Impacts of climate change on tsetse (Diptera: Glossinidae): water balance physiology and mechanistic modelling

by
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Declaration

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Abstract

Climate change will alter both temperature and moisture availability in the future and therefore will likely affect vector borne disease prevalence. Organisms faced with changes in weather can respond in a variety of ways and this complicates any predictions and inferences for these organisms with climate change. Cause-and-effect links between climate change, insect vector responses, and changes in risk of disease transmission are poorly established for most vector borne diseases. Tsetse (Diptera, Glossinidae) are important vectors of trypanosome parasites posing a major threat to human health and socio-economic welfare in Africa. Water balance plays an important role in determining activity patterns, energy budgets, survival and population dynamics and, hence, geographic distribution and abundance of insects. *Glossina* species occupy a wide range of habitats in Africa and are notable for their desiccation resistance in xeric environments. Yet, whether or not the different species, subgroups or ecotype groups differ in susceptibility to changes in weather remain undetermined.

The first main focus of my thesis was to test the effects of climate change on water balance traits (water loss rate, body water content and body lipid content) of adult tsetse flies. Four species from xeric and mesic habitats were exposed to a range of temperature (20 – 30 °C) and relative humidity (0 – 99 %) combinations. Water loss rates were significantly affected by measurement treatments, while body water content, body lipid content and mass were less affected and less variable across treatment combinations. The results provide support for mass-independent inter- and intra-specific variation in water loss rate and survival times. Therefore, water balance responses to variation in temperature and relative humidity are complex in *Glossina*, and this response varies within and among species, sub-groups and ecotypes in terms of magnitude and the direction of effect change.

Secondly, I apply a mechanistic distribution model for *G. pallidipes* to predict potential population responses to climate change. I validate the mechanistic model (NicheMapper^{TM}) results spatially and temporally using two methods. Both tests of the model showed that NicheMapper's predicted resting metabolic rate has great potential to capture various aspects of population dynamics and biogeography in *G. pallidipes*. Furthermore, I simulate the effect of phenotypic plasticity under different climate change scenarios and solve for the basic reproductive number of the trypanosomiasis disease (R_0) under a future climate scenario.

This integrated thesis provides strong evidence for a general decrease in optimal habitat for *G. pallidipes* under future climate change scenarios. However, it also provides strong support for a

1.85 fold increase in R_0 based on changes in biting frequency as a result of higher predicted metabolic rates in the future. This might suggest that the reduction in optimal habitat could be outweighed by the increase in R_0. The results demonstrate that an understanding of the physiological mechanism(s) influencing vectors of disease with climate change can provide insight into forecasting variation in vector abundance and disease risk.

Opsomming

Die invloed van klimaatsverandering op die temperatuur en vog beskikbaarheid mag moontlik insek-oordraagbare siektes in the toekoms beïnvloed. Organismes wat verandering in klimaat ervaar kan op verskillende maniere reageer en daarom is voorspelling en afleidings van die reaksies op klimaatsverandering nie eenvoudig nie. Boonop is die verband tussen klimaatsverandering, insek reaksies en veranderinge in die oordragsrisiko van siektes onbekend vir die meeste insek-oordraagbare siektes. Tsetse (Diptera: Glossinidae) is belangrike draers van trypanosoom parasiete wat ‘n bedreiging inhou vir mensegesondheid en sosio-ekonomiese welsyn in Afrika. Waterbalans speel ‘n belangrike rol in die energiebondel samestelling, aktiwiteitspatrone, oorlewing en populasie dinamika van insekte en, dus, die geografiese voorkoms en verspreiding van insekte. *Glossina* spesies kom in ‘n verskeidenheid habitatte in Afrika voor en is bekend daarvoor dat hulle weerstand bied teen uitdroging in droë habitatte. Maar, die mate waartoe die verskillende subgroepe, ekotiepegroepe en spesies kwesbaar is vir klimaatsverandering, is steeds onbekend.

Die eerste hooffokus van my tesis was om die uitwerking van klimaatsverandering op waterbalans-relevante uitkomste (tempo van waterverlies, waterinhoud en vetinhoud) van volwasse tsetse vlieë te bestudeer. Vier spesies van droë en klam habitatte is aan verskillende kombinasies van temperatuur (20 – 30 °C) en relatiewe humiditeit (0 – 99 %) blootgestel. Die tempo van waterverlies is betekenisvol deur die verskillende toetskombinasies beïnvloed, terwyl die water-inhoud, vetinhoud en liggaamsmassa tot ‘n minder mate beïnvloed is en minder gevarieer het tussen die toetskombinasies. Die resultate toon bewyse vir gewigs-onafhanklike inter- en intraspesie variasie in waterverlies tempo’s en oorlewingstyd. Die waterbalans uitkomste op variasie in temperatuur en relatiewe humiditeit is dus ingewikkeld in *Glossina*, en dit varieer binne en tussen spesies, subgroepe en ekotiepe in terme van die graad en rigting van effek verandering.

Tweedens pas ek ‘n meganistiese verspreidingsmodel toe vir *G. pallidipes* om die moontlike populasiereaksies met klimaatsverandering te voorspel. Ek toets die antwoorde van die model (NicheMapper^{TM}) oor tyd en skaal op twee verskillende maniere. Beide toetse het aangedui dat die NicheMapper voorspelde rustende metaboliese tempo die verskillende aspekte van *G. pallidipes* populasie dinamika en biogeografie goed beskryf. Ek simuleer die uitkomste van die fenotipiese veranderbaarheid van *G. pallidipes* onder ‘n verskeidenheid klimaatsverandering-uitkomste, en los

‘n model van die basiese ommekeer van trypanosomiasis (R_0) op onder ‘n klimatsverandering situasie in die toekoms.

Hierdie geïntegreerde tesis toon sterk bewyse dat die optimale habitat van *G. pallidipes* verminder met klimaatsverandering. Dit toon egter ook bewyse vir ‘n 1.85 keer toename in R_0 gebasseer op ‘n verhoging in die frekwensie van bytgeleenthede weens die hoër voorspelde metaboliese tempo van die vlieë in die toekoms. Laasgenoemde stel voor dat die afname in optimale habitat moontlik deur ‘n toename in R_0 oorheers sal word. Die resultate demonstreer dat beter begrip van die fisiologiese meganisme(s) wat parasiet-draers beïnvloed verdere insig kan voorsien in die toekomstige voorspelling van draer teenwoordigheid en siekte waarskynlikheid.

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Chapter 1

General introduction to climate change, water balance physiology and distribution modelling

1.1 Climate change and disease risk

Climate systems are changing and are predicted to change further in spatially variable ways (Walther *et al*. 2002; Hulme 2005; Tebaldi *et al.* 2006; Cowie 2007; IPCC 2007; Tebaldi and Sanso 2009; Sanderson *et al*. 2011). Annual precipitation, high and low temperature extremes and the duration of dry periods seem to fluctuate almost everywhere over the globe under simulated CO_2 doubling scenarios (Cubasch *et al.* 1995; Zwiers and Kharin 1998; Sugiyama *et al*. 2010; reviewed in Walther *et al*. 2002). Global climate change predictions are furthermore often characterized by high levels of uncertainty and spatial heterogeneity (Giorgi *et al*. 2001; Walther *et al*. 2002; Stainforth *et al*. 2007; Ganguly *et al*. 2009; Sanderson *et al*. 2011). However, downscaled models for regional climate are more straightforward, making local climate predictions more reliable (New *et al*. 2006; Cowie 2007; Lumsden *et al*. 2009; Shongwe *et al*. 2009).

Climate change models emphasize that more frequent and intense global change-type droughts could occur in the future, and suggest that drought may be a common expectation for much of Africa (Tebaldi and Sanso 2009; Walther *et al*. 2002). In southern Africa heat wave probability is predicted to increase more than three-fold (Lyon 2009). Comparatively, most projected increases in mean temperatures by 2075 in sub-Saharan Africa show virtually no overlap with current average values (Burke *et al*. 2009). Hotter and wetter scenarios are predicted in eastern- and western Africa (Giorgi *et al*. 2001; IPCC 2007), but drier monsoon-related scenarios are predicted in eastern–Africa (Zwiers and Kharin 1998; Easterling *et al.* 2000; Giorgi *et al*. 2001; Fortain *et al*. 2010). In contrast, hotter and drier conditions are expected to increase in southern Africa, especially during the austral summer months (Giorgi *et al*. 2001; Lyon 2009).

