Critical thermal limits depend on methodological context

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A full-factorial study of the effects of rates of temperature change and start temperatures was undertaken for both upper and lower critical thermal limits (CTLs) using the tsetse fly, *Glossina pallidipes*. Results show that rates of temperature change and start temperatures have highly significant effects on CTLs, although the duration of the experiment also has a major effect. Contrary to a widely held expectation, slower rates of temperature change (i.e. longer experimental duration) resulted in poorer thermal tolerance at both high and low temperatures. Thus, across treatments, a negative relationship existed between duration and upper CTL while a positive relationship existed between duration and lower CTL. Most importantly, for predicting tsetse distribution, *G. pallidipes* suffer loss of function at less severe temperatures under the most ecologically relevant experimental conditions for upper ($0.06°C\ min^{-1}$; 35°C start temperature) and lower CTL ($0.06°C\ min^{-1}$; 24°C start temperature). This suggests that the functional thermal range of *G. pallidipes* in the wild may be much narrower than previously suspected, approximately 20–40°C, and highlights their sensitivity to even moderate temperature variation. These effects are explained by limited plasticity of CTLs in this species over short time scales. The results of the present study have broad implications for understanding temperature tolerance in these and other terrestrial arthropods.

Keywords: temperature tolerance; phenotypic plasticity; rapid cold-hardening; acclimation rate; lethal limits; survival

1. INTRODUCTION

The ability of an organism to remain active under extreme conditions is a significant component of fitness (Loeschcke & Hoffmann 2007). Therefore, determining the limits to activity is an important first step in understanding the ways in which environmental variation affects fitness and the dynamics of a given population. Thermal limits have received much attention because their investigation provides insight into the manner in which climate shapes variation in the ecology, distribution and evolution of species (Janzen 1967; Pörtner 2001; Ghalambor *et al.* 2006; Chown & Terblanche 2007). Furthermore, upper temperature limits are positively related to optimal performance temperatures (Huey & Bennett 1987; Garland *et al.* 1991) and these limits are relatively simple to measure (Chown & Nicolson 2004). In consequence, factors that affect the assessment of thermal tolerance are significant (Lutterschmidt & Hutchison 1997a; Chown & Nicolson 2004). In insects, these include photoperiod (Lanciani *et al.* 1992), ontogeny (Hollingsworth & Bowler 1966; Rossolimo 1997), CO_2 anaesthesia (Nilson *et al.* 2006), temperature acclimation (e.g. Terblanche *et al.* 2005, 2006) and time of day (Sinclair *et al.* 2003; McMillan *et al.* 2005). Importantly, they also include elements of basic experimental design, such as the rate of temperature change (Worland 2005)

and exposure temperature (David *et al.* 2003; Rako & Hoffmann 2006).

Typically, limits are assessed using either dynamic or static methods (Lutterschmidt & Hutchison 1997a; Hoffmann *et al.* 2003). Briefly, the dynamic method involves changing temperature at a constant rate and assessing individual knockdown. By contrast, when using the static method, temperature is held constant and exposure duration varied (or duration can be held constant while temperature is varied) with assessments based on survival of a proportion of a sample. Techniques that assess recovery from coma are dynamic in the sense that recovery time is assessed following exposure to one or more coma-inducing temperatures (David *et al.* 2003). One dynamic technique that is widely used to resolve both high- and low-temperature thresholds is the determination of critical thermal limits (CTLs; for critique and reviews see Lutterschmidt & Hutchison 1997a,b; Beitinger *et al.* 2000; Chown & Nicolson 2004). CTL determinations involve heating or cooling an animal from a starting temperature until physiological failure (e.g. knockdown, loss of righting response, onset of muscle spasms). CTLs are considered ecologically relevant because they provide an indication of the activity range for a population under acute exposure conditions (Vannier 1994; Somero 2005), although animals may mediate effects of environmental temperature variation through behavioural adjustments (Huey *et al.* 2003). However, the extent to which experimental design of CTLs influences their outcome has not been systematically explored (Chown & Nicolson 2004).

