The Journal of Experimental Biology 207, 2361-2370
Published by The Company of Biologists 2004
doi:10.1242/jeb.01023

Upper thermal tolerance and oxygen limitation in terrestrial arthropods

C. Jaco Klok, Brent J. Sinclair and Steven L. Chown*

Spatial, Physiological and Conservation Ecology Group, Department of Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

*Author for correspondence (e-mail: slchown@sun.ac.za)

Accepted 8 April 2004

Summary

The hypothesis of oxygen limitation of thermal tolerance proposes that critical temperatures are set by a transition to anaerobic metabolism, and that upper and lower tolerances are therefore coupled. Moreover, this hypothesis has been dubbed a unifying general principle and extended from marine to terrestrial ectotherms. By contrast, in insects the upper and lower limits are decoupled, suggesting that the oxygen limitation hypothesis might not be as general as proposed. However, no direct tests of this hypothesis or its predictions have been undertaken in terrestrial species. We use a terrestrial isopod (*Armadillidium vulgare*) and a tenebrionid beetle (*Gonocephalum simplex*) to test the prediction that thermal tolerance should vary with oxygen partial pressure. Whilst in the isopod critical thermal maximum declined with declining oxygen concentration, this was not the case in the beetle. Efficient oxygen delivery *via* a tracheal system makes oxygen limitation of thermal tolerance, at a whole organism level, unlikely in insects. By contrast, oxygen limitation of thermal tolerances is expected to apply to species, like the isopod, in which the circulatory system contributes significantly to oxygen delivery. Because insects dominate terrestrial systems, oxygen limitation of thermal tolerance cannot be considered pervasive in this habitat, although it is a characteristic of marine species.

Key words: critical thermal limits, critical thermal maximum (CT_{max}), oxygen limitation, tracheated arthropods, marine, terrestrial

Introduction

Owing to the realization that many species will be incapable of migrating in response to modern climate change, largely as a consequence of habitat fragmentation (Warren et al., 2001), there is increasing interest both in the ways in which thermal tolerance evolves and the rate at which this can take place (Davis and Shaw, 2001; Hoffmann and Blows, 1993). Building on previous work (Cossins and Bowler, 1987; Prosser, 1986; Ushakov, 1964), Pörtner (Pörtner, 2001, 2002a; Pörtner et al., 2000) has developed a wide-ranging hypothesis suggesting that in complex metazoans, critical temperatures that affect fitness (i.e. survival and reproduction) are generally not set by cellular level responses (such as loss of protein or membrane function), but are rather set by a transition to unsustainable anaerobic metabolism. Thus, deleterious temperatures (referred to as the pejus by Pörtner) result from insufficient aerobic capacity of mitochondria at low temperatures, and a mismatch between excessive oxygen demand by mitochondria and insufficient oxygen uptake and distribution by ventilation and circulation at high temperatures. In other words, whole-animal metabolism is limited at both low and high temperatures, and this sets limits to animal performance. Seasonal and evolutionary adjustments to temperature are made by alterations of mitochondrial aerobic scope and these changes have concomitant effects on both high and low deleterious temperatures. In consequence, there is an inverse relationship between performance at high and low temperatures when measured in either a population across seasons, or among populations from habitats differing in their thermal regimes (Pörtner, 2001, 2002c; Pörtner et al., 2000). This inverse relationship is also apparent in interspecific comparisons, although there are differences between stenothermal polar species and those from other regions (see Pörtner, 2002a).

Pörtner (2001, 2002a) has argued that this oxygen limitation of thermal tolerance applies as much to terrestrial animals as it does to the marine species for which he marshalled most evidence. In support of this proposition, Pörtner (2001, 2002a) points out that less complex organisms, such as eukaryotes and prokaryotes, have high thermal tolerances owing to the simplicity of their organization relative to spiders, scorpions, turtles and endothermic vertebrates which, as metazoans, have a much increased organizational complexity, resulting in a considerable decrease in their thermal tolerances. The unicellular organisms have no need of complex circulatory and gas exchange mechanisms, and therefore oxygen delivery does not set limits to performance.

However, there is a growing body of evidence suggesting

that the thermal tolerances of terrestrial species might not be limited by the same mechanisms as those in marine species. The bulk of this evidence comes from insects, in which upper and lower lethal limits are not necessarily related. Rather, these limits are decoupled, such that alterations in low temperature tolerance do not usually result in a change in upper lethal limits. Such a decoupled response has been found in interspecific comparisons at global to regional spatial scales (Addo-Bediako et al., 2000; Chen et al., 1990; Gaston and Chown, 1999; Goto et al., 2000), and in intraspecific comparisons at somewhat smaller scales (Hercus et al., 2000; Klok and Chown, 2003; but see also Hoffmann et al., 2002), and has also been documented in the responses of *Drosophila* species to selection (Gilchrist et al., 1997; Hoffmann et al., 1997). There is also a generally greater acclimation response in lower than in upper lethal temperatures, although the extent of variability in both sets of traits is often small and insufficient for perfect compensation (Chown, 2001; Kingsolver and Huey, 1998; Klok and Chown, 2003). These findings strongly suggest that thermal tolerance in insects, and possibly in other terrestrial ectotherms (see discussion in Chown, 2001; Klok and Chown, 2003), is not limited by oxygen delivery. However, to date no direct test of Pörtner's oxygen limitation hypothesis or any of its predictions has been undertaken for terrestrial ectotherms.

Of the many predictions arising from Pörtner's hypothesis, one of the most significant is that hypoxia should result in a decline in critical temperatures (Pörtner, 2001, 2002b). A test of this prediction, for insects (or other terrestrial ectotherms), initially appears straightforward. However, quite how critical limits should be identified in insects is a potential obstacle. In the work discussed by Pörtner (2001, 2002a), critical limits are reflected in a decline in aerobic scope (or a measure thereof, such as changes in haemolymph O_2 concentration), whereas in insects critical limits are generally measured as knockdown temperature as temperatures are altered (Gibert and Huey, 2001; Huey et al., 1992; Klok and Chown, 2003), or as time to knockdown at a given temperature (Hoffmann et al., 1997). Whilst lethal limits and knockdown temperatures are related to some degree, selection experiments often reveal a large measure of independence, reflecting the fact that these traits are genetically independent (Hoffmann et al., 1997; Berrigan and Hoffmann, 1998; Berrigan, 2000). Even so, it is clear that knockdown methods are not conducive to understanding oxygen demand, nor are they entirely free from observer bias if the onset of muscular spasms must be assessed, as suggested by Lutterschmidt and Hutchison (1997). However, J. R. B. Lighton and R. J. Turner (personal communication; see also Lighton and Turner, 2004) have developed a technique, dubbed thermolimit respirometry, which enables a marriage of both conventional observation of the critical thermal maximum (CT_{max}) using detection of movement by means of infra-red diodes, and real-time respirometry, which provides a measure of metabolic rate as temperature changes. This method objectively pinpoints the exact temperature where activity ceases and closely links this with changes in CO_2 release patterns.

