa</i>	hr	Prescott Valley	7-01*	6
			<i>Heliaula rufa</i>	hr2	Arivaca	7-02* [†]	6
	Eritettigini		<i>Amphitornus coloradus</i>	ac	Prescott Valley	6-01* [†]	7
			<i>Eritettix simplex</i>	es	Prescott Valley	6-01 and 7-01*	5
			<i>Opeia obscura</i>	oo	Prescott Valley	7-01*	1
	Mermeriini		<i>Mermiria bivitatta</i>	mb	Arivaca	9-03 [‡]	1
			<i>Mermiria texana</i>	met	Arivaca	9-03 [‡]	1
	no tribe		<i>Acantherus piperatus</i>	ap	Arivaca	9-03 [‡]	4
	Parapomelini		<i>Parapomala pallida</i>	pp	Prescott Valley	7-01*	1
			<i>Parapomala pallida</i>	pp	Arivaca	9-03 [‡]	3
Oedipodinae	Hippiscini		<i>Hadrotettix trifasciatus</i>	ht	Prescott Valley	7-01*	4
			<i>Heliastus benjamini</i>	hb	Arivaca	9-03 [‡]	1
			<i>Leprus wheeleri</i>	lw	Arivaca	9-03 [‡]	1
			<i>Xanthippus corallipes</i>	xc	Prescott Valley	6-01* [†]	5
	Sphingonotini		<i>Conozoa carinata</i>	cc	Arivaca	9-03 [‡]	1
			<i>Trimerotropis pallidipennis</i>	tp	Prescott Valley	7-01*	1

*Animals used for the scaling study; [†]animals used for the P_c study; [‡]animals used for the ventilation frequency/tidal volume study.

length. Therefore, for this study, we estimated inspiratory abdominal volume and expiratory volume as follows:

$$\text{Abdominal volume} = (\text{abdominal height}/2)^2 \times \text{abdominal length} \times \pi. \quad (1)$$

We calculated tidal volume index as the difference between

the inspiratory and expiratory volumes, using an average of three breaths for each individual. For a variety of reasons, this calculated tidal volume should be considered only an estimate for individual species. First, abdominal length measurements were made on pinned specimens and averaged for a species. Second, our prior simultaneous measures of height, width and

length changes were made only on *Schistocerca americana* (Greenlee and Harrison, 1998), and it is possible that different species show different patterns of abdominal compression. Finally, convection in grasshoppers can be enhanced by non-abdominal movements such as neck pumping (Miller, 1960). We calculated our index of ventilation volume ($\mu\text{l min}^{-1}$) as ventilation frequency \times tidal volume.

Phylogenetic analysis and statistics

Typically, scaling relationships are determined by simple regressions, and we used this test here. However, this method assumes that each species mean is an independent point. Because closely related species could be expected to have similar body masses or measured responses to our experimental procedures, we needed to account for phylogenetic effects. The best way to account for ancestry is by using a known phylogenetic tree and calculating branch lengths to weight the relationships between variables (Harvey and Pagel, 1991; Garland, Jr and Adolph, 1994). However, for orthopterans, the phylogeny is largely unknown and untested, and exact branch lengths exist for few species. Therefore, we created a tree (animals identified by an asterisk in Table 1) from previously published works and from taxonomy. For relationships between family, subfamily and tribe, we used Otte and Nasrecki (Otte and Nasrecki, 1997), and for species relationships we used published molecular studies (Chapco et al., 1997; Chapco et al., 1999; Knowles and Otte, 2000). Then, we counted the number of branches at the level of family, subfamily, tribe, genus and species between each species pair as a measure of the distance between species (Fagan et al., 2002; Woods et al., 2004). These distance measures were compiled into a matrix, which was held constant, while we compared matrices created for the differences in body mass between each species pair to the differences in respiratory parameters between the same pairs, using partial Mantel tests (PASSAGE software) (Rosenberg, 2001). To test for significance of the correlation, one matrix was held constant while the other was randomized over 999 iterations, and those predicted values were compared to the observed Z statistic. Large values of Z indicate that large differences between species in one matrix were correlated with large differences in the other matrix. For other statistical analyses, we used SYSTAT 10.2.01. For all statistics, our within-experiment type I error was less than 5%. Values are means \pm standard errors (s.e.m.) throughout.