Disease outbreak is amongst the most important factors contributing to the socio-economic vulnerability of many African countries (IPCC 1997). Many parts of Africa remain understudied in terms of vulnerability to climatic changes and in addition, the interaction with recurrent droughts, poor economic status, and high population growth are likely to compound negative effects of climate change in Africa (IPCC 2001).

Trypanosome parasites (*Trypanosoma* spp.) are transmitted by tsetse flies (Diptera: Glossinidae) during blood meal feeding and infection causes disease in the human or animal hosts (Leak 1999; Maudlin 2006). As a neglected tropical disease, Human African Trypanosomiasis (HAT) and Animal African Trypanosomiases (AAT) have the potential to expand in geographic range with

climate change (Githeko *et al.* 2000). Outbreaks of HAT are difficult to control and are known to re-emerge once control measures cease (Barrett *et al.* 2003). Furthermore, despite their relatively slow dispersal rate, alarming re–invasion estimates of tsetse after the termination of control efforts have been illustrated (Hargrove 2000). HAT is a daily threat to 37 countries in sub-Saharan Africa, 22 of which are among the least developed countries in the world. At the turn of the 20th century sleeping sickness appeared as a large epidemic, referred to as the Ugandan outbreak (Forde 1902; Hide 1999; Welburn *et al.* 2001). *Trypanosoma* spp. have caused several more HAT epidemics throughout the 20th century (MacKichan 1944; Fèvre *et al.* 2004) and lead to approximately 17 500 new HAT cases annually (Leak 1999; WHO 2006). Tsetse–transmitted *T. congolence, T. vivax* and *T. brucei* affect 45 – 50 million cattle in at least 8 million km^2 of sub–Saharan Africa. In total, AAT leads to approximately ~ US$5 billion in direct (meat and milk) and indirect (agricultural production) losses in sub-Saharan Africa (Swallow 1997).

The temperature-dependent developmental stage of the trypanosome parasite are free swimming until it reach the salivary glands of the fly where it attach to the microvilli and multiply as attached trypomastigotes. The trypanosome parasite is transferred to the human or animal host through the saliva during tsetse blood meal feeding (Leak 1999, Figure 1.1).

International collaboration and expenditure on various control mechanisms to reduce the incidence of HAT and AAT, has been critical for sub-Saharan Africa (Jordan 1986; Brightwell *et al.* 2001; McDermott & Coleman 2001; Robinson *et al.* 2002; Barrett *et al.* 2003). The impacts of climate change, human population growth and expected disease control activities on HAT risk for 2050 was explored on a continental scale by McDermott and Coleman (2001). They predicted a decreased HAT risk in West Africa, associated with drier years. In contrast, predictions by Patz *et al*. (2000) suggested that climate change could lead to increased habitat suitability for species from dry environments and potentially enlarge range expansions into temperate regions. Moreover, evolutionary changes in water balance physiology, altering species abundance in general, could also lead to vector population range expansions (see e.g. Kearney *et al.* 2009; Kleynhans and Terblanche 2009) and this aspect should enjoy future attention.

1.2 Insect responses to climate change

Species distributions, community composition and ecosystem structures, amongst others, are predicted to change globally as a consequence of climate change (Parmesan 1996; Walther *et al.*

2002; Parmesan and Yohe 2003; Root *et al*. 2003; Thomas *et al*. 2004; Musolin 2007). Ectotherms are especially vulnerable to changes in ambient temperature as their body temperature are closely linked to ambient temperature and consequently their physiological traits in many cases are related to seasonal or regional climates (e.g. Chown and Gaston 1999; Chown and Nicolson 2004; Terblanche *et al.* 2006). Furthermore temperature affects many rate processes and fitness traits throughout the insect life cycle (Kingsolver and Huey 2008). In addition the thermal aspect of the environment has a marked effect on insect phenology (i.e. timing of seasonal activities), thus life history and population growth rate.

Indeed, insects can compensate for variation in weather by i) migrating out of an area or through changes in timing of behaviour, ii) adjusting physiological tolerances to climatic stress within a single generation or iii) evolving enhanced tolerance to climate extremes over several generations (reviewed in Chown *et al*. 2011). Some insects, for example true bugs (Hemiptera), often respond to climate variability by changing their community structure, behaviour, physiology, voltinism, phenology, abundance or geographic range (reviewed in Walther *et al*. 2002; Parmesan and Yohe 2003; Musolin 2007). If none of these three options are possible in local populations, extinction is highly likely (Berteaux *et al.* 2004; Huey *et al.* 2009).

Insect distributions can be limited by host plant availability (Strathdee *et al.* 1993; Bale *et al.* 2002) or competition with other species (e.g. Duyck *et al.* 2004). In addition, some species might be geographically limited by predation or parasitism, for example, the Holly Leaf-miner *Phytomyza ilicis* (Diptera: Agromysidae) and the parasitoid *Chrysocharis gemma* (Klok *et al.* 2003). However, the distribution limits of many insect species are set by physiological constraints such as their developmental temperature requirements (MacLean 1983), survival or tolerance of thermal extremes (Addo-Bediako *et al.* 2000), or water requirements (Addo-Bediako *et al.* 2001; Hoffmann *et al.* 2003a). For example, it is clear that physiological tolerance plays an important role in beetle (Coleoptera) geographic distribution (Calosi *et al.* 2010).

The consequence of physiological tolerance, habitat requirement, life history and biotic interaction have been studied in a range of insect taxa (Addo-Bediako *et al.* 2000; Chown and Nicolson 2004; Helmuth *et al.* 2005; Dillon *et al.* 2007; Deutsch *et al.* 2008; Calosi *et al.* 2010). In addition, the direct and indirect effects of climate change, in particular temperature and water availability, on performance, physiology and ecology of insects have been well studied (Parmesan and Yohe 2003; Chown and Nicolson 2004; Hulme 2005; Frazier *et al*. 2006; Chown and Terblanche 2007). An

example of indirect effects is the impact from changes in the spatiotemporal availability of natural resources, because of less rainfall, leading to a lower net primary productivity (Dodson *et al.*, 2000).

Deutsch *et al.* (2008) investigated the fitness effects of climate change around the optimal temperature (T_{opt}) of terrestrial insects relative to their latitudinal position. Their study concluded habitat temperatures (T_{hab}) above T_{opt} nearing the insects' critical thermal maximum (CT_{max}: the upper limit of organism performance) result in decreased relative fitness of organisms (Figure 1.2). By contrast, insects living at relatively low T_{hab}, such as regions nearer the poles, are theoretically likely to experience increased fitness with warming since any increase in T_{hab} will place them closer to their T_{opt}. Also, insects living close to T_{opt} will perform worse if T_{hab} were to increase in their environment by decreasing the amount of buffer temperature between T_{hab} and CT_{max}. However, insects living in the tropics have a lower "thermal safety margin" (the difference between T_{opt} and T_{hab}) indicating even a small increase in temperature might decrease their fitness (Deutsch *et al.* 2008). Thus, Deutsch *et al*. (2008) concluded that tropical insects are more vulnerable to climate change–related warming. By contrast higher latitude species are likely to experience elevated fitness.

Temperature affects physiological and life-history traits of insects often leading to altitudinal and latitudinal range changes (Konvicka *et al.* 2003; Wilson *et al*. 2005; Chen *et al.* 2009; Calosi *et al.* 2010). The thermal aspect of the environment has a marked effect on the phenology (i.e. timing of seasonal activities), thus life history as a function of growth rates. For example, phenological changes in butterflies in response to northward or upward shifted 'climate envelopes' consequently altering species distributions (Walther *et al.* 2002). Pole ward shifts in insect ranges can be as a response to increased temperature (Parmesan *et al.* 1999; Willig *et al.* 2003), for example, Japan, where the southern green stink bug (*Nezara viridula*), has shifted its range northwards by 70 km over the past 40 years in response to temperature increases of 1-2 °C in minimum temperatures (Musolin 2007; Yukawa *et al*. 2009; and see Parmesan 1996). Rapid long-term latitudinal and altitudinal range shifts of the winter pine processionary moth (*Thaumetopoea pityocampa*) is in response to warmer winters conducing an increased flight activity in newly emerged females (Battisti *et al.* 2006). This phenomenon highlights the importance of extreme events (and altered frequency in extremes) in the range formation of phytophagous insects. Theoretically, more extreme events might decrease insect performance by increasing the number of events above CT_{max} leading to a decrease in fitness (Figure 1.2).