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The few studies that have been done have mostly concerned critical thermal minima (CT_{min}), demonstrating that slow cooling rates lower the CT_{min}, thus improving acute low-temperature tolerance (Kelty & Lee 1999, 2001; Powell & Bale 2004, 2006; Overgaard *et al.* 2006). The general explanation for this improvement in tolerance is that slower rates provide sufficient time for hardening, a form of phenotypic plasticity (see Hoffmann *et al.* 2003) that protects cells from subsequent injury (Overgaard *et al.* 2006). However, despite expectations that plasticity in lower thermal limits is widespread in all organisms (Rako & Hoffmann 2006), theory suggests that acclimation responses should be much less common in tropical or polar species compared with their temperate counterparts (Tsuji 1988; Somero *et al.* 1996; Ghalambor *et al.* 2006; Chown & Terblanche 2007). Given that the tropics probably house more insect species than non-tropical regions (Gaston 1996; Hillebrand 2004), and the small number of studies restricted to temperate species, the generality of the rate effect on CT_{min} cannot be considered well established. Moreover, rate effects on critical thermal maxima (CT_{max}) have not been widely explored (Chown & Nicolson 2004; Mora & Maya 2006).

A further complication in the determination of CTLs is the question of the start temperature used before cooling or heating commences. Start temperature usually means the constant temperature period during which animals are held before heating or cooling to allow equilibration of body temperature with ambient temperature. Although some studies use the same acclimation and start temperature, these can differ (e.g. Powell & Bale 2006), although the influence of these alternatives has not always been clear. Although start temperature is usually explicitly recorded in descriptions of experimental design (e.g. Kelty & Lee 1999, 2001; Shermin & Levitis 2003), and is typically varied by investigators when species or populations from different thermal environments are examined (e.g. Brattstrom 1965), its effect on CTLs is poorly understood (but see Das *et al.* 2005). Indeed, an implicit assumption is made that start temperature has little effect on experimental outcome. However, start temperature may have a significant influence on CTL determinations, directly, and through its influence on the overall time (duration) an experimental group of individuals experience conditions that either affect physiological functioning or induce a physiological response (e.g. Terblanche *et al.* 2005; see also Rako & Hoffmann 2006).

If start temperature and rate of temperature change affect CTLs in similar ways, and predominantly via the effects on duration of exposure, it might be expected that slow rates and start temperatures distant from the CTL in question would result in an improvement of tolerance. Moreover, these experimental effects might be substantial as a consequence of the considerable plasticity in CTLs and other thermal tolerance traits shown by various species (Chen *et al.* 1990; Hoffmann *et al.* 2003; Klok & Chown 2003).

Given the above and that among-population and interspecific variation in CTLs have been used to test a variety of macrophysiological hypotheses (e.g. Gaston & Chown 1999; Addo-Bediako *et al.* 2000; Ghalambor *et al.* 2006), examination of the extent to which experimental conditions influence CTLs is important. Therefore, here, using the full-factorial design, we dissect the effects of rate and start temperature on CTLs in a widely distributed, economically important tropical insect species, the tsetse fly (*Glossina pallidipes*; Diptera, Glossinidae). Since the ways in which ambient temperature affect the population dynamics, distribution and abundance of tsetse are of considerable interest (Hargrove 2001, 2004; Rogers & Robinson 2004), we also explore the outcomes of this work in the context of microclimates encountered by *G. pallidipes* in natural conditions.

2. MATERIAL AND METHODS

Adult tsetse flies (*G. pallidipes*) were collected from South Luangwa National Park, Zambia during October 2006. Odour-baited biconical and Ngu traps were used to collect live flies in the morning (06.00–09.00 hours; as outlined in Terblanche *et al.* 2006). Flies were kept in an insulated container and returned to the field laboratory within 2 hours of the end of each morning's collection period. All experiments were conducted on freshly collected animals only.