Results

Scaling of \dot{M}_{CO_2} and P_c

Normoxic CO_2 emission rates scaled with body mass to the power 0.92 ± 0.07 ($r^2 = 0.90$, $P < 0.0001$) (Fig. 1) with masses ranging from 0.61 to 8.34 g. As P_{O_2} decreased, CO_2 emission rates remained fairly constant down to the P_c value (Fig. 2). There was no effect of exposure time on P_c for the smaller species, *M. sanguinipes* (Table 2) ($t = 0.7$, $P = 0.5$). However, the longer exposures resulted in a slightly higher P_c value for the larger species, *M. differentialis* (Table 2) ($t = -2.3$, $P = 0.049$). We found no relationship between the mean P_c and body mass

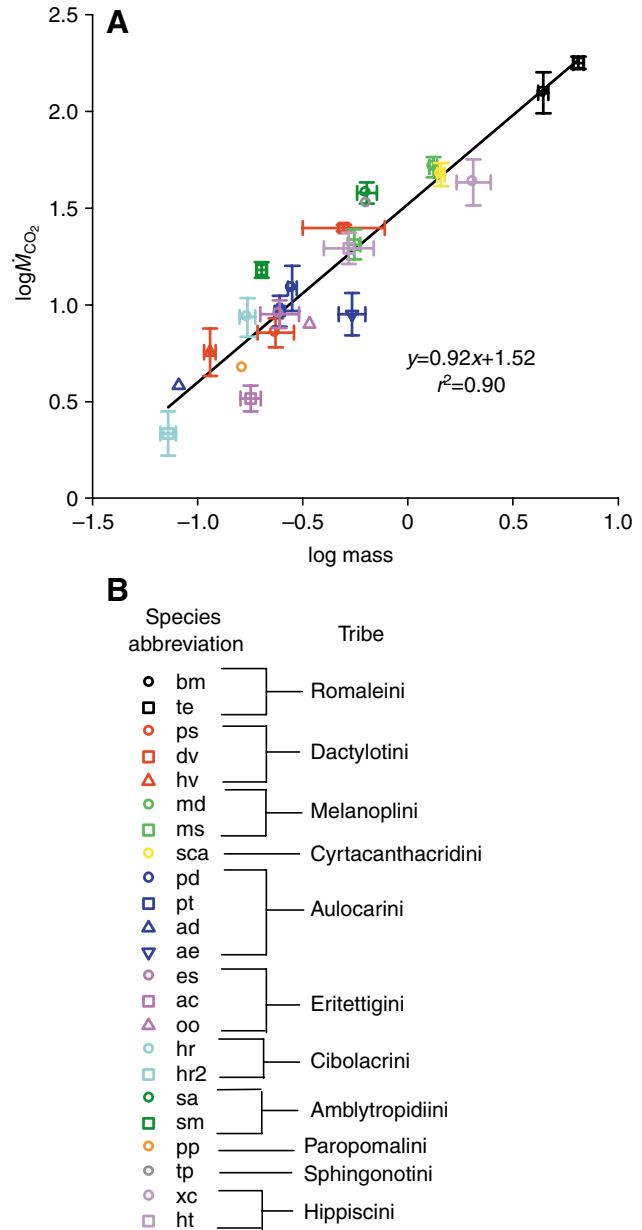


Fig. 1. (A) Log-log plot of species mean CO_2 emission rate \dot{M}_{CO_2} ($\mu\text{mol h}^{-1}$) vs species mean body mass (g). (B) Species in the same tribe have symbols of the same color.

using linear regression (Fig. 3) ($F_{1,11} = 1.1$, $P = 0.32$; average P_c across all species = 4.2 kPa). Similarly, when we controlled for phylogeny using the Mantel test, we found no significant relationship between P_c and body mass (Fig. 4; Table 3). There was also no correlation between phylogeny and body mass (Fig. 4) (Mantel test, correlation coefficient = -0.05, $P = 0.7$).