In this study we use thermolimit respirometry to test directly the prediction of the oxygen limitation hypothesis that hypoxia should result in a decline in the CT_{max}. We also predicted that if the CT_{max} is a function of failure in oxygen uptake and distribution by ventilation and circulation, then hyperoxia should lead to an increase in the CT_{max}. To test these predictions we use two terrestrial species – an isopod, which makes use of pleopodal exopodites for gas exchange, and subsequent transport of oxygen *via* haemocyanin in the circulatory system (Schmidt and Wägele, 2001), and a tenebrionid beetle, which, like all insects (Chapman, 1998), has an elaborate tracheal system for delivery of oxygen directly to its tissues and cells.

Materials and methods

Armadillidium vulgare (Latreille) (Isopoda: Armadillidiidae) (live mass: 0.0621±0.0018 g, mean ± S.E.M., N=10 for each treatment) and *Gonocephalum simplex* (Fabricius) (Coleoptera: Tenebrionidae) (live mass: 0.0523±0.0080 g, N=10 for each treatment) were collected 24 h prior to experiments from gardens on the University of Stellenbosch main campus during the austral mid-summer. Individuals were housed in the laboratory at 25±1°C with a natural photoperiod (16 h:8 h L:D) and were provided with soil and detritus from their microhabitats. In a pilot trial, CT_{max} was determined under normoxia by visual observation of knockdown temperatures in a water-jacketed set of Perspex chambers connected to a Grant (Cambridge, UK) LTD20 water bath programmed to increase temperature at 0.25°C min^{-1} following initial equilibration for 15 min at 30°C.

For the main experiments, a Sable Systems (Las Vegas, NV, USA) flow-through CO_2 respirometry system was used to record gas exchange characteristics (LiCor 6262 CO_2/H_2O infra-red gas analyser; IRGA) and motor activity (AD1 activity detector; Sable Systems) (see Lighton, 1988). Compressed synthetic air (21% O_2 and balance N_2) was passed through soda lime and Drierite columns to remove CO_2 and H_2O. From there the scrubbed air flowed through a mass flow controller (Sidetrak; Monterey, CA, USA) set to regulate gas flow at 75 ml min^{-1} into an automatic baselining system, the 5 ml cuvette containing the animal, and finally the IRGA. Sable Systems DATACAN V software was used for data capture and control of the respirometry system. The cuvette and activity detector were placed inside a waterproof container and immersed in a programmable water bath (Grant LTD20) set to equilibrate the animal at 30°C for 15 min in synchrony with the respirometry system's baseline and gas equilibration procedures. Thereafter the water bath increased the temperature at 0.25°C min^{-1} to several degrees past the CT_{max} (all animals were dead by this point). A 40-SWG copper-constantan thermocouple connected to a Grant Squirrel SQ800 datalogger was used to monitor the cuvette's internal temperature in synchrony with DATACAN V. Owing to the isopods' sensitivity to desiccation, the synthetic air was rehumidified by inserting a LiCor LI610 dew point generator

in the stream. At the start of a recording (from 30°C) a dew point of 20°C (2.347 kPa saturation vapour pressure) was set. The dew point was increased to 25°C (3.181 kPa) after the water bath reached 40°C.

Subsequent to the normoxic (21% O_2) CT_{max} determination, thermolimit respirometry was repeated in three separate trials using hyperoxic air (40% O_2), mildly hypoxic air (10% O_2), and extremely hypoxic air (2.5% O_2). Oxygen concentrations were manipulated using two Sidetrak mass flow control units, providing N_2 and O_2 respectively, and connected in parallel so that they could be adjusted to provide the required concentration at a combined steady flow rate of 75 ml min^{-1}. Oxygen concentrations were monitored using an AMETEK S3A-II Oxygen Analyzer (Paoli, PA, USA). To allow accurate regulation of 2.5% O_2, the total flow rate was increased to 200 ml min^{-1}.

Determination of the CT_{max} *and data analysis*

DATACAN V analysis software was used to extract thermolimit respirometry data from the recordings of every individual at the four O_2 concentrations. Prior to data analyses the temperature data were combined and aligned with the CO_2 release and activity data from the Sable Systems recordings, using the time stamp of the instruments.

The critical thermal maximum was defined dually in terms of the species' motor activity, monitored by the AD1, and respiratory breakdown, based on $\dot{V}_{CO_2}$ (Fig. 1). From activity data the CT_{max}, for both the isopods and the beetles, was recorded as the last temperature where a movement was detected by the AD1 (Fig. 1). In the isopods, the respiratory signal that corresponded closely with the activity-based CT_{max} point was a brief spike in the CO_2 emission. Because of their diffusion-based gas exchange *via* their pleopodal exopodites, the isopods gave generally smooth CO_2 recordings and the characteristic spike at the onset of CT_{max} could be easily identified (Fig. 1). In the beetles, spiracular activity resulted in rapidly fluctuating CO_2 emissions at temperatures preceding the CT_{max}. However, their respirometry CT_{max} spike can be distinguished from preceding spikes by a smooth CO_2 decline following the CT_{max} onset, signifying the complete cessation of spiracular activity (Fig. 1). This cessation of spiracular activity therefore corresponds closely with the overall cessation of motor activity measured by the infrared activity detector.

In addition to the CT_{max}, we also measured the temperature of maximum metabolic activity (T_{MetMax}). This was the point at which CO_2 production reached its peak, and there was an inflection in the curve (indicated on Fig. 1). The deleterious temperature range was between the T_{MetMax} and the CT_{max}, where metabolic rate declined with increasing temperature (Fig. 1).

Least-squares linear regression was used to determine the relationship between $\log\dot{V}_{CO_2}$ and temperature, and the regression coefficients of these lines were compared among O_2 treatments using analysis of covariance (ANCOVA). Analyses of variance (ANOVA) were used to compare maximum $\dot{V}_{CO_2}$ and CT_{max} at the four O_2 concentrations in each of the test species.

Results

Metabolic patterns during thermolimit respirometry

From the starting temperature, 30°C, the animals experience benign thermal conditions, and thus metabolic rates remained stable, with no evidence of discontinuous gas exchange cycles in the beetle. At increasing temperatures the metabolic rates reach a maximum point analogous to the optimum range, as defined by Pörtner (2001, 2002a) (Fig. 1). This point, the T_{MetMax}, indicates a definite inflection in the metabolic rate curve from which the metabolic rate declines markedly for a few degrees – the deleterious temperature range – before another break appears, characterised by a brief CO_2 spike in the recording (Figs 1, 2A, 3A) signifying the respirometry CT_{max}. The animals did not recover once they reached the post-CO_2 decline (Fig. 1) and we regarded them as functionally dead. After death there is a brief rise in CO_2 emission as residual CO_2 is released from the body at the increasing temperature, followed by a steady and smooth decline until CO_2 emission rates approach zero.