The degree of variation between physiological traits is not equal within and between species and populations (Chown 2001, 2002). Variation in physiological traits can extend to larger geographic scales (e.g. continental or global) (Addo-Bediako *et al.* 2001; Chown 2002; Hoffmann *et al.* 2003b; Marron *et al.* 2003). Among inter–specific and inter-population differences are cold hardiness (Chen *et al.* 1990), desiccation resistance or tolerance (Edney 1977; Hadley 1994; Addo-Bediako *et al.* 2001), upper lethal temperature limits (Kimura *et al.* 1994), metabolic rates (Schultz *et al.* 1992), cuticular hydrocarbons (Gibbs *et al.* 1991) and intra–individualistic variation in discontinuous gas exchange cycles (Chown 2002). Generally, water loss rates are higher when higher metabolic rates are achieved (Edney 1977; Hadley 1994; Harrison and Roberts 2000), although, whether metabolic rate is modulated to reduce respiratory water loss remains controversial (Edney 1977; Chown 2002).

Smaller insects have a greater surface area to volume ratio increasing heat and water loss by convection or transpiration (Stone and Willmer 1989; Hadley 1994). Furthermore, three empirical patterns exist in the association between fitness, body size and body temperatures of insects. First, larger body sizes are frequently associated with greater fitness within populations; the 'bigger is better' pattern of fitness in relation to phenotypic variation (Kingsolver and Huey 2008). In tsetse for example, larger individuals prove more desiccation tolerant than smaller flies (Bursell 1959; Spicer and Gaston 1999). Second, development at higher temperatures usually leads to small adult size and is known as the 'hotter is smaller' pattern, indicating phenotypic plasticity of a genotype. Finally, higher optimal temperature usually leads to greater maximal performance at that temperature, explaining an evolved variation in reaction norm among genotypes or between species; the 'hotter is better' pattern (Kingsolver and Huey 2008). Among these survival mechanisms, physiological responses to variation in temperature (Angilletta 2009) and moisture availability has proved essential in insect survival (Gibbs *et al.* 1997; Le Lagadec *et al.* 1998; Hoffmann *et al.* 2001; Marron *et al.* 2003; Terblanche *et al.* 2005; Jurenka *et al.* 2007).

It is clear that insect water balance physiology is related to habitat moisture availability. Variation in water balance physiology is such that species or populations from xeric environments are either more desiccation resistant or more desiccation tolerant than those from wet (mesic) environments, suggesting evolutionary adaptation as an underlying mechanism (Le Lagadec *et al.* 1998; Gibbs and Matzkin 2001). Insects must strike a balance between water lost and water gained to ensure that their water reserves are not depleted to lethal levels; insects must therefore maintain their water contents within their critical water limits (Bursell 1964). Insects employ three main physiological

mechanisms to survive dehydrating conditions (Bursell 1964; Hadley 1994; Gibbs *et al.* 1997; Danks 2000; Marron *et al.* 2003; reviewed in Chown and Nicolson 2004). Briefly, these are to i) reduce water loss rates, ii) increase body water content or iii) increase desiccation survival time. These mechanisms are detailed in Chapter 2.

Moisture availability is a critical factor determining insect distribution, reproduction or development (Hadley 1994; Tauber *et al.* 1998; Addo-Bediako *et al.* 2001; Hawkins *et al.* 2003; Chown and Nicolson 2004). However, the primary physiological means by which insects respond to their environments, along with the contribution of water balance traits to distribution and abundance of the species deserves more attention (Feder 1987; Spicer and Gaston 1999). Furthermore, basal (not plastic) physiological water balance response traits have not been thoroughly conceived in the context of insect disease vectors (Hulme 1996; Martens *et al.* 1999; Terblanche *et al.* 2006) and are clearly important for predicting likely impacts of climate change on disease distribution and risk.

Acclimation is broadly considered as phenotypic alteration in physiology to an environmental change within the laboratory (Huey *et al.* 1999) while acclimatization by contrast is a phenotypic alteration in physiology to an environmental change in the natural environment (Schmidt-Nielsen 1997). Both are regarded as plastic, reversible responses (Chown and Terblanche 2007). The ability to anticipate a stressful condition is thought to provide fitness advantages (Ghalambor *et al.* 2007) although this is debated from a number of perspectives, mostly related to the beneficial acclimation hypothesis (Chown and Terblanche 2007). On the other hand, genetic adaptations taking place across generations which result from climate variation are less controversial, though only a few examples have been empirically found to date (e.g. Ayres and Scriber 1994; Balanyá *et al.* 2006). Phenotypic plasticity may be a physiological response that occurs via evolutionary change (West-Eberhard 2003; Ghalambor *et al.* 2007). Huey and Berrigan (1996) provided a useful framework for understanding phenotypic plasticity in the context of evolutionary physiology. The ability to acclimate to an environment might increase survival over short-term extreme changes, for example rapid cold hardening in *Drosophila* (Kelty and Lee 1999). The hypothesis for acclimation would normally indicate that individuals exposed to a pre-treatment would consequently perform better in the conditions under which it was acclimated, phrased as ‘beneficial acclimation’ (Huey and Berrigan 1996; Deere and Chown 2006; Terblanche and Kleynhans 2009). However, acclimation may not necessarily result in a fitness benefit (e.g. Leroi *et al.* 1994). The conditions experienced may therefore result in a performance decline if the previous exposure is somehow detrimental, phrased as ‘deleterious acclimation’ (Loeschcke and Hoffmann 2002). Moreover, some animals

may simply be at an advantage under selected extreme conditions due to evolutionary adaptation and secondary constraint(s) and do poorly under all other conditions. Alternatively, animals may have no response ('no phenotypic plasticity') to a particular range of conditions. The latter also represents a null model for phenotypic plasticity (see discussions in Seebacher 2005; Angilletta *et al.* 2006; Deere and Chown 2006).

1.3 Tsetse: a model organism

As a disease vector, tsetse contributes significantly to the socio-economic burden as well as human and animal welfare of Africa (Kristjanson *et al.* 1999; Aksoy 2000; Allsopp 2001; Robinson *et al.* 2002; Maudlin 2006). Tsetse flies have a unique form of reproduction. They show adenotrophic viviparity in which adult females carry their young *in utero* for the duration of embryonic and larval development. The larval-instars are constantly supplied with nutrients in the form of a 'milk' substance (Leak 1999). Adult tsetse feed solely on blood (i.e. haematophagous), which is nutritionally rich enough to support this reproductive strategy. The tsetse life cycle (Figure 1.3) is extremely temperature dependent. At 24 °C, an adult female fly produces an egg every 9 – 10 days (Leak 1999). The egg hatches and the 1st 2nd and 3rd instar larva is carried in the female uterus. The 3rd instar is deposited into light sandy soil where it pupates and emerges as an adult after ~ 30 days, at 24 ºC (Figure 1.3).

Based on sensitivity and acclimation responses to ambient environmental moisture, vegetation and habitat the ~ 33 species and subspecies of tsetse can be categorized into three main ecotype groups; those adapted to the forest (*fusca* group), forest and riverine habitats (*palpalis* group) and savannah (*morsitans* group) (Rogers 2000; Leak 1999). Both temperature and moisture are important predictors of geographic distribution and abundance, although the relative importance of each abiotic factor probably differs between the pupal and adult life stages (Rogers and Randolph 1986; Rogers and Robinson 2004; Hargrove 2004; Kleynhans and Terblanche 2009). Early physiological investigations suggested an important role for water balance in tsetse puparia (Buxton and Lewis 1934; Bursell 1958) and the sensitivity of adult flies to temperature (Hargrove 2004). Temperature has major effects on birth-, development-rate and mortality with a strong non-linear relation between adult mortality and ambient temperature (Hargrove 2001, 2004). Recent work on *G. pallidipes* has shown that adult flies respond to acclimation temperature by increasing water loss rate (WLR) and body water content (BWC) almost two-fold under cool temperatures and decreasing WLR and BWC when maintained at higher temperatures (Terblanche *et al.* 2006).

Moreover, recent inter-specific investigations revealed that WLR is the most likely trait of water balance that has responded to habitat moisture availability in puparia (Kleynhans and Terblanche 2009). By contrast survival time, BWC and body size were all shown to be less important in relation to several climatic variables. In addition, Kleynhans and Terblanche (2009) showed that across species WLR is significantly positively correlated to precipitation in puparia even after phylogenetic–adjustment.