Ventilation frequency, tidal volume index and ventilation volume

Hypoxia response

Three animals had unmeasurable tidal volumes and, therefore, were deleted from this analysis. In general, exposure

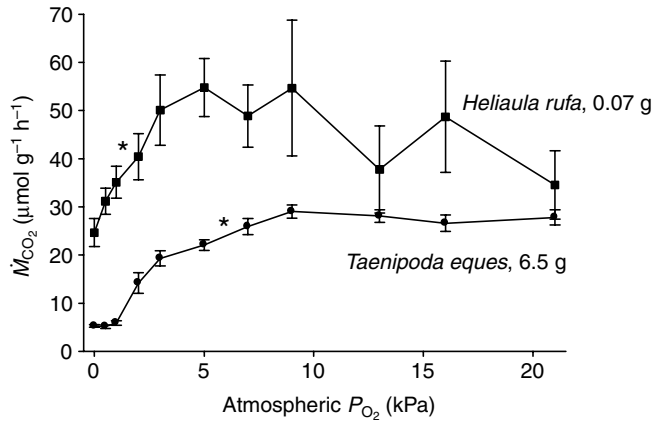


Fig. 2. CO_2 emission rate (\dot{M}_{CO_2}) as a function of atmospheric P_{O_2} for two species of grasshopper. Asterisks mark the P_c for each species.

to hypoxia stimulated ventilatory activity. Ventilatory frequencies (breaths min^{-1}) approximately doubled during hypoxia (repeated-measures ANOVA, $F_{1,12}=33.0$, $P<0.001$). Tidal volume ($\mu\text{l breath}^{-1}$) also increased from normoxia (24.0 ± 4.2) to hypoxia [38.3 ± 9.2 ; repeated-measures General Linear Model (GLM), $F_{1,11}=5.4$, $P=0.04$]. The response of ventilation volume to hypoxia varied for animals with different masses (significant mass \times atmosphere interaction, repeated measures GLM, $F_{1,11}=5.374$, $P<0.001$).

Scaling of ventilatory parameters

With species means considered to be independent data points, ventilation frequency did not significantly scale with body mass during normoxia, but did increase significantly with mass in 5% O_2 (Fig. 5; Table 3). However, ventilation frequencies in both 21 and 5 kPa O_2 were positively correlated with body mass when phylogeny was held constant (Table 3; Fig. 5). Using linear regressions, both normoxic and hypoxic tidal volumes scaled with mass to the 0.71 and 0.69 power, respectively (Table 3). When phylogeny was taken into account, tidal volume in normoxia was not correlated with body mass. However accounting for phylogenetic relatedness did not change the positive correlation between hypoxic tidal volume and body mass (Table 3). Ventilation volume ($\mu\text{l min}^{-1}$) increased significantly with mass under both normoxic and hypoxic conditions, with slopes of 1.0 and 0.93, respectively (Fig. 6; Table 3). This pattern was also observed when we corrected for phylogeny.

Table 2. Comparison of long-term and short-term hypoxia exposures in two species of grasshopper

Species	Exposure time	Mean P_c	\dot{M}_{CO_2} ($\mu\text{mol g}^{-1} \text{h}^{-1}$) at the P_c
<i>Melanoplus differentialis</i>	3 min	3.1	41.0 ± 3.8
	1 h	7.2*	24.0 ± 2.3
<i>Melanoplus sanguinipes</i>	3 min	6.1	43.2 ± 6.13
	1 h	4.2	27.6 ± 4.4

*Long-term exposure differed significantly from short-term exposure.

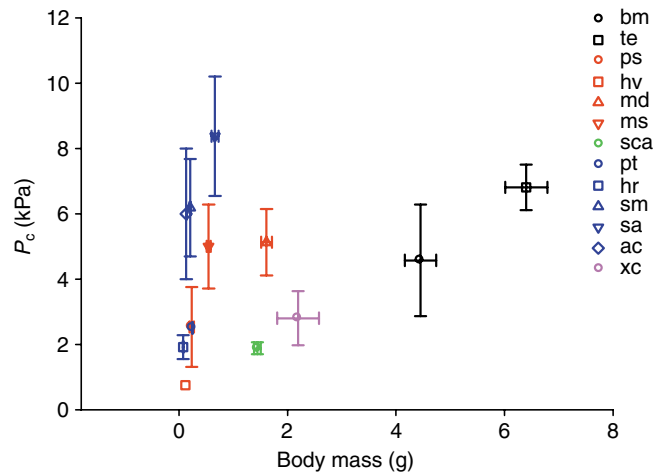


Fig. 3. Species mean P_c vs species mean body mass. Animals of the same subfamily have the same color of symbol (abbreviations as in Fig. 1).