Pilot trials indicated that during normoxia both the respirometry and activity CT_{max} values were identical to the knockdown CT_{max} estimated visually for both species (Isopod 44.4±0.4°C, mean ± S.E.M., ANOVA $F_{(1,27)}$=0.001, P>0.98; beetle 48.8±0.3°C ANOVA $F_{(1,18)}$=1.017, P>0.32). Therefore, identifying the CT_{max} values based on activity recordings and on respirometry recordings was straightforward and consistent for both *Armadillidium vulgare* and *Gonocephalum simplex*.

In *A. vulgare*, the CO_2 traces were smooth (Fig. 2), as might be expected for a species with no means of physically regulating gas exchange. The regression coefficients of the rate–temperature relationships decreased with declining oxygen concentration, from 40% to 21% O_2, but were idiosyncratic thereafter (Table 1). At 2.5% O_2, the large majority (7 out of 10) of the individuals investigated showed a decline in metabolic rate (Fig. 2C) with increasing temperature, suggesting that this species was under considerable stress and is a metabolic conformer (*sensu* Herreid, 1980), with metabolic rate declining in response to hypoxia. This was reflected in the significant decline of T_{MetMax} with declining oxygen concentration (Table 1). There was a decrease in both activity and respirometry CT_{max} with declining oxygen concentration, at least at and below normoxia (Table 2). At values between 21% and 40% O_2 there was no change in CT_{max} (Table 2).

Although the regression coefficients of the relationships between $\dot{V}_{CO_2}$ and temperature differed with O_2 concentration, albeit in inconsistent directions (Table 1), the majority of the respiratory and thermal parameters in *G. simplex* differed from *A. vulgare*. Excluding extreme hypoxia, T_{MetMax} did not differ with $\%O_2$. The CO_2 traces also showed similar, marked fluctuations, suggesting spiracular opening and closing, probably as a consequence of animal activity (Fig. 3). At 2.5% O_2, the CO_2 trace was generally much smoother,

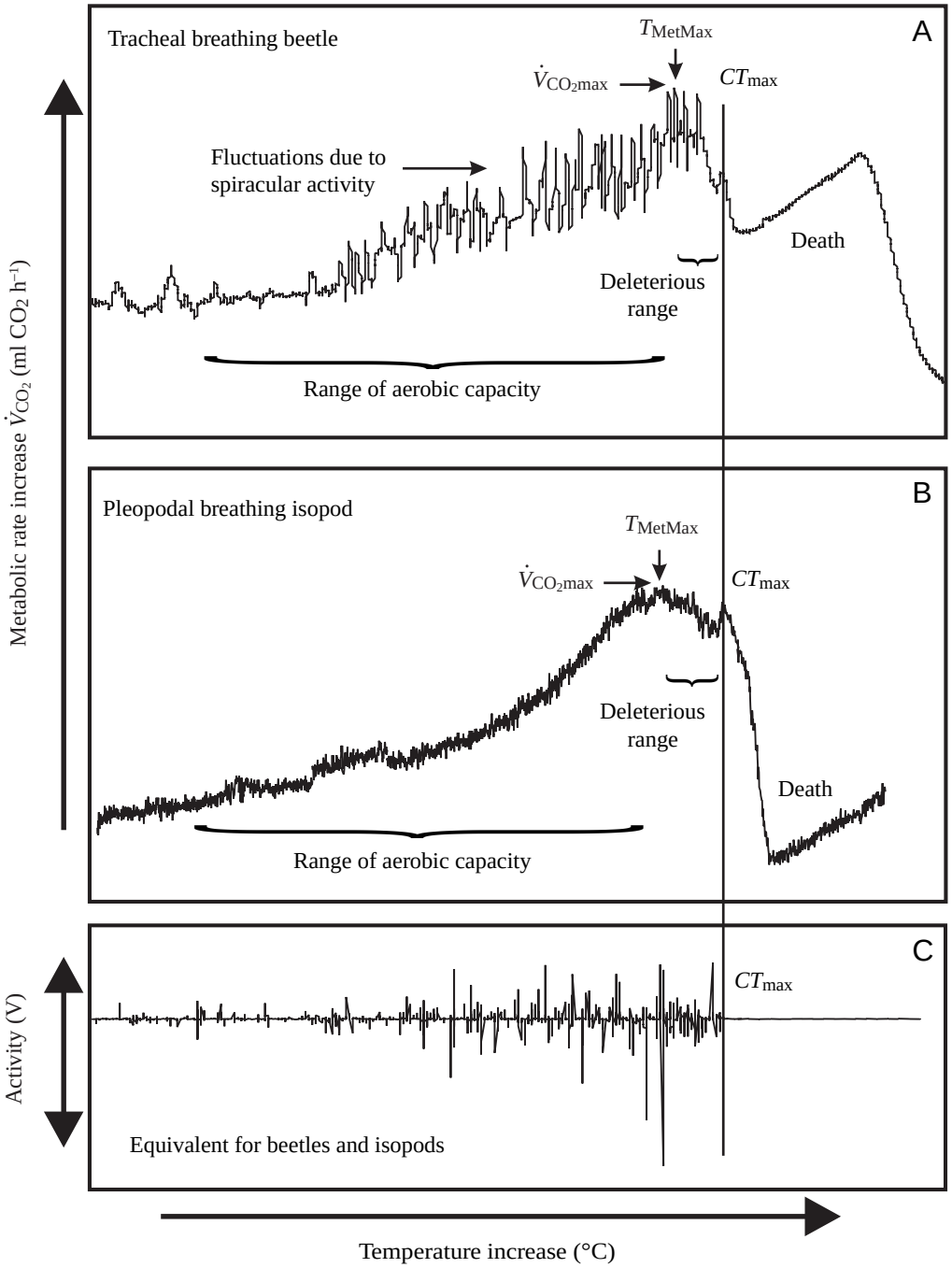

Fig. 1 Standardized data output of thermolimit respirometry outlined as (A,B) changes in metabolic rate ($\dot{V}_{CO_2}$; ml CO_2 h^{-1}) and (C) activity (V) for a tracheal breathing beetle (A,C) and a pleopodal breathing terrestrial isopod (B,C) at increasing temperatures starting at 30°C. Analogous to the law of tolerance (Schwerdfeger, 1977; Shelford, 1931) as interpreted by Pörtner (2001, 2002a), the CO_2 profiles characterise the rise in metabolic rate in both species across their range of aerobic capacity (above 30°C), culminating in a maximum metabolic rate $\dot{V}_{CO_2max}$ corresponding to a temperature here termed the T_{MetMax} (equivalent to Pörtner's T_{pII} – upper pejus temperature). Beyond the T_{MetMax} (=T_{pII}) increasing temperatures cause the onset of a progressive decrease in metabolic rate. This short temperature range is called the deleterious range. This metabolic rate decline culminates in the respirometry CT_{max}, signalled by a brief spike in CO_2 emission. With temperature increases beyond the CT_{max}, metabolic breakdown continues and eventually leads to death and the subsequent release of residual CO_2 from the body. Motor activity also increases with increasing temperature and these responses are equivalent in both test species. The cessation of coordinated motor function characterises onset of the activity CT_{max} (*sensu* Lutterschmidt and Hutchison, 1997). The respirometry CT_{max} often corresponds closely with the activity CT_{max} (see vertical line). Furthermore, respiratory recordings in the tracheal breather show spiracular activity and this ceases in concert with cessation in coordinated motor function shown in the activity recordings (J. R. B. Lighton and R. J. Turner, personal communication; see also Lighton and Turner, 2004). The primarily diffusive pleopodal breathing isopod does not show this.