The abiotic variables affected by climate change such as land surface temperature and saturation deficit, show strong relations to tsetse distribution, abundance, physiology and life-history (Bursell 1957; Rogers and Randolph 1991; Rogers 2000; Hargrove 2004; Rogers and Robinson 2004). Current predictions of tsetse distribution however are severely compromised by the general lack of information on the susceptibility of different tsetse species to different climate change scenarios. In addition, whether significant ecotype (mesic vs. xeric) variation exists among species is not well established.

1.4 Mechanistic distribution modelling

The principles of biophysical ecology have been used to link physiology, behaviour and ecology of species to spatial environmental data in order to better understand and predict key ecological processes affecting distribution and abundance in different habitats (Gilman *et al.* 2006; Kearney *et al.* 2009; Kearney and Porter 2009). Modelling, as opposed to observational dataset compilation, might be a more effective way of predicting changes in disease risk, especially when focussing on a wide area with multiple ecological interactions (Rogers 2000). The need for physiological integration with biophysical modelling has been strongly emphasised in the past (Helmuth *et al.* 2005) and it can facilitate better understanding of the distribution of disease vectors and changes in transmission risks (Kovats *et al*. 2001) with climate change.

Correlative and mechanistic models are capable of explaining much of the variation in species distribution (Beerling *et al.* 1995; Porter and Mitchell 2006; Pearson and Dawson 2003; Thomas *et al*. 2004). From a physiological perspective the correlative modelling approach has been questioned for three main reasons (Davis and Shaw 2001; Helmuth *et al*. 2005; Hulme 2005). First, the spatial variation in population responses to the environment is often not considered (Davis and Shaw 2001). Secondly, the effect of phenotypic plasticity (rapid alterations to phenotypes in the form of e.g. developmental plasticity or seasonal variation) is typically ignored (Helmuth *et al*. 2005).

Lastly, the potential outcome of multivariate climatic constraints is generally not considered (Rogers and Randolph 2000). Hulme (2005) stresses the fact that correlative models, although providing a good indication of potential abundance under climate change impacts, may not be reliable as planning tools or even in the identification of knowledge gaps. Thus far, correlative bioclimatic envelope models have been used to statistically link spatial data to species distribution records, assuming that current distribution is limited only by climate (see Table 1 in Kearney and Porter 2009 for a comparison of methods in spatial modelling). The correlative approach furthermore requires prior knowledge of a species' distribution. In comparison, a mechanistic (e.g. steady–state energy balance model) links functional traits of the organism to the environment (Kearney 2006), thereby predicting a species fundamental niche as opposed to the realised niche (Kearney and Porter 2009). Although mechanistic models are data intense and time consuming, no prior data of current species distribution is essential or incorporated in any way (Kearney and Porter 2009).

1.5 Aims of this thesis

There are two broad aims of this thesis. First, I undertake laboratory simulations of several climate change scenarios on a range of *Glossina* species in the adult life stage to better comprehend species-level water balance responses. Second, I incorporate water balance and other physiological, behavioural and morphological responses of *G. pallidipes* into a mechanistic distribution model of geographic range.

Specifically, in Chapter 2, I determine the effect of changes in humidity and temperature on basal WLR of *G. brevipalpis*, *G. morsitans centralis*, *G. pallidipes* and *G. palpalis gambiensis*. Moreover, I investigate the influence of the interaction between changes in humidity and temperature on water balance traits under ecologically relevant conditions. In Chapter 3, using a bottom-up, steady-state energy balance modelling approach, I investigate the likely impacts of climate change on the future distribution of *G. pallidipes* and potential impacts on the basal reproductive number (R_0) of the trypanosome disease. This integrated Chapter uses empirical physiological data to "ground truth" the results from the mechanistic model in a spatially explicit manner. The results of this thesis will provide general insights into processes determining population dynamics of several *Glossina* species under climate change scenarios, and should inform management and control practise for disease intervention in the future.

1.6 References

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1.7 Figures

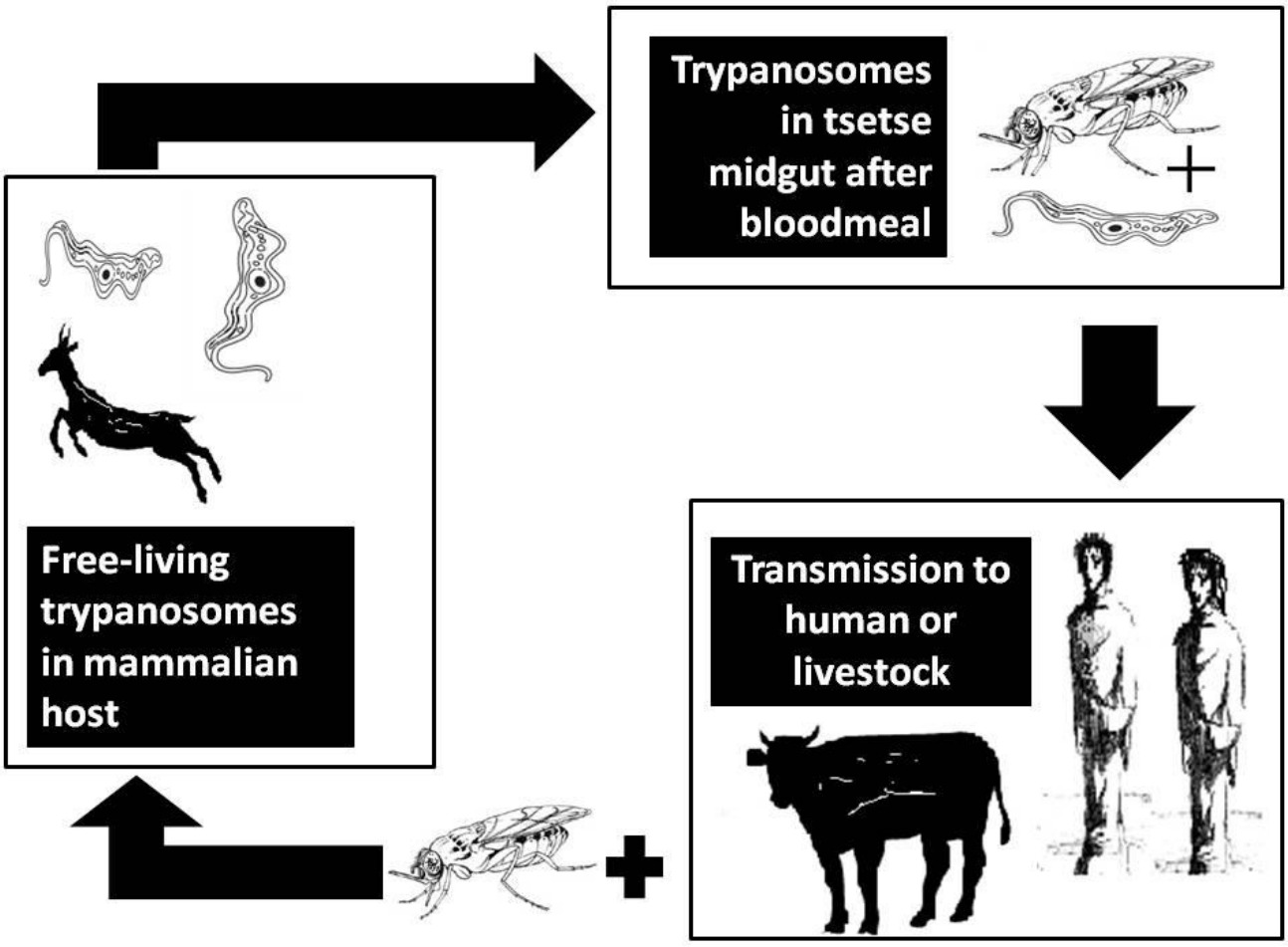

Figure 1.1. Simplified trypanosome life cycle showing the trypanosome free-living in the mammal host from where it is taken up into the tsetse during blood meal feeding. The trypanosomes are transported into the human or life stock host through the saliva during blood meal feeding.