Maximal tracheal system conductance

Maximal tracheal conductance was calculated from $\dot{M}_{\text{CO}_2} \times P_c^{-1}$, where \dot{M}_{CO_2} is the CO_2 emission at the P_c (Greenlee and Harrison, 2004a). This calculation assumes that at the P_c , the animal is maximizing gas exchange capacity (spiracles maximally open, tracheal fluid removed, etc.) and that at the P_c , mitochondrial P_{O_2} is indistinguishable from 0 kPa. Maximal tracheal conductance scaled with mass^{0.73} (Fig. 7; Table 3). Mass-specific conductance decreased with mass, when analyzed with both linear regression and partial Mantel tests (Table 3).

Discussion

Body size effects on P_c

Contrary to a prediction based on the hypothesis that oxygen delivery is more challenging for larger insects, we found that there was no effect of body size on the safety margin for gas exchange in resting grasshoppers (Figs 3, 4). There was considerable variation in P_c among species and within species (O_2 range: 2–17 kPa). One possible explanation for the variation is that the age of these field-collected animals was unknown, and previous work has shown that developmental stage can significantly affect P_c (Greenlee and Harrison, 2004b).

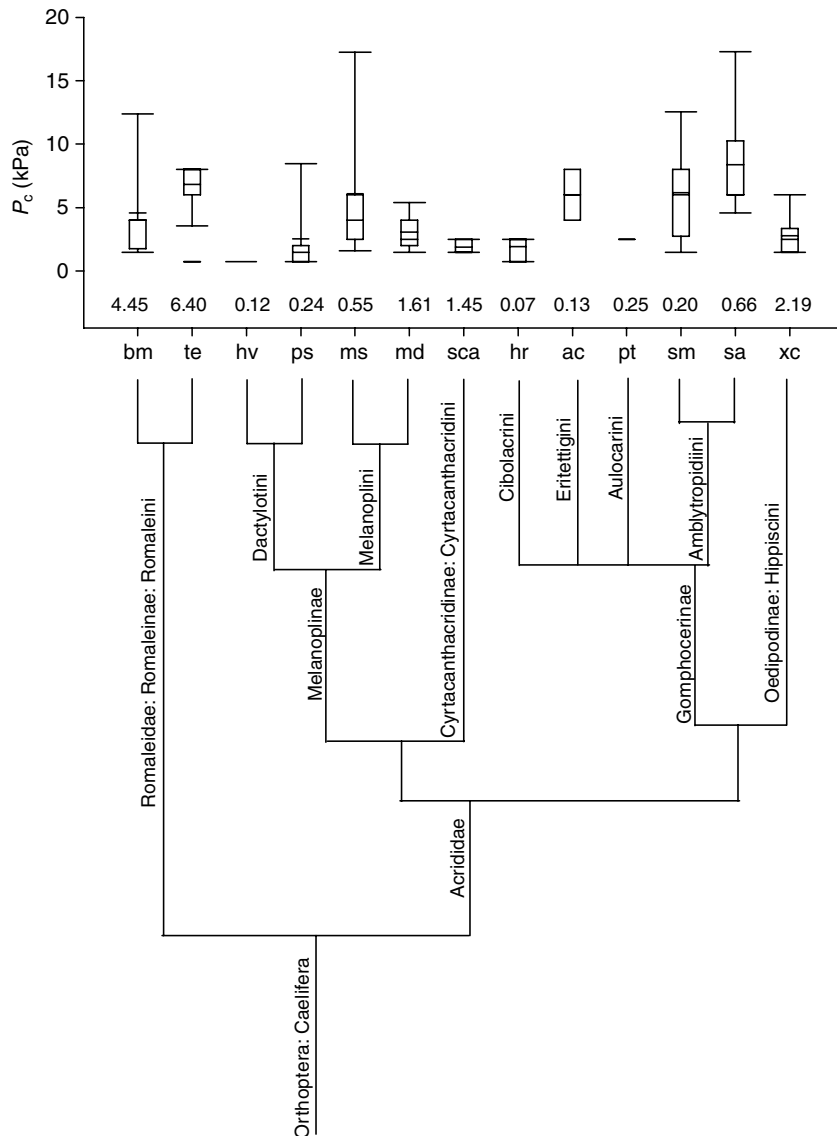


Fig. 4. Box plot of P_c for 13 species of grasshopper. Numbers at the bottom of the graph indicate mean body mass (g) for that species. The boundaries of each box mark the 25th percentile and the 75th percentile. The line within each box indicates the median P_c value. Error bars mark the 10th and 90th percentiles. The tree below lists family, subfamily and tribe for each species. Species abbreviations as in Fig. 1.