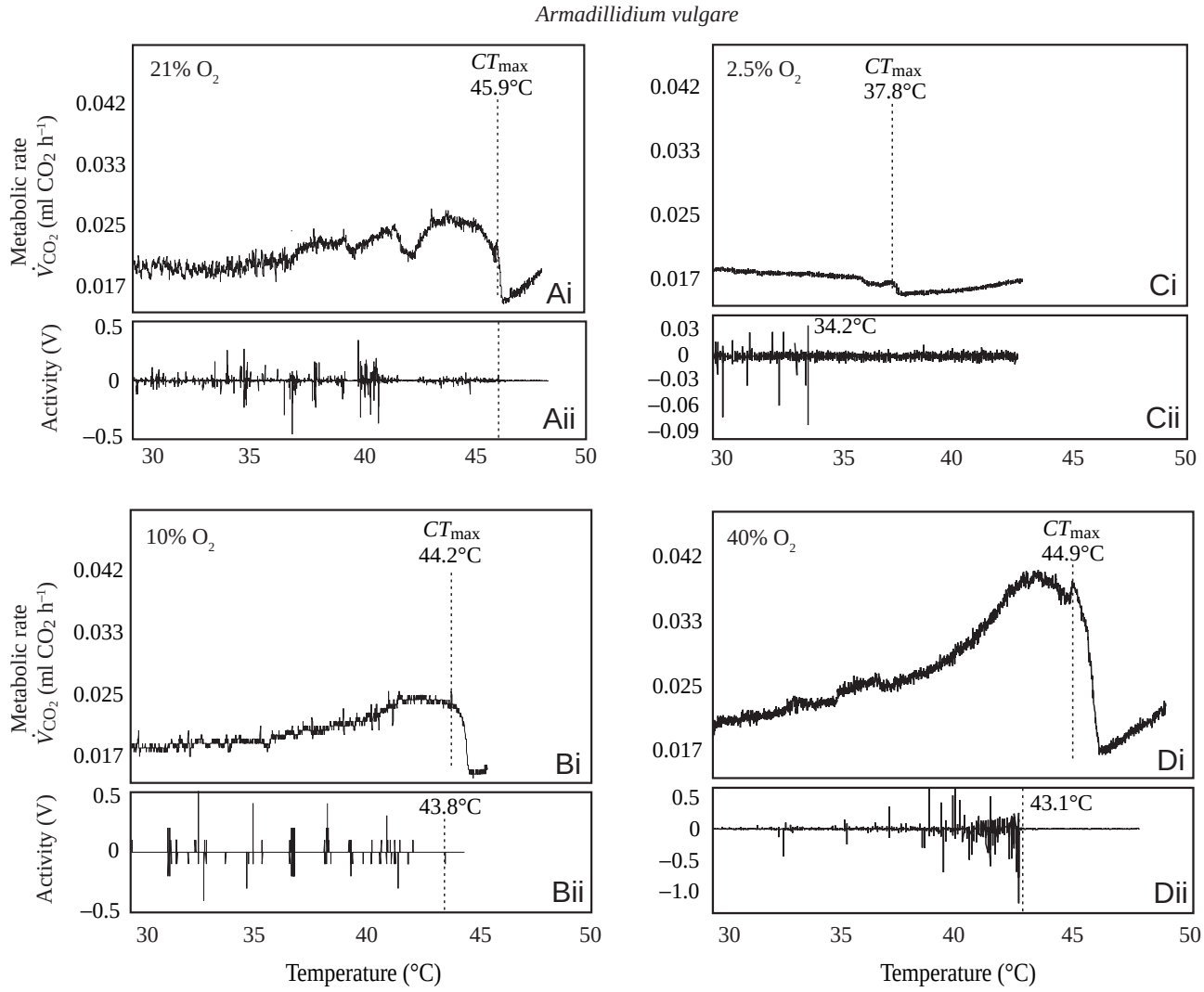

Fig. 2. Representative data for the thermal limit respirometry experiments on *Armadillidium vulgare*. (A) 21% oxygen, (B) 10%, (C) 2.5%, (D) 40%. (Ai–Di) Metabolic rate $\dot{V}_{CO_2}$, (Aii–Dii) activity in arbitrary units (V), where both negative and positive values indicate activity.

corresponding to reduced activity levels (Fig. 3C) and maximum spiracle opening to ensure sufficient O_2 uptake at that low O_2 concentration. Moreover, metabolic rates were significantly lower than those at the other concentrations, as was the regression coefficient of the rate temperature relationship (Table 1). In other words, oxygen delivery only becomes problematic when the concentration declines from 10% to 2.5% O_2. The respirometry CT_{max} was unaffected by oxygen concentration, although the beetles tended to maintain very low activity levels at 2.5% O_2, resulting in a lower, but probably artefactual, activity CT_{max} (Table 2).

Discussion

If oxygen limitation of thermal tolerance is a unifying principle at temperatures above freezing (Pörtner, 2001, 2002a), then the predictions of the hypothesis should apply to all ectothermic animals, or at least to the vast majority of them. In this particular instance, CT_{max} should decline with hypoxia and increase with hyperoxia. The two terrestrial species we investigated showed very different responses to changing oxygen partial pressures. In the isopod there was a significant decline in CT_{max} with hypoxia, whereas there was no significant change in CT_{max} in the tenebrionid beetle. Moreover, it was clear that there was a considerable effect of hypoxia on maximum metabolic rates in the isopod, whereas the effect was noticeable only at 2.5% O_2 in the tenebrionid, and then did not cause a pronounced decline in the CT_{max}. This difference can readily be explained by the dissimilarity of the gas exchange and transport systems of the two species. Terrestrial isopods such as *A. vulgare* exchange gasses by means of pleopod exopodites, from where oxygen is transported to the areas where it is required by haemolymph, containing the respiratory pigment haemocyanin (Ruppert and Barnes, 1994; Schmidt and Wägele, 2001). In this respect *A. vulgare* is functionally similar to many of the marine species investigated by Pörtner and his colleagues (e.g. Frederich and Pörtner, 2000; Mark et al., 2002; Peck et al., 2002; Sommer et

Table 1. *Maximum metabolic rate (T_{MetMax}) and regression coefficients of the relationship between temperature and log metabolic rate up to the T_{MetMax} at each of four oxygen concentrations in the isopod* A. vulgare *and the beetle* G. simplex