Microhabitat temperature data were logged for 10 days at three sites using calibrated iButton data loggers, with a resolution of 0.5°C (8-bit Model DS1923, Dallas, TX, USA). These sites were located on shaded trees and had been identified by previous inspection as sites used by resting flies during all daylight hours.

Effects of age, gender and feeding status on critical limits in *G. pallidipes* are negligible (table S1 in the electronic supplementary material; Terblanche *et al.* 2006). Therefore, these characteristics were not determined prior to the trials. Ten animals were placed individually into chambers in an insulated, water-jacketed isolation chamber system connected to a programmable water bath (as in Terblanche *et al.* 2006). A thermocouple (type T, 36 SWG) was inserted into a control chamber to monitor chamber temperature. In preliminary trials, we have recorded body temperature (T_b) changes of up to 10°C within 2 min as T_b tracks ambient temperature change prior to a CTL determination. Furthermore, T_b does not lag behind chamber temperatures during CTL experiments at a heating/cooling rate of 0.25°C min^{-1} (figure S1 in the electronic supplementary material). Starting temperatures were selected on the basis of previous assessments of CTLs in East African populations of this species (Terblanche *et al.* 2006). A full-factorial 3×3 design was used to assess the effects of rate and start temperature on CT_{min} and CT_{max}. Start temperatures of 16, 20 and 24°C and cooling rates of 0.06, 0.12 and 0.25°C min^{-1} were used for the CT_{min} experiments. For CT_{max} experiments, start temperatures of 35, 38 and 41°C and heating rates of 0.06, 0.12 and 0.25°C min^{-1} were used. After 12 min of equilibration at one of the three starting temperatures (periods of 10 min or less are frequently used in CTL assessments, e.g. Kelty & Lee 2001), flies were heated or cooled at one of the three rates. The point of critical thermal minimum (CT_{min}) was defined as the temperature of loss of coordinated muscle function, and critical thermal maximum (CT_{max}) was defined as the temperature of onset of muscle spasms (as in Terblanche *et al.* 2006). To avoid observer bias and diurnal effects, a single observer (J.A.D.) undertook all of the experimental work between 10.00 and 14.00 hours. Sample sizes varied between 10 and 20 for each of the nine rate×start temperature combinations in the CT_{max} experiment, and $n=20$ for each unique combination in the CT_{min} experiment. Ambient laboratory temperatures ranged from 30 to 35°C between
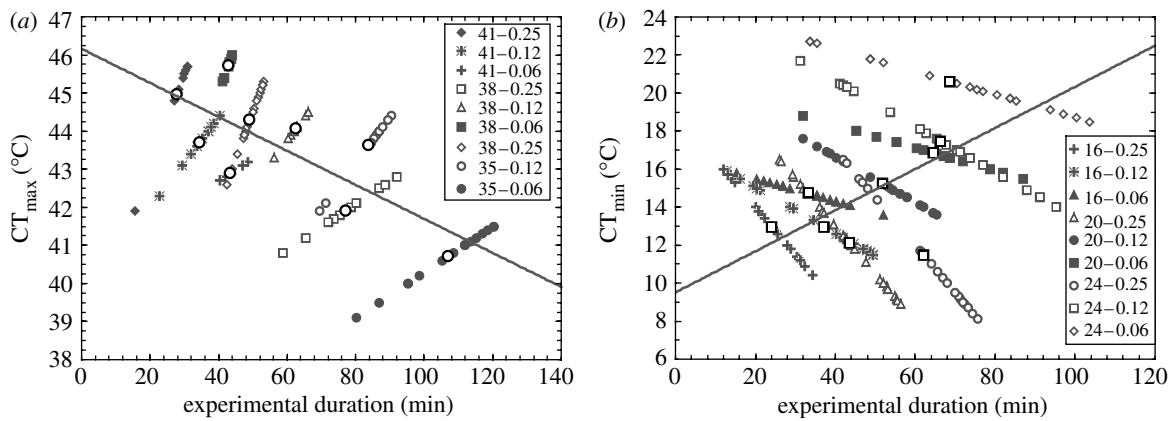
Figure 1. Scatter plot of experimental duration against (*a*) critical thermal maxima (CT_{max}) and (*b*) critical thermal minima (CT_{min}). Each treatment is plotted as a unique series. In the figure, the first number refers to the start temperature and the second number indicates the rate of temperature change during the experiment. Means of each treatment are indicated as open circles (CT_{max}) or filled squares (CT_{min}). Note the colinearity within treatments, but the opposite relationship among treatments for both CT_{min} and CT_{max}. The lines shown were fitted by least-squares regression using the means for each treatment, to illustrate the differences in direction of relationship within and among treatments.