Interestingly, longer exposures (1 h vs 3 min) to hypoxia increased the P_c in the larger *M. differentialis*, from 3.1 to 7.2 kPa (Table 2). Similarly, in a previous study using *Schistocerca americana*, we found that adults exposed to longer periods of hypoxia had slightly higher P_c values and lower CO_2 emission rates (Greenlee and Harrison, 2004a). These data suggest that some grasshoppers may have difficulty sustaining metabolism over long hypoxic periods, perhaps because these species rely on anaerobic ATP synthesis or internal oxygen stores to maintain metabolic rates in short term hypoxia. The smaller Melanopline (*M. sanguinipes*) showed no difference in P_c between short or long exposures, but, regardless of duration of exposure, P_c values for the larger

species did not differ statistically from those of the smaller species. Therefore, though our data suggest that longer hypoxic exposures increase P_c , there remains no statistical evidence for a reduced safety margin for oxygen delivery in larger resting grasshopper species.

Mechanisms for hypoxia tolerance

How do grasshoppers maintain metabolic rates in hypoxia? Many animals in low oxygen atmospheres increase ventilation frequencies, tidal volumes, or both, resulting in increased ventilation volume (Frappell et al., 1992). Across species, we found evidence for both mechanisms in grasshoppers, with ventilation frequency, tidal volume and ventilation volume all significantly increased by exposure to hypoxia (Figs 5, 6, Table 2). Since ventilation volumes increased threefold as oxygen levels dropped fourfold, other mechanisms are likely to be involved in the preservation of gas exchange. These mechanisms may include decreased tracheolar fluid levels and/or tissue P_{O_2} levels. Drops in tissue P_{O_2} seem most likely to have occurred, since conductance from the tracheae to the tissue does not increase significantly until P_{O_2} values drop below 5 kPa in grasshoppers (Greenlee and Harrison, 1998).

Ventilatory compensation for larger body size in normoxia

Typically, as vertebrates get bigger ventilation frequency decreases, and tidal volume increases isometrically (Peters, 1983). Together the scaling coefficients of these parameters (-0.25 and 1, respectively) sum to the scaling coefficient for absolute metabolic rate (0.75) (Schmidt-Nielsen, 1984). Thus, as vertebrates increase in body mass, mass-specific metabolic needs decrease, and ventilatory frequencies decrease in accordance with those requirements. No prior study has examined such scaling during active ventilation in insects. In contrast to the pattern found in vertebrates, ventilation frequency tended to increase with mass (during hypoxia, and during normoxia when phylogeny was accounted for), and tidal volumes increased, but less than isometrically (not significantly when phylogeny was controlled, scaling with $\text{mass}^{0.7}$). However, as in vertebrates, ventilation volumes and metabolic rates scaled similarly with $\text{mass}^{(1 \text{ and } 0.93)}$. Thus, larger grasshoppers match ventilation to metabolic oxygen need, resulting in similar safety margins for oxygen delivery, at least at rest.

These scaling patterns for abdominal pumping in grasshoppers differed from the scaling of frequencies and volumes of gas emission found for insects exchanging gases discontinuously.