	T_{MetMax} (ml CO_2 h^{-1})				
Oxygen concentration (%)	Mean	Minimum	Maximum	*N*	Regression coefficient
Armadillidium vulgare					
2.5	0.0403±0.0015[A,1]	0.0293	0.0469	10	0.0176±0.0082[A,*,2]
10	0.0550±0.0030[B]	0.0424	0.0703	10	0.0274±0.0012[B]
21	0.0576±0.0024[B]	0.0497	0.0731	9	0.0181±0.0010[C]
40	0.0794±0.0064[C]	0.0581	0.1156	10	0.0228±0.0013[D]
Gonocephalum simplex					
2.5	0.0733±0.0320[A,3]	0.0547	0.1320	10	0.0217±0.0005[A,4]
10	0.1554±0.0085[B]	0.1124	0.1920	10	0.0256±0.0007[B]
21	0.1495±0.0128[B]	0.0867	0.2219	10	0.0348±0.0015[C]
40	0.1724±0.0100[B]	0.1347	0.2317	10	0.0292±0.0011[D]

Values are means ± S.E.M.
In all cases different letters denote significant differences in the values based on ANOVA or ANCOVA – Tukey's HSD test.
[1]ANOVA $F_{(3,35)}$=17.66, P<0.0001; [2]ANCOVA for homogeneity of slopes $F_{(3,2900)}$=7.952, P<0.0001; [3]ANOVA $F_{(3,36)}$=19.87, P<0.0001; [4]ANCOVA for homogeneity of slopes $F_{(3,6687)}$=36.319, P<0.0001.
*N=3; most other individuals showed a steady decline in metabolic rate with increasing temperature.

Table 2. *Activity and respirometry critical thermal maxima at each of four oxygen concentrations in the isopod* A. vulgare *and the beetle* G. simplex

	CT_{max} (°C)			
Oxygen concentration (%)	Mean ± S.E.M.	Minimum	Maximum	*N*
Armadillidium vulgare	Activity			
2.5	34.6±0.4[A,1]	33.4	36.5	8
10	42.1±0.6[B]	38.7	45.7	10
21	44.4±0.5[C]	41.7	47.0	10
40	43.2±0.5[B,C]	38.8	44.7	10
	Respirometry			
2.5	38.7±0.3[A,2]	37.7	40.4	10
10	42.4 ±0.6[B]	38.7	44.7	10
21	44.7±0.6[C]	41.1	46.6	10
40	44.9±0.2[C]	43.7	46.1	10
Gonocephalum simplex	Activity			
2.5	38.4±2.0[A,3]	30.2	50.1	9
10	49.3±0.3[B]	48.0	51.2	10
21	49.3±0.4[B]	46.3	50.6	10
40	49.2±0.2[B]	49.7	50.1	10
	Respirometry			
2.5	50.1±0.2[4]	48.9	51.8	10
10	49.2 ±0.2	48.4	50.8	10
21	49.3±0.4	46.6	51.0	10
40	49.0±0.2	48.4	50.1	10

In all cases different letters denote significant differences in the values based on ANOVA and Tukey's HSD test.
[1]ANOVA $F_{(3,33)}$=59.72, P<0.0001; [2]ANOVA $F_{(3,35)}$=43.34, P<0.0001; [3]ANOVA $F_{(3,35)}$=29.95, P<0.0001; [4]ANOVA $F_{(3,36)}$=2.89, P=0.05.

al., 1997; van Dijk et al., 1999). Moreover, it is also similar to the terrestrial species from which evidence has been cited in support of the oxygen limitation hypothesis (Pörtner, 2002a). Spiders and scorpions generally exchange gasses using book lungs and circulation is *via* the haemolymph using respiratory pigments (Bridges et al., 1997; Burmester, 2002; Paul et al., 1987), whilst in the terrestrial vertebrate models that Pörtner discussed, gas exchange and circulation are also based on two separate systems. It is no surprise in animals using a two-stage system for gas exchange and its subsequent delivery that at high temperatures a mismatch between excessive oxygen demand by mitochondria and insufficient oxygen uptake and distribution should arise (Pörtner, 2001, 2002a).

By contrast, insects have a very different system of gas exchange and delivery. In general, oxygen moves along a pathway from the spiracles through the main tracheal tubes (*via* convection or diffusion) to the tracheoles, where it diffuses to the mitochondria (Buck, 1962; Kestler, 1985; Nation, 2002). Some diffusion from the large tracheae to the surrounding tissues or haemolymph also takes place, although it probably accounts for no more than 25% of the total because of the small partial pressure difference between the tracheal lumen and the surrounding tissues (Schmitz and Perry, 1999). Carbon dioxide does not follow the same route in the opposite direction. Rather, it is thought to enter the tracheal system at all points from the tissues and haemolymph, where it is buffered as bicarbonate (Bridges and Scheid, 1982; Schmitz and Perry, 1999). Thus, O_2 delivery is highly efficient. Indeed, most insects can be considered metabolic regulators in the sense that oxygen consumption remains constant with declining O_2 concentration until a critical oxygen tension of about 5–10% O_2 is reached (generally lower in adults) (Loudon, 1988). Only at oxygen tensions below this critical point does metabolic rate decline. However, many species remain apparently unaffected