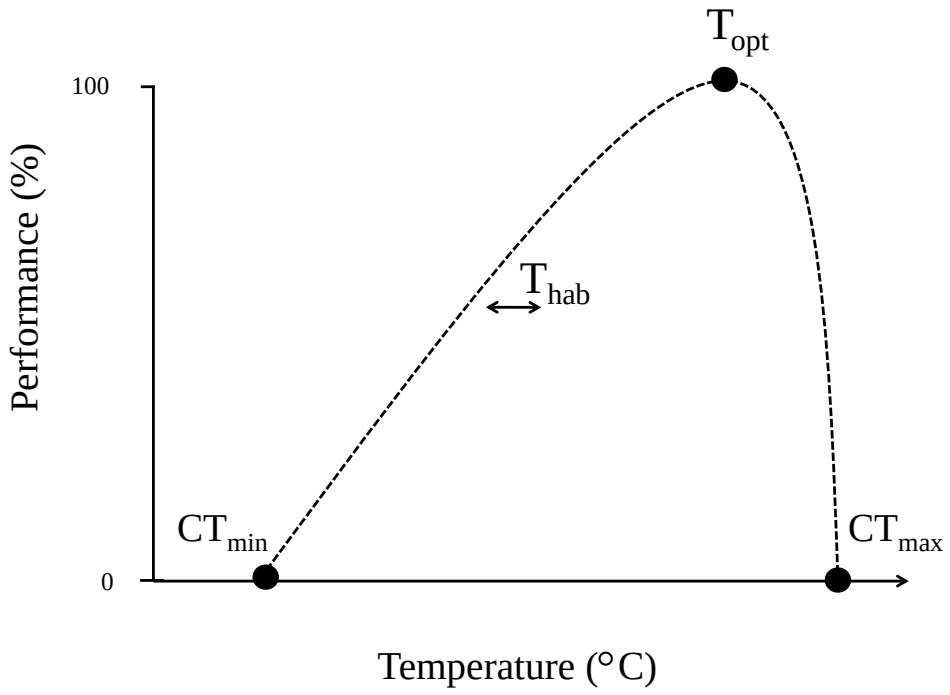

Figure 1.2. A generalized ectotherm thermal performance curve. Relative performance decreases from an optimum temperature (T_{opt}), where performance is maximised, to a lower limit, the Critical Thermal Minimum (CT_{min}). At temperatures greater than T_{opt} there is a more rapid decline in performance to Critical Thermal Maximum (CT_{max}). Changes in the thermal aspect of climate are presented as changes in habitat temperature (T_{hab}) along the x-axis.

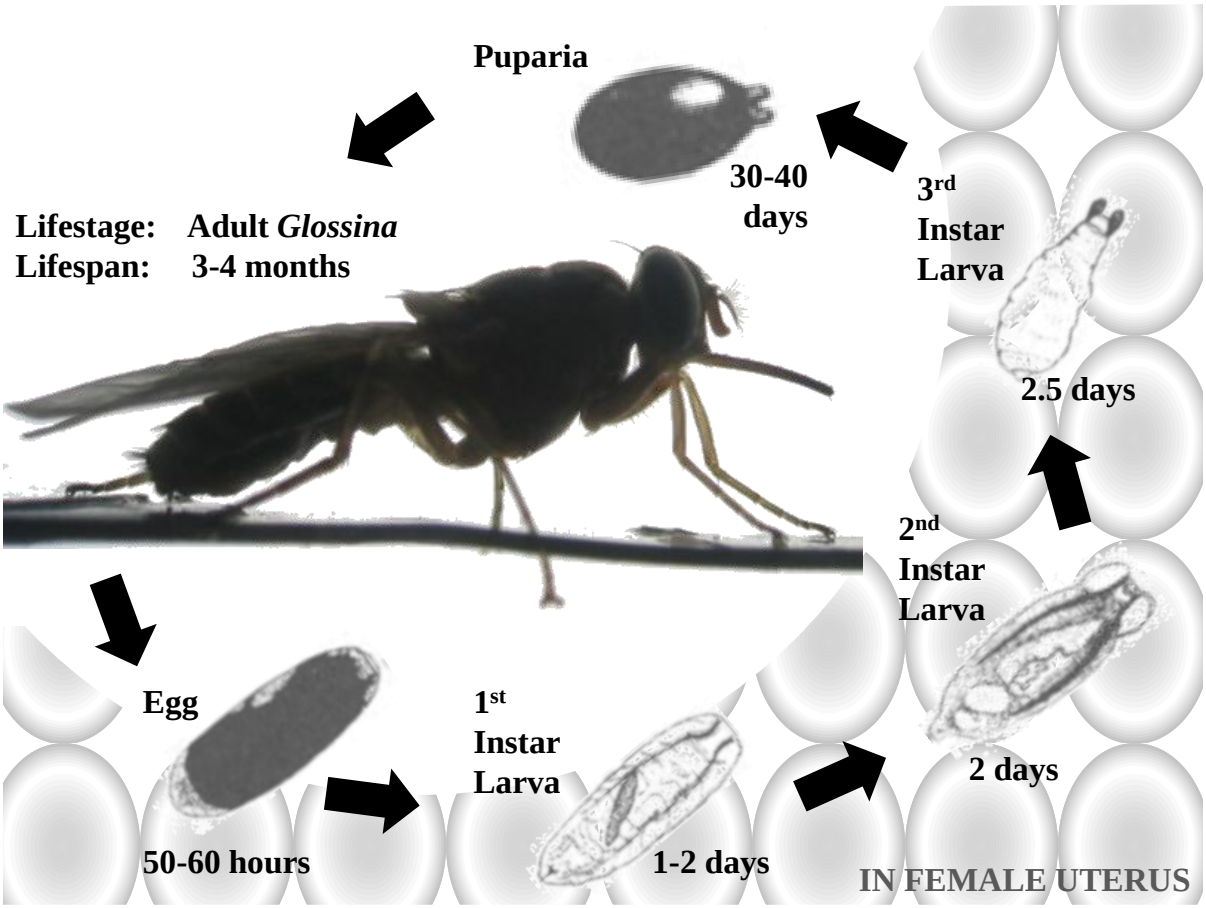

Figure 1.3. A simplified tsetse life cycle, at 24 ºC. A female adult lifespan lasts 3 – 4 months. The adult fly produces an egg every 9 – 10 days. The egg hatches to the 1st 2nd and 3rd instar larva in the female uterus. The 3rd instar is deposited into light sandy soil where it pupates and emerges as an adult after approximately 30 days.

Chapter 2

Complex interactions between temperature and relative humidity on water balance of adult tsetse (Glossinidae, Diptera): implications for climate change

This chapter has been published in *Frontiers in Physiology*, Vol. 2, Entitled: "*Complex interactions between temperature and relative humidity on water balance of adult tsetse (Glossinidae: Diptera): implications for climate change*" by E. Kleynhans and J. S. Terblanche (October 2011)

2.1 Introduction

Water balance plays a critical role in determining insect fitness, geographic range and in the case of disease vectors, disease dynamics in the face of climate change (Danks 2000; Chown and Nicolson 2004; Chown *et al*. 2011). Climate change is typically interpreted in terms of variation in mean ambient temperature. However, it is increasingly apparent that regional variation in temperature and humidity are likely to be significant in understanding the impacts on insect populations (Easterling *et al.* 2000; Tebaldi and Sanso 2009; Benoit and Denlinger 2010). Terrestrial insects face considerable challenges in balancing water gained and lost largely due to their high surface area to volume ratio (Hadley 1994).

Four main physiological mechanisms provide resistance or tolerance to dehydration in xeric (dry) environments. First, cuticular hydrocarbons form a barrier of hydrophobic bonds that increase desiccation resistance (Edney 1977; Jurenka *et al.* 2007; Bazinet *et al*. 2010). Insects can thus exploit this low cuticular permeability by reducing metabolic rate (e.g. through quiescence, alteration of gas exchange patterns or water conservation in the sub-elytral chamber) (e.g. Terblanche *et al*. 2010; reviewed in Chown *et al*. 2011; but see also discussion in Woods and Smith 2010). This is further maximized by behavioural avoidance of desiccating conditions, such as limiting activity to a time of day when desiccation is least likely (e.g. Kessler and Guerin 2008). However, for each species an environment-specific transition temperature may exist beyond which cuticular lipids melt, resulting in rapidly increased cuticular permeability and ultimately accelerated water loss (Benoit 2010; Gibbs 2011). Second, insects increase the quantity of water available in their bodies to resist dehydration by i) ingestion of free standing water or nectar, ii) absorbing water from the ambient environment, iii) the conversion of metabolic water or iv) increasing body size over evolutionary time–scales (reviewed in Hadley 1994; Benoit and Denlinger 2010; Chown *et al*. 2011; Yoder *et al*. 2011). Higher carbohydrate content can increase bound water reserves and has been associated with desiccation resistance in some insects (see e.g. Gibbs *et al*. 1997; Marron *et al*. 2003). The complementary provision of water through metabolism may include altering metabolic fuels, e.g. from lipids to carbohydrates, to yield higher metabolic water or decreased mass-specific metabolic rate to limit respiratory water loss (e.g. Marron *et al*. 2003; Terblanche *et al*. 2010, but see discussions in Woods and Smith 2010). Third, insects enhance desiccation tolerance by production of cellular compounds that maintain cell function under dehydration stress (e.g. heat shock proteins, Lopez-Martinez *et al*. 2009; Benoit and Denlinger 2010). Finally, rapid, intra-individual phenotypic adjustments are a wide-spread physiological mechanism employed to

enhance desiccation tolerance or resistance under dehydrating conditions (e.g. Woods and Harrison 2001; Hoffmann *et al*. 2003; Terblanche and Kleynhans 2009; Bazinet *et al*. 2010; Terblanche *et al.* 2010; reviewed in e.g. Chown *et al*. 2011).