Table 3. *Scaling of respiratory parameters with body mass*

Respiratory variable (<i>N</i>)	Mantel test results		Linear regression coefficients			
	Correlation	<i>P</i>	<i>m</i>	<i>b</i>	<i>P</i>	<i>r</i> ²
<i>P_c</i> (13)	n.s.		n.s.			
Ventilation frequency	0.43	0.0003	n.s.			
21 kPa O ₂ (15)						
5 kPa O ₂ (15)	0.68	0.001	0.23±0.08	1.8±0.03	0.02	0.34
Tidal volume (μl)	n.s.	–	0.71±0.24	1.4±0.09	0.01	0.44
21 kPa O ₂ (13)						
5 kPa O ₂ (15)	0.43	0.001	0.69±0.28	1.5±0.09	0.03	0.32
Ventilation volume (μl min ⁻¹)	0.57	0.001	1.00±0.20	2.93±0.07	0.001	0.69
21 kPa O ₂ (13)						
5 kPa O ₂ (15)	0.59	0.001	0.93±0.26	3.35±0.09	0.03	0.49
Tracheal system conductance (μmol kPa ⁻¹ h ⁻¹) (13)	0.81	0.001	0.73±0.1	2.4±0.15	0.00	0.5
Mass-specific conductance (μmol kPa ⁻¹ g ⁻¹ h ⁻¹) (13)	–0.56	0.001	–0.28±0.1	2.4±0.02	0.02	0.41

Values of *m* and *b* are means ± s.e.m.

Correlation coefficients (calculated from partial Mantel tests) between body mass *M_b* (in g) and respiratory variables when corrected for phylogeny.

Regression equations were calculated as: log(ventilatory parameter)=*m*(log*M_b*)+*b*, where *m*=slope, *b*=*y*-intercept. n.s., not significant.

During discontinuous gas exchange (DGC), frequencies of spiracular opening did not vary with mass, and the volume of CO₂ emitted per burst scaled isometrically with mass in Tenebrionid beetles (Lighton, 1991). In discontinuously ventilating cerambycid beetles, spiracular opening frequency was not correlated with mass and volume of CO₂ during the open phase scaled with mass^{0.37} (Chappell and Rogowitz, 2000). In dung beetles, frequency of spiracular bursts increased with mass (scaling exponent=0.56) and the volume of CO₂ emitted per burst scaled with mass^{0.83} (Davis et al., 1999). Finally, across seven weevil species noted to be cyclically, but not discontinuously, ventilating, frequency of CO₂ bursts did not scale with body mass, although the volume of each burst scaled with mass^{0.65} (Klok and Chown, 2005). Together, these data suggest that patterns of mass-specific scaling of ventilatory parameters are highly diverse in insects and may differ depending on the mode of ventilation.

The effect of body size on ventilatory patterns in this interspecific study also contrasted with our previous work with developing *S. americana* grasshoppers. During the ontogenetic study (Greenlee and Harrison, 2004a), we found clear evidence for increased tidal volumes and increasing use of convection with age/size. Early instar

juvenile grasshoppers have negligible tidal volumes and are likely to be more reliant upon diffusion for gas exchange (Greenlee and Harrison, 2004b). In this study, while the increased ventilation frequency with mass suggests increased use of convection in larger species, the lower mass-specific tidal volumes in larger animals, and the observation that ventilation