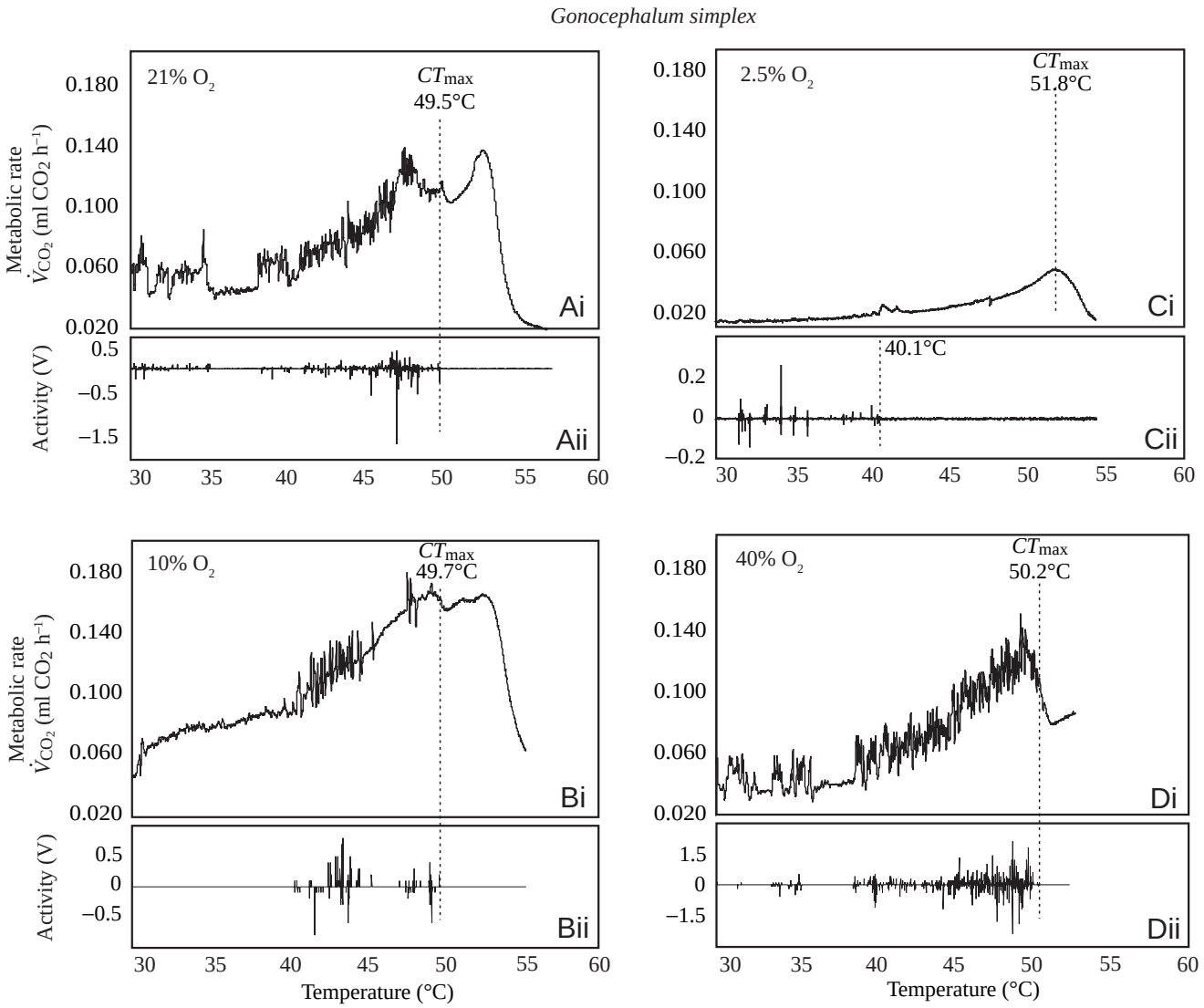

Fig. 3. Representative data for the thermal limit respirometry experiments on *Gonocephalum simplex*. (A) 21% oxygen, (B) 10%, (C) 2.5%, (D) 40%. (Ai–Di) Metabolic rate $\dot{V}_{CO_2}$, (Aii–Dii) activity in arbitrary units (V), where both negative and positive values indicate activity.

at low oxygen partial pressures, maintaining gas exchange and activity below these levels (Holter and Spangenberg, 1997; Chown and Holter, 2000). This suggests that oxygen limitation is unlikely for insects under a wide variety of conditions, and would certainly not limit thermotolerance, at least not under the conditions generally experienced in terrestrial habitats. Our data on the tenebrionid beetle *G. simplex* certainly support this idea, and indicate that oxygen limitation of thermal tolerance does not take place: hypoxia has no effect on CT_{max}.

One counterargument might be that our assessments of CT_{max} were undertaken over short timescales and that the deleterious limits only become apparent over longer periods (Pörtner, 2001, 2002a). In other words, had we continued our trials for a longer period, oxygen limitation of thermal tolerance would have become apparent. In our view this is unlikely. Insects are known to be highly responsive to changing oxygen availability and can compensate for longer-term changes in gas concentrations in both physiological and developmental ways (Frazier et al., 2001; Loudon, 1988; Wigglesworth, 1935). Even in flying insects, flight metabolism is generally only sensitive to ambient oxygen partial pressures below 10% O_2 (Harrison and Lighton, 1998; Joos et al., 1997). Thus, it seems unlikely that a mismatch between oxygen supply and demand sets thermal limits in insects. In addition, in contrast to stenothermal subtidal marine habitats, microclimates can and do reach upper lethal temperatures for terrestrial arthropods (e.g. Neargarder et al., 2003; Roberts and Feder, 1999; Wehner et al., 1992), and approach lethal limits in intertidal environments (Helmuth et al., 2002).

The question of what accounts for thermal limits in insects then remains. At high temperatures, thermal limits are probably the consequence of differing responses to heat injury at the cellular level. High temperature injury generally results from disruption of membrane structure, and problems associated

with protein folding. This causes a breakdown in the function of membranes, especially synaptic membranes, alterations in the cell microenvironment, DNA lesions and perturbation of protein structure (Feder, 1999; Somero, 1995). The importance of responses to cellular level thermal damage for whole-organismal survival is clearly reflected in geographic variation in heat shock protein expression associated with variation in environmental temperatures to which the organisms are exposed (Bettencourt et al., 2002; Dahlhoff and Rank, 2000; Neargarder et al., 2003; Sørensen et al., 2001). In addition, thermal resistance of the nervous system has been correlated with concentrations of membrane polyunsaturated fatty acids (PUFAs) in vertebrates (Logue et al., 2000; Hulbert, 2003), and could also play a significant role in insects. In turn, membrane damage affects development, neural functioning, muscular contraction and several other processes at higher organizational levels (Denlinger and Yocum, 1998). Such determinants of CT_{max} at the cellular level in insects would make the CT_{max} independent of oxygen availability, as we have observed. Moreover, the absence of a response of CT_{max} to hyperoxia in our experiments also suggests that cellular level damage sets the CT_{max} – no amount of improved oxygen delivery can alter the value upwards. Intriguingly, this was also the case in the isopod, suggesting that a combination of higher level organizational constraints and cellular level resistance to thermal injury might set the upper thermal limit (see also Sokolova and Pörtner, 2003).

However, it seems that insufficient aerobic capacity of mitochondria at low temperature might well be important in setting lower critical limits. In freeze-tolerant *Pringleophaga marioni* (Lepidoptera, Tineidae) caterpillars there is a precipitous decline in metabolic rate at the critical thermal minimum (Sinclair et al., 2004). Moreover, in both honey bees and *Drosophila*, decreasing temperature results in a steady decline in the resting potential of flight muscle neurons. The critical thermal minimum appears to be the temperature at which the Na^+/K^+-ATPase pump can no longer maintain nerve cell polarisation to a level where action potentials could be produced (Hosler et al., 2000). Thus, lack of ATP owing to insufficient aerobic metabolism in the mitochondria might well set lower limits in insects. This is in keeping with Pörtner's hypothesis (Pörtner, 2001), and also supports the idea that mechanisms underlying the higher and lower critical thermal limits in tracheated terrestrial ectotherms are different (Chown, 2001).