Generally, insects from xeric environments are either more desiccation resistant or desiccation tolerant than insects from mesic environments (Edney 1967; Hadley 1994; Le Lagadec *et al.* 1998; Gibbs and Matzkin 2001; Hoffmann *et al.* 2003; Matzkin *et al*. 2009). However, insects from stable mesic environments might experience lower dehydration stress, resulting in an increased water loss rate (WLR) when exposed to xeric conditions (e.g. Yoder *et al*. 2011). Water balance traits can drive evolutionary processes, especially in insects associated with xeric environments, resulting in physiological diversification among individuals, populations or species (Gibbs *et al.* 1997; Hoffmann and Harshman 1999; Woods and Harrison 2001; Chown and Terblanche 2007; Gefen and Gibbs 2009; Kleynhans and Terblanche 2009; Matzkin *et al*. 2009; Simard *et al*. 2009; Benoit and Denlinger 2010). Water balance trait variation can also extend to larger geographic scales (e.g. continental or global) (Addo-Bediako *et al.* 2001; Chown 2002; Hoffmann *et al.* 2003; Marron *et al.* 2003; Kleynhans and Terblanche 2009).

Tsetse (genus *Glossina*) consists of 22 species which occupy a wide range of habitats in sub-Saharan Africa. Given their critical importance as a major vector of tropical human and animal disease (trypanosomiases), understanding the physiological drivers of tsetse water balance is crucial for predicting disease dynamics under climate change scenarios. Tsetse are classified into three major subgroups (ecotypes) on the basis of morphological differences in the structure of the male genitalia (Leak 1999; Rogers and Randolph 1986). The three subgroups can also be differentiated by variation in physiology, behaviour and ecological characteristics related to sensitivity to moisture availability (Bursell 1960). Tsetse population dynamics is affected by temperature and relative humidity variation in the field (Hargrove 2004; Rogers and Robinson 2004; Terblanche *et al*. 2008). Marked variation in daily and seasonal temperature and relative humidity has been measured in tsetse-occupied habitats (e.g. Hargrove 2001, 2004; Jurenka *et al*. 2007). Tsetse can experience mean temperatures between 20 – 30 °C within a single species' geographic range (e.g. Terblanche *et al*. 2006), with 1.2 to 5.6 °C variation occurring seasonally (Jurenka *et al*. 2007) and varying by up to 20 °C daily within a single location (Terblanche *et al*. 2008). Saturation deficits between 1 – 36 mB have been observed (e.g. Fig. 7.3 in Hargrove 2004) while relative humidity can also vary substantially at either low or high mean temperatures (Jurenka *et al*. 2007). Thus, a wide range of saturation deficits can be experienced over the average flies' lifetime, across a species' geographic range, or among species. Early studies concluded that adult water balance was not an

important factor in the occupancy of xeric environments (Bursell 1959), although water balance physiology is clearly important to the pupal stage (Bursell 1958; Kleynhans and Terblanche 2009). This contrasts strongly with much of the modern water balance literature (e.g. Terblanche *et al*. 2006; reviewed in Chown *et al.* 2011) and this conclusion may have been reached prematurely without due consideration of plastic physiological responses and the range of potential conditions that affect water loss rates (see also Terblanche and Kleynhans 2009). Moreover, it is likely that the entire *Glossina* genus could be adapted to xeric environments thereby masking interspecific variation measured under a single set of conditions. Certainly much of the early comparative work on tsetse water balance physiology focused on inter-specific comparisons made from measurements undertaken under a single or a few controlled relative humidity conditions at a single temperature (e.g. Bursell 1959). Adult tsetse feed solely on blood which leads to them facing a unique set of challenges in suppressing dehydration in the off–host environment and maintaining a positive water balance while actively seeking a blood meal, mates or producing offspring (see discussions in Benoit and Denlinger 2010).

Here I measure aspects of water balance physiology in adults of four ecologically diverse species of tsetse under a range of environmentally-relevant combinations of temperature and relative humidity. I aim to answer three main questions regarding water balance physiology of tsetse. (1) How does the water balance physiology of a species respond to changes in temperature and relative humidity? (2) Does the interaction between temperature and relative humidity elicit a consistent physiological response among individuals and among species in terms of magnitude and direction of change? This can be rephrased to ask if water balance responses to temperature and relative humidity are transferrable between species or ecotypes. (3) What conclusions can be drawn regarding the interplay of temperature and relative humidity on intra-specific survival times? If species, traits, or ecotypes respond similarly, then this would allow predicted climate change impacts to be generalized across tsetse species, or one could use data from a single species to forecast environmental effects across species. However, in combination, temperature and relative humidity may behave synergistically eliciting complex physiological responses (Gibbs *et al*. 1997; Gibbs and Matzkin 2001; Chown *et al*. 2011). If, on the other hand, tsetse species differ in their water balance physiology and responses to varying moisture and temperature, then forecasting climate change responses for the range of species will be considerably more complex.

2.2 Materials and methods

2.2.1 Study organisms

I measured traits of water balance physiology in adults of *Glossina brevipalpis* Newstead (mesic), *G. morsitans centralis* Westwood (xeric), *Glossina pallidipes* Austen (xeric) and *G. palpalis gambiensis* Vanderplank (mesic) (Diptera, Glossinidae). Pupae were received from laboratory colonies maintained at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, International Atomic Energy Agency, Vienna, Austria, or from a laboratory colony maintained at the Onderstepoort Veterinary Institute, Pretoria, South Africa (*G. brevipalpis*). The pupae (22–25 days old) were received via air cargo in well insulated non–airtight containers kept under controlled temperature conditions during transportation (typically < 2 days of transport, temperature range 18 – 24 °C controlled by two phase changing agents). Species were chosen to represent the three main tsetse subgroups: *fusca* (*G. brevipalpis*), *palpalis* (*G. p. gambiensis*) and *morsitans* (*G. m. centralis* and *G. pallidipes*). The *fusca* group resides in forests (mesic) and is the most ancestral of the three groups. The *palpalis* group is also associated with mesic habitats and the *morsitans* group with xeric savannahs.

Upon arrival, puparia were kept in a dark climate chamber (Labcon, South Africa) at 25 ºC and 76 % relative humidity until emergence. Once the flies emerged, they were transferred into mesh cages and fed three times, every second day, using defibrinated, infection free bovine blood on a membrane–tray system (similar to methods described previously in Terblanche *et al*. 2004, 2005). After the third blood meal, adults were kept under controlled conditions (optimal, standard rearing conditions: 25 °C, 76 % relative humidity) for a further 24 hours before experiments began. Water lost by excretion was negligible (see Bursell 1960). Each treatment group (n = 30) had emerged on the same day to standardise age in each treatment. Treatments were conducted in random order.

2.2.2 Experimental treatments

Water balance traits were determined at three temperatures: 21, 25, or 29 ± 1.0 °C; and three relative humidities: < 5 (0 %), 73 – 77 (76 %), or > 95 (99 %). Treatments and corresponding saturation deficits include the following temperature (in °C), relative humidity (in %) combinations: 21,99 (CW); 25,99 (IW); 29,99 (HW); 21,76 (CI); 25,76 (II); 29,76 (HI); 21,0 (CD); 25,0 (ID) and 29,0 (HD) where C = cold, W = wet, I = intermediate, H = hot and D = dry. Saturation deficit is an index of the evaporative capability of the air calculated as:

$$SD = SVP - AVP \quad \text{(Equation 1)}$$

where SD is the saturation deficit (in mB), SVP is the saturated vapor pressure (in mB) and AVP is the actual vapour pressure (in mB). The saturation vapour pressure (Equation 1) is a function of temperature (in °C) and the actual vapour pressure is a function of relative humidity (in %). Temperature and relative humidity were recorded during all experiments using Thermocron iButtons (DS1402D–DR8, Dallas Semiconductors; ± 0.5 °C and 1 % accuracy at a 10 minute sampling rate).