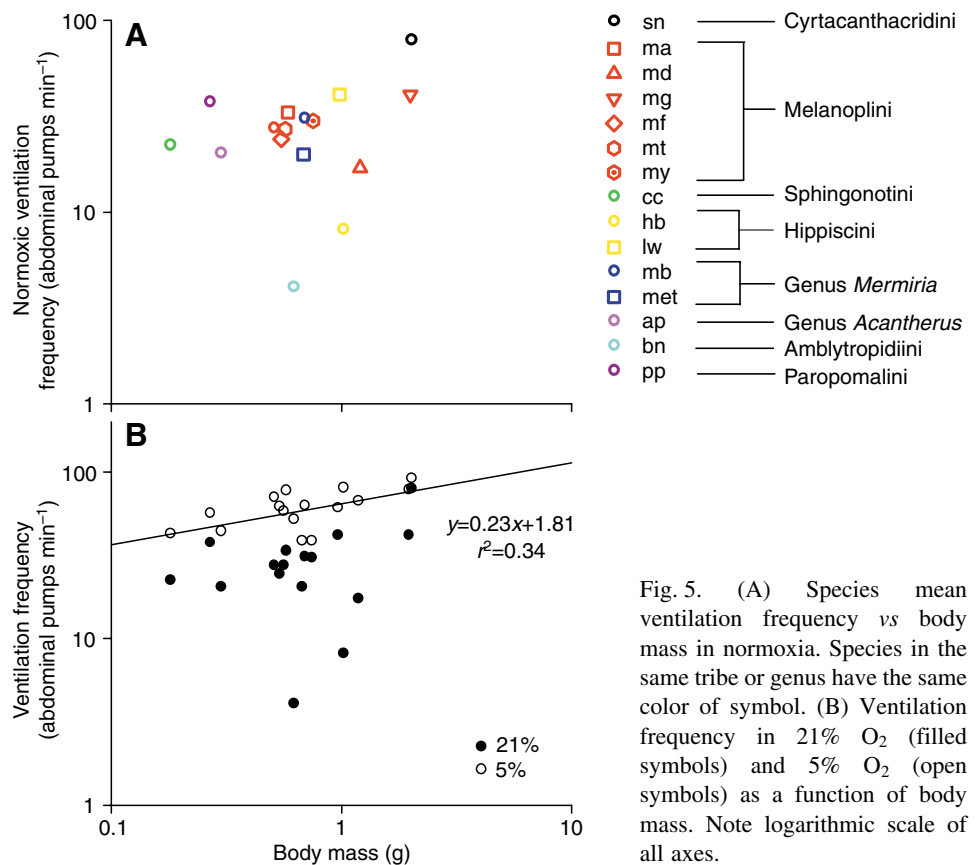


Fig. 5. (A) Species mean ventilation frequency vs body mass in normoxia. Species in the same tribe or genus have the same color of symbol. (B) Ventilation frequency in 21% O₂ (filled symbols) and 5% O₂ (open symbols) as a function of body mass. Note logarithmic scale of all axes.

volume scaled similarly with metabolic rates across grasshopper species, suggests that use of convective gas exchange is similar across these species, as found for vertebrate interspecific comparisons (Stahl, 1967; Lasiewski and Calder, 1971).

Does atmospheric O₂ limit the maximal body size of insects?

At least in these grasshoppers, we found no evidence that larger insects have smaller safety margins for O₂ delivery. Perhaps we would see decreased safety margins if we looked at the largest extant insects; the largest grasshopper species now alive exceed 10 g (Carbonell, 1984) and the largest beetles approach 100 g (Williams, 2001). There is evidence that larger insects have decreased safety margins during times of high energy use, such as during flight or terrestrial locomotion (Rascón and Harrison, 2005; Harrison et al., 2006), so perhaps examination might find a positive body size effect on P_c during flight. However, the only study to examine P_c during locomotion in insects found that oxygen delivery capacities increase strongly with age/size during jumping of grasshoppers (Kirkton et al., 2005). Thus the evidence to date suggests that larger insects overcome potential diffusion limitations by matching tracheal system conductance to tissue needs, thus maintaining constant safety margins for O₂ delivery across size.

Even though we found no evidence for decreasing safety margins for oxygen delivery in larger insects, maximal insect body size could still be affected by atmospheric oxygen levels. For example, increased O₂ availability could increase growth rates and body size, as in the mealworm, *Tenebrio molitor*, (Loudon, 1988; Greenberg and Ar, 1996) and fruitflies (Frazier et al., 2001). Additionally, it is conceivable that even if natural selection operates to maintain constant safety margins for gas exchange across insect sizes, that the ability of convection to compensate for large size might reach some limit. For example, if convective gas exchange is increased by increasing the volume of tracheal air sacs across species, as may occur in developing grasshoppers (Greenlee and Harrison, 2004a; Lease et al., 2006), it is conceivable that at some large size the volume of air sacs required might exceed available internal space. In support of this argument, the largest living beetles have been reported to have a huge fraction of their body filled with tracheae and air sacs (Miller, 1966). Higher atmospheric oxygen levels might then facilitate gigantic insects by allowing similarly sized tracheae