Table 1. Outcome of a type III general linear model showing the confounding effect of experimental duration (duration) on CT_{max} and CT_{min} in *G. pallidipes*. Rate of temperature change (rate) and start temperature (temp) are included as might typically be done using such an approach (see text for detail). (Rate and temp were defined as categorical variables while duration was included as a continuous covariate.)

effect	SS	d.f.	F	p	estimate $\pm$ s.e.
CT_{max}					
intercept	1901.1	1	14 101.7	<0.0001	
duration	33.3	1	247.3	<0.0001	0.086 ± 0.006
rate	119.1	2	441.6	<0.0001	
temp	54.5	2	202.1	<0.0001	
rate$\times$temp	44.5	4	82.6	<0.0001	
error	12.9	96			
CT_{min}					
intercept	5413.8	1	6139.8	<0.0001	
duration	450.0	1	510.4	<0.0001	-0.115 ± 0.005
rate	1238.1	2	702.1	<0.0001	
temp	703.5	2	398.9	<0.0001	
rate$\times$temp	270.7	4	76.7	<0.0001	
error	158.7	180			

trials and experimental days and were within the range of recorded microclimate temperatures.

Prior to analysis, data were inspected for normality and equality of variances using Shapiro–Wilk and Hartley–Bartlett tests, respectively. In most cases data were normally distributed (15/18 cases) and had similar variance (16/18 cases). Exclusion of outliers corrected these issues in the remainder thereof. Initial analyses were performed using a full-factorial ANOVA implemented in STATISTICA v. 7 (Statsoft, Tulsa, OK, USA) and normality of residuals confirmed in each ANOVA. The effects of start temperature and rate on upper and lower CTLs were investigated using an ordered-factor ANOVA implemented in SAS (v. 9.0, SAS Institute, Cary, NC, USA).

Duration of exposure is thought to be a major proximate cause of variation in CTLs. On the one hand it is thought that increasing exposure duration might compound stress and therefore reduce tolerance (Cossins & Bowler 1987; Sømme 1999). On the other hand, prolonged exposures to sublethal temperatures might improve stress resistance (see §1). Therefore, the role of duration in altering CTLs in the nine different treatments was examined to determine if it might at least

partially account for the effects of rate and start temperature. At first, it might appear simple to do so by including duration, as calculated from the start of a given CT trial to the thermal limit identified for each individual, as a covariate in a general linear model. However, owing to the way the CTL is determined, it is colinear with duration for each rate and start temperature group. For example, in an upper CTL experiment, a higher CTL necessarily means that the animal has been warming for a longer period. In consequence, a general linear model including duration as a covariate cannot be used to investigate its effects. In other words, the physiological effects of duration of exposure as a consequence of different rates and start temperatures cannot be separated from the within-treatment or experimental effect of duration. The latter will always be positive for upper CTLs and negative for lower CTLs, as scatter plots of duration versus CTL (figure 1), and a general linear model incorporating duration in this manner (table 1), demonstrate. However, from a physiological consequences perspective, the relationship between duration and CTL might either be positive, negative or non-existent.