Individual adult flies were placed separately into numbered 50 ml pill vials with ventilation holes to achieve complete desiccation. Pill vials were randomly placed within airtight plastic 500 ml desiccation jars. A 100 ml vial inside the jar contained silica gel (0 %), saturated sodium chloride (NaCl) (76 %) or Millipore filtered, doubly-distilled water (99 %) to control for relative humidity (see methods in Terblanche and Kleynhans 2009). Care was taken to ensure that all treatment groups were handled for the same duration during transfer from the climate chamber to the vials and spent a similar amount of time outside the desiccating conditions during weighing (~ 20 – 30 minutes per group). Gender ratios were not strictly controlled but accounting for size in statistical analyses eliminated sex effects on water balance physiology (see e.g. Terblanche *et al*. 2006).

2.2.3 Physiological water balance traits

Treatments were performed for 24 hours in the dark to suppress activity. Bursell (1957a) concluded that relative humidity and hunger state affected tsetse activity. However, Terblanche *et al*. (2006) suggest that differences in activity levels do not account for water loss rate variation among populations or temperature treatments. Activity might affect the measured WLR estimates in response to stressful conditions (see Gibbs *et al*. 2003) or acclimation (see Hadley 1994; Terblanche *et al*. 2006). However, during experimental treatments weighing the adult flies did not show any significant differences in observed activity levels between treatments or species (and see Terblanche *et al*. 2006). I calculated WLR, body water content (BWC) and body lipid content (BLC) gravimetrically. Weighing took place for each fly individually on an electronic microbalance to 0.1 mg (Mettler Toledo AX504). Body mass was recorded at the start of the experiment (Mb_i) and after exposure to the controlled temperature and relative humidity treatments (Mb_a). WLR was calculated by subtracting Mb_a from Mb_i and dividing the difference by the treatment time (in h). Tsetse were dried to a constant mass at 50–60 °C for approximately 72 h and re-weighed to determine dry mass (DM). The BWC was calculated as the difference between Mb_i and DM. Finally, body lipids were

extracted using three chloroform: methanol (1: 1 ratio) washes, once per day, baking dry at 50–60 °C for approximately 72 h after the final wash and subsequent weighing. BLC was the difference between the mass after lipid extraction and DM.

2.2.4 Survival time estimation

Survival time was estimated given that the conversion rate of lipids to metabolic water is 1.08 and the critical lipid content is 4.2 % of DM (Bursell 1959). Furthermore, survival time is a function of all available water reserves and the rate at which water is lost, calculated as:

$$S = ((1.08 \times (BLC - (0.042 \times DM)) + (BWC - \partial)) \div WLR \quad \text{(Equation 2)}$$

where S is survival time (in hours), BLC is body lipid content (in mg), DM is dry mass, BWC is the body water content (in mg), ∂ is a constant critical body water content and WLR is water loss rate (in mg/h). The critical body water content is given by Bursell (1959) as 0.0657 mg for *G. brevipalpis*, 0.0645 mg for *G. m. centralis*, 0.0661 mg for *G. pallidipes* and 0.0656 mg for *G. p. gambiensis*. To our knowledge, tsetse have an exclusively proline-driven metabolism and their capacity for carbohydrate metabolism is limited (Bursell *et al*. 1974; Bursell 1977; Norden and Paterson 1969). Therefore, it is unlikely that tsetse rely on carbohydrate-bound water to increase body water content, and it is also unlikely that changes in metabolic pathways (e.g. from carbohydrates to lipids) are used to increase net water content and thereby increase survival times. Consequently, these latter factors were omitted from the estimation of survival time.

2.2.5 Statistical analysis

The response of WLR, BWC and BLC in response to variation in temperature, relative humidity or the interaction of temperature and relative humidity was analysed using type 3 generalized linear models (GLZ) (Proc Genmod; SAS Enterprise Guide version 4.1, SAS Institute, Inc. Cary, North Carolina) with normal errors and an identity link function. Survival time was analysed in a similar manner to investigate the survival time in response to variation in species, temperature and relative humidity. In all GLZ analyses, deviance was scaled to 1 to correct for overdispersion. The four tsetse species exhibit substantial intra-specific size variation. To control for variation among treatment groups, Mb_i of each tsetse was incorporated as a covariate as a standard measure of individual size. I used STATISTICA (v. 10, Statsoft, Tulsa, USA) to calculate the proportional or least-square mean results for graphs. Overlap in 95 % CLs was used to test for statistically significant homogeneity within and among treatment groups.

2.3 Results

2.3.1 Water loss rate

In all four species examined, WLRs were significantly affected by body mass of individuals, test temperature and relative humidity (Table 2.1; Figure 2.1 A). *Glossina brevipalpis* (mesic), *G. m. centralis* (xeric) and *G. p. gambiensis* (mesic) also showed significant interaction effects between temperature and relative humidity, although this was not the case for *G. pallidipes* (xeric). After adjustment for the mean mass across all four species (mean Mb_i = 33.99 mg), *G. brevipalpis* lost significantly more water at 29 °C, 0 and 76 % relative humidities relative to all other species measured at these conditions. By contrast, *G. p. gambiensis* lost water significantly slower than at least one other species across all the treatment conditions. (Figure 2.1 A). Even after adjustment for body size within each species (i.e. among treatment groups), there was significant variation in WLR across treatments within ecotype groups (Figure 2.2). Specifically, WLR of xeric species differed significantly among five of the nine experimental treatments and mesic species differed significantly in all but three experimental treatments (Figure 2.2).

All species lost water slower than expected at low saturation deficits and higher than expected at higher saturation deficits (Figure 2.3 A–D). Generally, WLR for *G. m. centralis* were lower at lower saturation deficits (Figure 2.3 B). Furthermore, *G. brevipalpis* showed a general positive association between WLR and temperature and a negative association between WLR and relative humidity. WLR increased significantly with higher temperature at 0 % relative humidity and decreased significantly with increased relative humidity at 25 and 29 °C. In *G. m. centralis* at 25 °C and 0 % relative humidity, WLR was significantly higher relative to the other treatments. *G. pallidipes* showed the same general pattern, losing significantly more water at 25 °C than at other temperatures, irrespective of relative humidity. For this species, WLR was significantly higher at 0 % relative humidity than at all other values, irrespective of temperature. *G. p. gambiensis* lost water significantly faster at 29 °C, 0 % relative humidity compared to all other treatments. In summary, within–species variation in WLR is significantly influenced by temperature and relative humidity in *G. brevipalpis* (*fusca* group, mesic ecotype). At an intermediate temperature (25 °C), *G. m. centralis* (*morsitans* group, xeric ecotype) WLR increases with decreased relative humidity. WLR is significantly higher at 0 % relative humidity in *G. pallidipes* (*morsitans* group) and higher temperatures (29 °C) at 0 %, and 76 % relative humidity treatments results in a significant increase in WLR of *G. p. gambiensis* (*palpalis* group, mesic ecotype).

2.3.2 Body water and lipid content

As expected, initial body mass generally did not vary among treatments within each species (Figure 2.1 D). Mass-independent variation in BWC with temperature and relative humidity was significant for all species, although these effects were less pronounced than in the case of WLR (Table 2.1; Figure 2.1 B). The main effect of relative humidity did not significantly affect BWC in *G. m. centralis* (Table 2.1). Generally, *G. pallidipes* had a lower BWC (~ 12.5 %) relative to all other species across treatments (Figure 2.1 B). *G. brevipalpis* had significantly lower BWC than *G. p. gambiensis* at 29 °C, 0 % and 76 % relative humidity. There was a significant interaction effect between temperature and relative humidity on BLC in *G. brevipalpis* and *G. pallidipes*, but no significant interaction effect in *G. m. centralis* or *G. p. gambiensis*. Only the main effect of temperature was significant for *G. p. gambiensis* (Table 2.1). In *G. pallidipes*, BLC was significantly lower at 29 °C relative to 21 °C across relative humidity treatments. By contrast BLC was significantly higher in *G. brevipalpis* at 29 °C than at 21 or 25 °C across relative humidity treatments with the exception of 99 % relative humidity where no significant differences in BLC were observed (Figure 2.1 C).