Our results therefore suggest that oxygen limitation of upper thermal tolerance is unlikely in insects, and probably also in other tracheated arthropods. They also suggest an explanation for the observation that, in insects, upper and lower lethal limits are generally decoupled (Chown, 2001), whereas this is not the case in most marine species (Pörtner, 2001). Thus, the question remains as to whether oxygen limitation of thermal tolerance can be considered a unifying general principle. This depends very much on one's perspective. From an entomological perspective, oxygen limitation is not pervasive: most terrestrial animal species are tracheated arthropods with two sets of wings (Samways, 1994). In this regard, terrestrial species are very different to those from marine environments. However, from a phylogenetic perspective it is clear that the members of most other higher taxa, with their two-stage oxygen delivery systems, probably face oxygen limitation of thermal tolerance. At present, too few species have been examined to verify these ideas, but clearly Pörtner's hypothesis makes several testable predictions that would cast considerable light on the apparent dichotomy.

John Lighton and Robbin Turner are thanked for insightful discussions concerning the interpretation of thermolimit respirometry and for providing access to unpublished information. Sue Jackson and Richard Mercer and two anonymous referees provided useful and insightful comments on an earlier version of the manuscript. C.J.K. was supported by a National Institutes of Health Grant AI-5245601, B.J.S. by a New Zealand Science and Technology Post-Doctoral Fellowship, and S.L.C. by National Institutes of Health Grant AI-5245601 and by National Research Foundation Grant GUN 2053804.

References

Addo-Bediako, A., Chown, S. L. and Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proc. R. Soc. Lond. B* **267**, 739-745.

Berrigan, D. (2000). Correlations between measures of thermal stress within and between species. *Oikos* **89**, 301-304.

Berrigan, D. and Hoffmann, A. A. (1998). Correlations between measures of heat resistance and acclimation in two species of *Drosophila* and their hybrids. *Biol. J. Linn. Soc.* **64**, 449-462.

Bettencourt, B. R., Kim, I., Hoffmann, A. A. and Feder, M. E. (2002). Response to natural and laboratory selection at the *Drosophila* hsp70 genes. *Evolution* **56**, 1796-1801.

Bridges, C. R., le Roux, J. M. and van Aardt, W. J. (1997). Ecophysiological adaptations to dry thermal environments measured in two unrestrained Namibian scorpions, *Parabuthus villosus* (Buthidae) and *Opisthophthalmus flavescens* (Scorpionidae). *Physiol. Zool.* **70**, 244-256.

Bridges, C. R. and Scheid, P. (1982). Buffering and CO_2 dissociation of body fluids in the pupa of the silkworm moth, *Hyalophora cecropia*. *Resp. Physiol.* **48**, 183-197.

Buck, J. (1962). Some physical aspects of insect respiration. *Annu. Rev. Entomol.* **7**, 27-56.

Burmester, T. (2002). Origin and evolution of arthropod hemocyanins and related proteins. *J. Comp. Physiol. B* **172**, 95-107.

Chapman, R. F. (1998). *The Insects. Structure and Function*. 4th ed. Cambridge: Cambridge University Press.

Chen, C.-P., Lee, R. E., Jr and Denlinger, D. L. (1990). A comparison of the responses of tropical and temperate flies (Diptera: Sarcophagidae) to cold and heat stress. *J. Comp. Physiol.* **160**, 543-547.

Chown, S. L. (2001). Physiological variation in insects: hierarchical levels and implications. *J. Insect Physiol.* **47**, 649-660.

Chown, S. L. and Holter, P. (2000). Discontinuous gas exchange cycles in *Aphodius fossor* (Scarabaeidae): a test of hypotheses concerning origins and mechanisms. *J. Exp. Biol.* **203**, 397-403.

Cossins, A. R. and Bowler, K. (1987). *Temperature Biology of Animals*. London: Chapman and Hall.

Dahlhoff, E. P. and Rank, N. E. (2000). Functional and physiological consequences of genetic variation at phosphoglucose isomerase: Heat shock protein expression is related to enzyme genotype in a montane beetle. *Proc. Natl. Acad. Sci. USA* **97**, 10056-10061.

Davis, M. B. and Shaw, R. G. (2001). Range shifts and adaptive responses to quaternary climate change. *Science* **292**, 673-679.

Denlinger, D. L. and Yocum, G. D. (1998). Physiology of heat sensitivity. In *Temperature Sensitivity in Insects and Application in Integrated Pest Management* (ed. D. L. Denlinger), pp. 7-53. Boulder: Westview Press.

Feder, M. E. (1999). Engineering candidate genes in studies of adaptation: the heat-shock protein HSP70 in *Drosophila melanogaster*. *Am. Nat. Suppl.* **154**, s55-s66.

Frazier, M. R., Woods, H. A. and Harrison, J. (2001). Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiol. Biochem. Zool.* **74**, 641-650.

Frederich, M. and Pörtner, H. O. (2000). Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. *Am. J. Physiol. Reg. Int. Comp. Physiol.* **279**, R1531-R1538.

Gaston, K. J. and Chown, S. L. (1999). Elevation and climatic tolerance: a test using dung beetles. *Oikos* **86**, 584-590.

Gibert, P. and Huey, R. B. (2001). Chill-coma temperature in *Drosophila*: effects of developmental temperature, latitude, and phylogeny. *Physiol. Biochem. Zool.* **74**, 429-434.

Gilchrist, G. W., Huey, R. B. and Partridge, L. (1997). Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. *Physiol. Zool.* **70**, 403-414.

Goto, S. G., Kitamura, H. W. and Kimura, M. T. (2000). Phylogenetic relationships and climatic adaptations in the *Drosophila takahashii* and *montium* species subgroups. *Mol. Phylogenet. Evol.* **15**, 147-156.

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

Helmuth, B., Harley, C. D. G., Halpin, P. M., O'Donnell, M., Hofmann, G. E. and Blanchette, C. A. (2002). Climate change and latitudinal patterns of intertidal thermal stress. *Science* **298**, 1015-1017.

Hercus, M. J., Berrigan, D., Blows, M. W., Magiafoglou, A. and Hoffmann, A. A. (2000). Resistance to temperature extremes between and within life cycle stages in *Drosophila serrata*, *D. birchii* and their hybrids: intraspecific and interspecific comparisons. *Biol. J. Linn. Soc.* **71**, 403-416.

Herreid, C. F. (1980). Hypoxia in invertebrates. *Comp. Biochem. Physiol.* **67A**, 311-320.

Hoffmann, A. A., Anderson, A. and Hallas, R. (2002). Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecol. Lett.* **5**, 614-618.

Hoffmann, A. A. and Blows, M. W. (1993). Evolutionary genetics and climate change: will animals adapt to global warming? In *Biotic Interactions and Global Change* (ed. R. B. Huey), pp. 165-178. Sunderland: Sinauer.

Hoffmann, A. A., Dagher, H., Hercus, M. and Berrigan, D. (1997). Comparing different measures of heat resistance in selected lines of *Drosophila melanogaster*. *J. Insect Physiol.* **43**, 393-405.

Holter, P. and Spangenberg, A. (1997). Oxygen uptake in coprophilous beetles (*Aphodius*, *Geotrupes*, *Sphaeridium*) at low oxygen and high carbon dioxide concentrations. *Physiol. Entomol.* **22**, 339-343.