One way of disentangling the effects of the experimental duration for each individual from the physiological
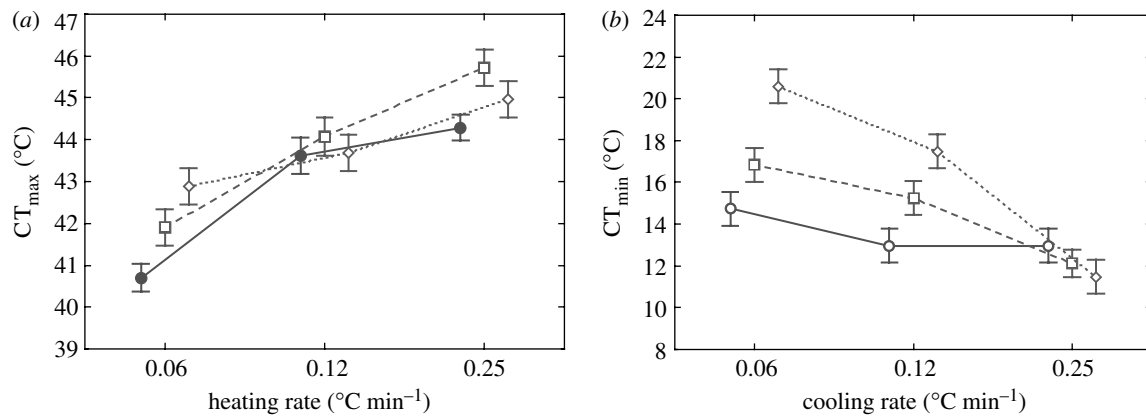
Figure 2. (*a*) Mean critical thermal maxima (CT_{max}) values for three heating rates (0.06, 0.12 and 0.25°C min^{-1}) using start temperatures of 35 (filled circles), 38 (open squares) and 41°C (open diamonds). (*b*) Mean critical thermal minima (CT_{min}) values for three heating rates (0.06, 0.12 and 0.25°C min^{-1}) using start temperatures of 16 (open circles), 20 (open squares) and 24°C (open diamonds).

Table 2. Outcome of an ordered-factor ANOVA (*proc GLM* in SAS) investigating the effects of start temperature and rate of temperature change on critical thermal maxima (CT_{max}) and critical thermal minima (CT_{min}) in wild *G. pallidipes*. (Estimates are given only for the linear effects.)

trait	effect	d.f.	*F*	*p*-value	estimate $\pm$s.e.
CT_{max}	start temperature	2	187.89	<0.0001	0.977 ± 0.164
	heating rate	2	26.30	<0.0001	3.156 ± 0.164
	start$\times$rate	4	8.64	<0.0001	0.919 ± 0.679
	error	97			
CT_{min}	start temperature	2	129.85	<0.0001	2.957 ± 0.335
	cooling rate	2	39.57	<0.0001	-5.201 ± 0.325
	start$\times$rate	4	23.35	<0.0001	13.400 ± 1.421
	error	181			

consequences of longer exposures to stressful temperatures is to examine the relationships between the mean CTL and mean duration for each treatment. In doing so, the colinearity is removed (e.g. upper CTL cannot simultaneously be positively and negatively related to duration). Therefore, we adopted this approach and examined the correlation between mean CTL and mean duration of exposure over the nine trials separately for the CT_{max} and the CT_{min} experiments. An additional general linear model including mean duration, mean CTL, and rate and start temperature was also implemented to distinguish the effects of duration from those of rate and start temperature. Here, a type I (additive) sums of squares model was used because the significance of the incremental increase in the sums of squares with each added effect provides an indication of the importance of that effect. The order of effects in the type I model was duration of experiment, rate of temperature change and start temperature.

3. RESULTS
The treatments resulted in greater than 5°C variation in CT_{max} and greater than 10°C variation in CT_{min} (figure 2). Rate of temperature change, start temperature and their interaction had significant effects on CT_{min} and CT_{max} (table 2). As figure 2 shows, faster rates resulted in greater thermal tolerances. Moreover, when start temperatures were high, upper CTL values were the largest, while when they were low, lower CTLs were the lowest. The significant interaction term indicates that start temperature had a striking influence on the extent to which rate affected CTLs (table 2). Because the start temperatures

most distant from the mean CTL appeared to have the largest effects, it seemed probable that exposure duration had a crucial role in altering the CTLs determined in each of the nine treatments.