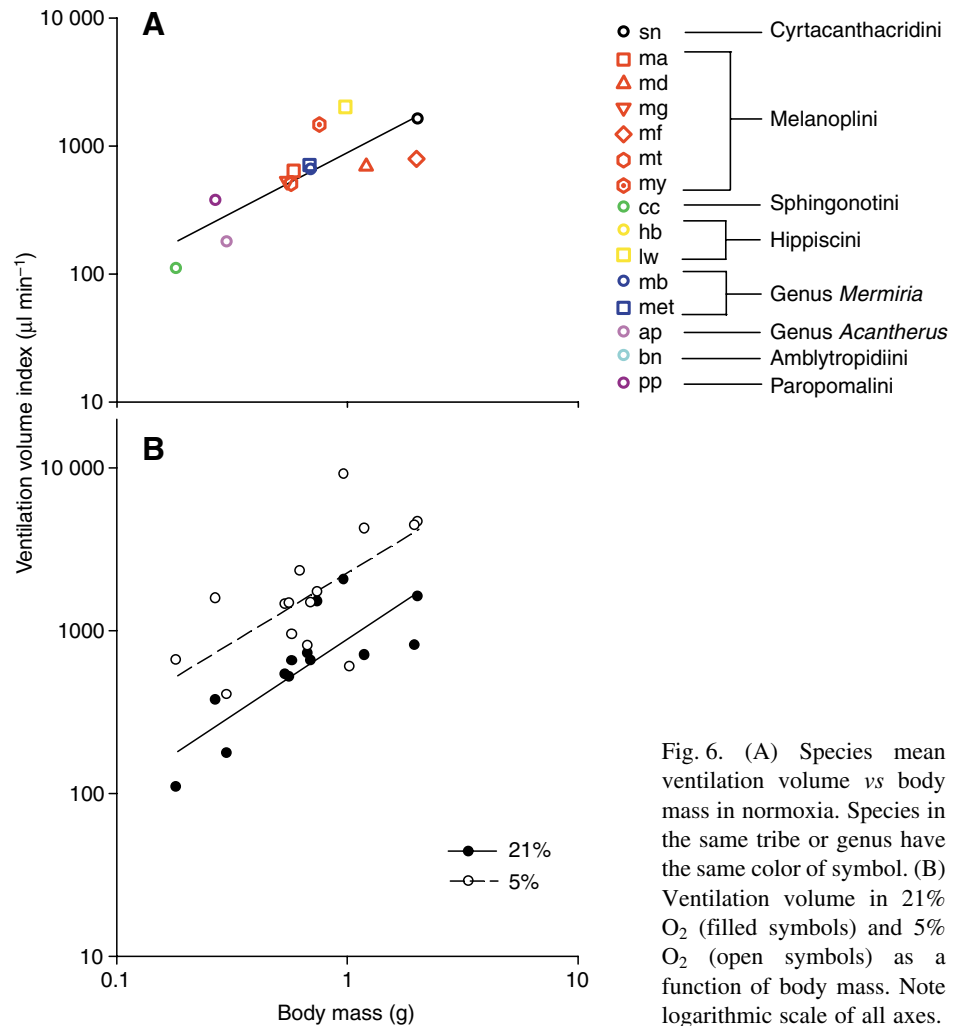


Fig. 6. (A) Species mean ventilation volume vs body mass in normoxia. Species in the same tribe or genus have the same color of symbol. (B) Ventilation volume in 21% O₂ (filled symbols) and 5% O₂ (open symbols) as a function of body mass. Note logarithmic scale of all axes.

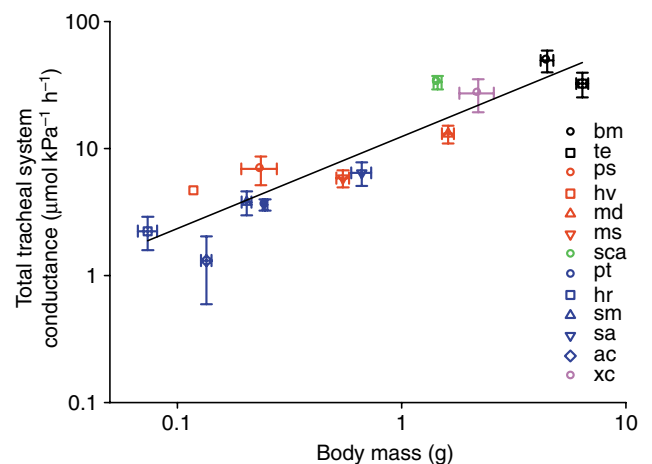


Fig. 7. Mass-specific total tracheal system conductance as a function of body mass across grasshopper species. Regression equation: $y=0.72x+1.09$, $r^2=0.85$. Species in the same tribe have symbols of the same color (abbreviations as in Fig. 1). Note logarithmic scale of both axes.

and air sacs to deliver more oxygen, since tracheal proliferation and dimensions are decreased by hyperoxic rearing (Jarecki et al., 1999; Henry and Harrison, 2004).

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