Hosler, J. S., Burns, J. E. and Esch, H. E. (2000). Flight muscle resting potential and species-specific differences in chill-coma. *J. Insect Physiol.* **46**, 621-627.

Huey, R. B., Crill, W. D., Kingsolver, J. G. and Weber, K. E. (1992). A method for rapid measurement of heat or cold resistance of small insects. *Funct. Ecol.* **6**, 489-494.

Hulbert, A. J. (2003). Life, death and membrane bilayers. *J. Exp. Biol.* **206**, 2303-2311.

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.

Kestler, P. (1985). Respiration and respiratory water loss. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 137-186. Berlin: Springer.

Kingsolver, J. G. and Huey, R. B. (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *Am. Zool.* **38**, 545-560.

Klok, C. J. and Chown, S. L. (2003). Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biol. J. Linn. Soc.* **78**, 401-414.

Lighton, J. R. B. (1988). Discontinuous CO_2 emission in a small insect, the formicine ant *Camponotus vicinus*. *J. Exp. Biol.* **134**, 363-376.

Lighton, J. R. B. and Turner, R. J. (2004). Thermolimit respirometry: an objective assessment of critical thermal maxima in two sympatric desert harvester ants, *Pogonomyrmex rugosus* and *P. californicus*. *J. Exp. Biol.* **207**, 1903-1913.

Logue, J. A., De Vries, A. L., Fodor, E. and Cossins, A. R. (2000). Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure. *J. Exp. Biol.* **203**, 2105-2115.

Loudon, C. (1988). Development of *Tenebrio molitor* in low oxygen levels. *J. Insect Physiol.* **34**, 97-103.

Lutterschmidt, W. I. and Hutchison, V. H. (1997). The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Can. J. Zool.* **75**, 1553-1560.

Mark, F. C., Bock, C. E. and Pörtner, H. O. (2002). Oxygen limited thermal tolerance in Antarctic fish investigated by magnetic resonance imaging (MRI) and spectroscopy (^{31}P-MRS). *Am. J. Physiol.* **283**, R1245-R1262.

Nation, J. L. (2002). *Insect Physiology and Biochemistry*. Boca Raton: CRC Press.

Neargarder, G., Dahlhoff, E. P. and Rank, N. E. (2003). Variation in thermal tolerance is linked to phosphoglucose isomerase genotype in a montane leaf beetle. *Funct. Ecol.* **17**, 213-221.

Paul, R., Fincke, T. and Linzen, B. (1987). Respiration in the tarantula *Eurypelma californicum*: evidence for diffusion lungs. *J. Comp. Physiol. B* **157**, 209-217.

Peck, L., Pörtner, H. O. and Hardewig, I. (2002). Metabolic demand, oxygen supply, and critical temperatures in the Antarctic bivalve, *Laternula elliptica*. *Physiol. Biochem. Zool.* **75**, 123-133.

Pörtner, H. O. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137-146.

Pörtner, H. O. (2002a). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Biochem. Physiol.* **132A**, 739-761.

Pörtner, H. O. (2002b). Environmental and functional limits to muscular exercise and body size in marine invertebrate athletes. *Comp. Biochem. Physiol.* **133A**, 303-321.

Pörtner, H. O. (2002c). Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. *J. Exp. Biol.* **205**, 2217-2230.

Pörtner, H. O., van Dijk, P. L. M., Hardewig, I. and Sommer, A. (2000). Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In *Antarctic Ecosystems: Models for Wider Ecological Understanding* (ed. C. H. Williams), pp. 109-122. Christchurch: New Zealand Natural Sciences.

Prosser, C. L. (1986). *Adaptational Biology. Molecules to Organisms*. New York: John Wiley & Sons.

Roberts, S. P. and Feder, M. E. (1999). Natural hyperthermia and expression of the heat shock protein Hsp70 affect developmental abnormalities in *Drosophila melanogaster*. *Oecologia* **121**, 323-329.

Ruppert, E. E. and Barnes, R. D. (1994). *Invertebrate Zoology*, 6th edn. Fort Worth: Saunders College Publishing.

Samways, M. J. (1994). *Insect Conservation Biology*. London: Chapman and Hall.

Schmidt, C. and Wägele, J. W. (2001). Morphology and evolution of respiratory structure in the pleopod exopodites of terrestrial Isopoda (Crustacea, Isopoda, Oniscidea). *Acta Zool.* **82**, 315-330.

Schmitz, A. and Perry, S. F. (1999). Stereological determination of tracheal volume and diffusing capacity of the tracheal walls in the stick insect *Carausius morosus* (Phasmatodea, Lonchodidae). *Physiol. Biochem. Zool.* **72**, 205-218.

Schwerdfeger, F. (1977). *Ökologie der Tiere*: Autökologie. Hamburg: Paul Parey.

Shelford, V. E. (1931). Some concepts in bioecology. *Ecology* **12**, 455-467.

Sinclair, B. J., Klok, C. J and Chown, S. L. (2004). Metabolism of the sub-Antarctic caterpillar *Pringleophaga marioni* during cooling, freezing and thawing. *J. Exp. Biol.* **207**, 1287-1294.

Sokolova, I. M. and Pörtner, H. O. (2003). Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: Littorinidae) from different latitudes. *J. Exp. Biol.* **206**, 195-207.

Somero, G. N. (1995). Proteins and temperature. *Annu. Rev. Physiol.* **57**, 43-68.

Sommer, A., Klein, B. and Pörtner, H. O. (1997). Temperature induced anaerobiosis in two populations of the polychaete worm *Arenicola marina* (L.). *J. Comp. Physiol. B* **167**, 25-35.

Sørensen, J. G., Dahlgaard, J. and Loeschcke, V. (2001). Genetic variation in thermal tolerance among natural populations of *Drosophila buzzatii*: down regulation of Hsp70 expression and variation in heat stress resistance traits. *Funct. Ecol.* **15**, 289-296.

Ushakov, B. P. (1964). Thermostability of cells and proteins of poikilotherms and its significance in speciation. *Physiol. Rev.* **44**, 518-560.

van Dijk, P. L. M., Hardewig, I. and Pörtner, H. O. (1999). Physiological disturbances at critically high temperatures: a comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae). *J. Exp. Biol.* **202**, 3611-3621.

Warren, M. S., Hill, J. K., Thomas, J. A., Asher, J., Fox, R., Huntley, B., Roy, D. B., Telfer, M. G., Jeffcoate, S., Harding, P. et al. (2001). Rapid responses of British butterflies to opposing forces of climate and habitat change. *Nature* **414**, 65-69.

Wehner, R., Marsh, A. C. and Wehner, S. (1992). Desert ants on a thermal tightrope. *Nature* **357**, 586-587.

Wigglesworth, V. B. (1935). The regulation of respiration in the flea, *Xenopsylla cheopsis*, Roths. (Pulicidae). *Proc. R. Soc. Lond. B* **118**, 397-419.