Correlation analyses using the means of each treatment demonstrate that this is the case. The relationship between mean CT_{max} and mean exposure duration was significantly negative ($r=-0.759$, $p=0.011$, $n=9$) whereas it was positive between mean CT_{min} and mean exposure duration. The latter relationship was marginally non-significant when including the 24°C and 0.25°C min^{-1} slightly outlying treatment ($r=0.598$, $p=0.068$, $n=9$), and highly significant following its exclusion ($r=0.829$, $p=0.006$, $n=8$). Thus, the longer duration of exposure to experimental conditions results in poorer thermal tolerance at both upper and lower temperatures. The type I general linear model confirmed the significance of duration and rate, but suggested that start temperature added little further explanatory power (table 3).

In the field, mean shaded cooling rate was 0.020 ± 0.007°C min^{-1} (mean$\pm$s.d.) while mean shaded heating rate over the same period was 0.050 ± 0.010°C min^{-1} across the three fly resting sites that were assessed. Thus, the 0.06°C min^{-1} experimental rate of change is the most ecologically relevant of the three rates used in the laboratory trials. Similarly, inspection of the microclimate data (figure 3) suggested that the 24 and 35°C start temperatures for lower and upper CTL, respectively, are most ecologically relevant to tsetse in their natural habitat.

Table 3. Outcome of a general linear model using type I sums of squares showing the incremental effects of experimental duration (duration), rate of temperature change (rate) and start temperature on critical thermal minima (CT_{min}) and maxima (CT_{max}) in *G. pallidipes* using mean values for each treatment group.

effect	SS	d.f.	MS	F	p	estimate ±s.e.
CT_{max}						
intercept	17 060.4	1	17 060.4	25335.1	<0.0001	
duration	10.8	1	10.8	16.1	0.010	−0.032±0.036
rate	4.6	1	4.6	6.8	0.048	9.608±7.464
start temp	0.1	1	0.1	0.1	0.811	−0.073±0.291
error	3.4	5	0.7			
CT_{min}						
intercept	2008.3	1	2008.3	712.0	<0.0001	
duration	25.0	1	25.0	8.9	0.031	−0.125±0.139
rate	24.8	1	24.8	8.8	0.031	−34.816±11.419
start temp	6.0	1	6.0	2.1	0.203	0.905±0.619
error	14.1	5	2.8			

4. DISCUSSION

Several points, critical for all CTL investigations of ectotherms, have emerged from this study. Perhaps the most obvious outcome is that the range of variation caused by changes in experimental protocol is as wide as that found in many investigations of geographical or acclimation-induced variation in CTLs. For example, in the same species, Terblanche *et al.* (2006) found approximately 3.3°C variation of CT_{min} and 0.5°C variation of CT_{max} induced by acclimation temperatures ranging from 20 to 30°C over a 10-day period. In temperate weevils, Klok & Chown (2003) found approximately 2.6°C variation of CT_{min} and approximately 2.4°C variation of CT_{max} induced by 15°C range of acclimation temperatures. The present findings do not mean that the results of these and other past studies are confounded because in these investigations standardized protocols are typically used. However, they do suggest that additional thought needs to be given to distinguishing environmental signal from experimental noise in broader comparisons (see discussion in Chown *et al.* (2003); Hodkinson (2003)). Nonetheless, many of the conclusions from previous broad scale comparisons are likely to remain unaltered. For example, as has been found in many previous interspecific, intraspecific and acclimation-based investigations (reviewed in Chown (2001) and Chown & Terblanche (2007)), the present work revealed greater plasticity in lower thermal limits (overall range 10°C) than in upper thermal limits (overall range 5°C).

Perhaps more importantly, this study demonstrated that both start temperature and rate of temperature change affect CTLs, and that their effects interact, probably as a consequence of the duration of exposure. Indeed, when mean duration of exposure, rate of temperature change and start temperature were entered sequentially into the type I general linear model, the latter variable was not significant, and in the CT_{max} trials rate of change was only marginally significant. These findings make it clear that just as recovery from coma experiments have to take the knockdown temperature into account (David *et al.* 2003; Rako & Hoffmann 2006), so too do CTL experiments have to take rate and start temperature into account. They also confirm the fact, well known from other thermobiological investigations (Cossins & Bowler 1987; Sømme 1999; Ramløv 2000), that exposure
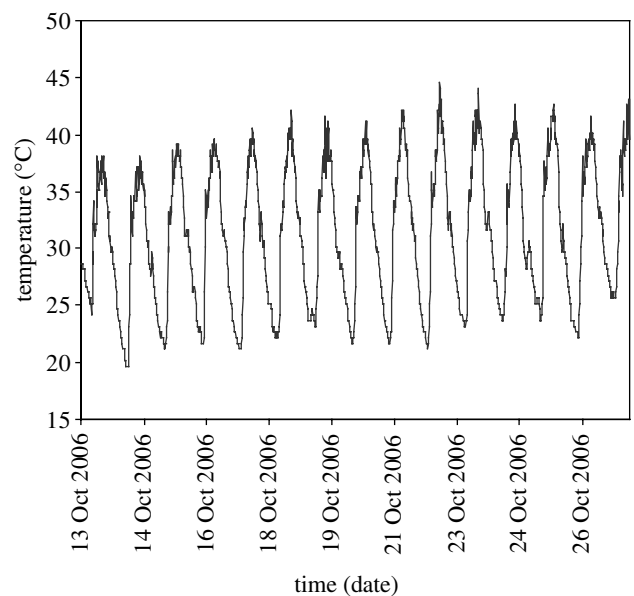

Figure 3. Average shade temperature for the habitat representative of *G. pallidipes* thermal refugia, gathered over two weeks during October 2006 (South Luangwa National Park, Zambia). Data obtained using thermochron iButtons sampling at 10 min frequency.

duration×temperature interactions ultimately determine mortality rates. Moreover, they emphasize that rates of temperature change affect critical limits, but that the extent of this influence depends on whether upper or lower critical limits are being examined, thus emphasizing the differences between these traits and their underlying mechanisms (see Chown & Nicolson 2004).

While a few previous studies have shown that duration of exposure has an effect on thermal tolerance, this effect is typically the opposite of what was documented in this study (although see Overgaard *et al.* (2006) for a more complex picture). Here, CTLs range declined as exposure duration increased. That is, CT_{min} increased and CT_{max} declined with prolonged exposure duration. By contrast, previous studies of CT_{min} in *Drosophila melanogaster* (Kelty & Lee 1999, 2001) and the grain aphid *Sitobion avenae* (Powell & Bale 2006) have shown that as the duration of exposure increases (rate of temperature change is decreased), so the insects are able to mount an

increasingly effective physiological response to the stress and CT_{min} declines. The mechanisms underlying rapid cold-hardening (RCH; Lee *et al.* 1987, 2006) are thought to lie at the heart of this response (Kelty & Lee 2001), although clearly too low a rate of change can prove deleterious at least as far as survival is concerned (Overgaard *et al.* 2006). What influence exposure duration has on CT_{max} is less clear, largely because very few studies have been undertaken in insects (Chown & Nicolson 2004). In one of the few (and in this case incidental) examinations of this trait, Kay & Whitford (1978) noted that during pilot trials using the honeypot ant *Myrmecocystus depilis* (Hymenoptera, Formicidae), an increase of heating rate from 1 to 2°C min^{-1} resulted in a decline in CT_{max} of approximately 2°C. Once again, *G. pallidipes* showed the opposite response, with CT_{max} increasing as exposure duration declined. These latter results are similar to those obtained in studies of fish, where a general trend exists for higher CT_{max} values at faster heating rates (e.g. Cocking 1959 and see discussions in Beitinger *et al.* 2000; Mora & Maya 2006).

Why these differences in the response of CTLs to exposure duration should exist between *G. pallidipes* and the other species studied is not entirely clear. In the case of CT_{max}, the effects of rate variation might not differ to any large extent among species, but the absence of comparative information makes it difficult to tell whether or not this is probable. In the case of CT_{min}, the differences may well be a reflection of the absence of RCH in a tropical species from a relatively stable thermal environment. In other words, prolonged exposure to stressful conditions simply increases mortality and does not elicit a hardening response. More generally, tropical species are thought to show less phenotypic plasticity than their temperate counterparts that encounter a wider range of temperatures (Janzen 1967; Ghalambor *et al.* 2006; Chown & Terblanche 2007). Although few studies have sought RCH in tropical species (Chown & Nicolson 2004), broader investigations of thermal tolerance have shown relatively little scope for short-term change in lower thermal tolerance in tropical insect species versus their temperate counterparts (e.g. Chen *et al.* 1990). In the case of *G. pallidipes*, adults are able to mount an acclimation response in CT_{min} over a week-long period (Terblanche *et al.* 2006), but it is clear that over a period of hours they are unable to do so (Terblanche *et al.*, in press). Whether or not this is a reflection of a poor acclimation response in tropical species is difficult to determine, especially given the ability of *G. pallidipes* to respond to acclimation at longer time scales. What may turn out to be more significant than temperature range *per se* is the timing of the temperature shift. Thus, *G. pallidipes* encounters low temperatures that are likely to limit activity only at night when it is least active (Brady 1988), but the need for rapid physiological response under these conditions is difficult to construe. Clearly, the significance of potential interactions between behavioural and physiological responses needs to be explored further (see also Huey *et al.* 2003). Nonetheless, it is apparent that in species which are capable of rapidly cold hardening, cooling rates will typically be positively correlated with CT_{min} assuming that these cooling rates are ecologically relevant over daily time scales. By contrast, in species that are unable to rapidly cold harden (low levels of phenotypic plasticity)

a negative correlation might be expected between CT_{min} and cooling rates. Presumably similar patterns would be characteristic of CT_{max}.

The final significant feature of this study is that at ecologically relevant rates of temperature change, CTLs provide a strong indication that physiological limits to activity might mediate the negative effects of environmental temperatures on tsetse population dynamics. Mark-recapture studies have demonstrated a strong relationship between temperature and mortality rate in *G. pallidipes* (Hargrove 2004) and other tsetse species (Hargrove 2001). However, previous investigations of acute physiological limits to activity, using a 0.25°C min^{-1} rate of temperature change and 16 or 35°C as start temperatures, revealed CTLs of 10–45°C (Terblanche *et al.* 2006). The CT_{max} value (CT_{max}: 44–45°C) lies well beyond the point at which temperature-dependent mortality occurs in the field (100% mortality: approx. 40°C; Hargrove 2004) suggesting that CT_{max} plays little part in mediating temperature effects on mortality. By contrast, at the ecologically relevant heating rate of 0.06°C min^{-1} the CT_{max} value of approximately 40°C is not only close to the absolute temperature limits experienced in the field in this study (figure 3, see also table S2 in the electronic supplementary material for macroclimatic data), but also closely matches the temperature at which mortality is 100%, determined from field mark-recapture studies in a similar south-central African location (Hargrove 2004). Therefore, these results provide one, but not necessarily the only, causal explanation for why temperature is such an excellent predictor of tsetse population dynamics (Hargrove 2004), abundance and distribution (Rogers & Robinson 2004). The marked sensitivity of tsetse to even moderate temperature variation makes prospects for a bottom-up, mechanistic model of the relationship between climate change and tsetse distribution and abundance (see Crozier & Dwyer (2006) for a similar approach) much better than had previously appeared to be the case.

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NOTICE OF CORRECTION

The acknowledgements are now presented in the correct form.

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