2.3.3 Survival time

The estimated (predicted) time to death by water and lipid exhaustion is a function (see Equation 2) of all three traits of water balance (WLR, BWC and BLC) and provides a good indication of water balance, assuming negligible activity effects. Lower WLR, higher BWC and higher BLC should all potentially result in increased predicted survival time (see e.g. discussion in Chown *et al*. 2011). Body mass had a significant influence on survival time as predicted by the model (Table 2.2, covariate mean mass: 33.99 mg) and the predicted survival time varied among species and treatments when adjusted for differences in body size (Table 2.2). Moreover, the interaction effects of temperature and humidity also influenced the predicted survival time significantly (Table 2.2, Figure 2.4). However, since I was interested in the ecologically relevant variation in the predicted survival time among species, I also investigated the predicted survival time without adjusting for body mass (Figure 2.4). This suggested that the precise temperature and relative humidity combinations affected survival of the different species and ecotype groups in a complex manner, and was not simply a consequence of body size variation. The slope of the relationship between temperature and the predicted survival time was generally negative. However, within species slopes differed and were occasionally positive with increased temperature, for example, at 99 % relative humidity in *G. m. centralis*. *Glossina brevipalpis* survived significantly longer than all other species

at 21 °C compared to the other temperature treatments at 0 and 76 % relative humidity and at 99 % relative humidity across all temperature treatments. *Glossina p. gambiensis* had significantly lower survival time relative to all species tested at 21 °C, 76 %; 29 °C, 76 % and all temperature treatments, at 99 % relative humidity.

G. brevipalpis (mesic) survived significantly longer than *G. p. gambiensis* (mesic) and *G. m. centralis* (xeric) survived significantly longer than *G. pallidipes* (xeric) at 29 °C, 0 % relative humidity. This suggests within-ecotype variation and higher resistance to desiccation through water balance traits in hot, dry environments in *G. brevipalpis* (mesic) and *G. m. centralis* (xeric). Furthermore, *G. brevipalpis* survived significantly longer than *G. p. gambiensis*, except at 25 °C, 0 % relative humidity which suggests variation in desiccation resistance within the mesic ecotype.

2.4 Discussion

Here, I measured water balance traits (WLR, BWC and BLC) in adult flies across a range of temperature (20 – 30 °C) and relative humidity (0 – 99 %) combinations in four tsetse species from both xeric and mesic habitats. In addition, we applied a simplified mathematical model to calculate (predict) survival time of a resting fly as a function of the measured water balance traits. One of the major outcomes of this work is that the results showed that the exact conditions under which the measurements of WLR were made either amplified or reduced variation within and among species, with the greatest differences found at low relative humidity and varying temperature (Figure 2.1). One might reason that this is simply a consequence of changes in saturation deficit. However, as the subsequent analyses showed, this was not simply a consequence of saturation deficit changing since WLRs were sometimes higher (and lower) than expected for a given saturation deficit (Figure 2.3). In sum, the results therefore suggest that both cuticular permeability, perhaps owing to temperature-dependent cuticular lipid phase changes (Gibbs 2011), and saturation deficit together set WLR under a particular set of environmental conditions.

Water loss rates (WLR) were significantly affected by measurement under different temperature and relative humidity combinations, while body water content, body lipid content and mass were less strongly affected. The marked variation in WLR suggests that hot, dry environments are sub-optimal for *G. brevipalpis* (Figure 2.1 A). Furthermore, *G. brevipalpis* lost water slower than all other species in moist treatment conditions (Figure 2.2), which suggests the species is desiccation resistant and that there is within-ecotype variation in WLR responses. Furthermore, WLRs found here are similar to previous measurements by Terblanche *et al*. (2006) on *G. pallidipes* (24 °C, 0 %

relative humidity) and Bursell (1957a, b) on *G. morsitans* (25 °C, 99 % relative humidity) if compared under similar measurement conditions. These results provide support for mass–independent inter- and intra-specific variation in WLR and survival times. Therefore, water balance responses to variation in temperature and relative humidity are complex in *Glossina*, and this response varies within and among species, sub–groups and ecotypes in terms of both magnitude of effects and the direction of change.

Variation in the response of BWC suggests within-ecotype variation between mesic species and tighter regulation of water balance in hot, dry environments in *G. p. gambiensis*. There was no consistent pattern in BLC across treatments for each species, as might be expected given the short duration of the treatments and Bursell's (1959) finding that relative humidity does not have a major effect on the amount of lipid in a fly. On average, 0.8 mg fat is metabolically oxidised in 16 h, producing 0.86 mg water (Bursell 1959). Lipid consumption increases in dry air as a consequence of the orthokinetic reaction to relative humidity and is directly positively correlated with tsetse metabolism (Bursell 1957b). Thus the BLC variation observed in these experiments is likely to be a consequence of the temperature dependence of metabolic rate (e.g. Terblanche and Chown 2007). However, it has been argued that lipid reserves should not become depleted in dry air before death occurs by desiccation in any species of tsetse (Jack 1939; Bursell 1959).

Species from mesic environments did not always survive the longest in high relative humidity conditions and those from xeric environments did not always perform best at low relative humidity perhaps suggesting complex evolutionary trade-offs among currently occupied environments. However, *G. p. gambiensis* survived all experimental conditions poorly, which is in agreement with its mesic classification. In particular, at 99 % relative humidity and at any temperature survival time was in the range of 30 – 40 hours, a value approximately half that of *G. brevipalpis*. Thus, it seems that *G. brevipalpis* has at least partly overcome its mesic habitat restriction on the basis of its increased body size (Figure 2.1 D; Figure 2.4). Activity levels could confound our simplified estimates of survival time, especially under field conditions. However, the survival time calculations presented here consider water loss for the resting state of the adult fly for the purpose of inter-species comparison. We are therefore of the opinion that our estimates of survival time reflect the maximum likely survival time and probably overestimate survival time in the wild where a higher activity, higher water loss rate and, consequently, lower survival time might be expected, especially over longer durations. Thus, species-specific, temperature-dependent activity levels could in the future be included to make the survival time estimates more accurate.

Different effects of temperature and relative humidity within and among experimental conditions and species suggests cuticular permeability and saturation deficit are likely to be key factors in forecasting tsetse water balance responses to climate variability. Climate change is often viewed in terms of its projected effects on ambient temperature only (e.g. Deutsch *et al*. 2008) although it is becoming clear that such an approach may provide limited insights into realized ectotherm responses (e.g. Bonebrake and Mastrandrea 2010; Clusella-Trullas *et al*. 2011). However, relative humidity is also likely to be affected, based in part on projected rainfall, fog and cloud cover changes (e.g. Adler *et al.* 2008; Zhou *et al.* 2009; Tebaldi and Sanso 2009). Ultimately, both relative humidity and temperature could change in different ways depending on the exact geographic location (see e.g. Walther *et al.* 2002; Knapp *et al.* 2008; Bonebrake and Mastrandrea 2010; Fung *et al*. 2011 and discussions in Clusella-Trullas *et al*. 2011; Chown *et al.* 2011). This study shows that due consideration of the synergistic impacts of temperature and moisture availability is important since the two potential stressors interact in fairly unexpected ways to affect measured water loss rates. Moreover, given the survival time estimation results presented here, this interaction may have far-reaching implications for tsetse population dynamics, and hence, vector responses to climate change. In particular, in the four tsetse species we examined, which represented three different subgroups and two ecotypes, species WLR did not respond similarly to temperature and relative humidity variation. Perhaps the most surprising outcome is the difference in water loss rates and survival time under desiccating conditions for two xeric species and two mesic species (*contra* Hoffmann *et al*. 2003), which one might have expected to respond relatively similarly, at least in the form and direction of the responses (though see also Terblanche and Kleynhans 2009), especially after accounting for size effects. Here, we show that extrapolating water balance responses to temperature and relative humidity among species or even among ecotypes should probably be undertaken with caution. Future efforts to model tsetse species responses to climate change may therefore require species–specific information if water balance physiology is to be incorporated into mechanistic models (see e.g. Kearney *et al.* 2009). This will likely increase the accuracy of predictions for the vector, thereby enabling better disease intervention planning in future.

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2.6 Figures

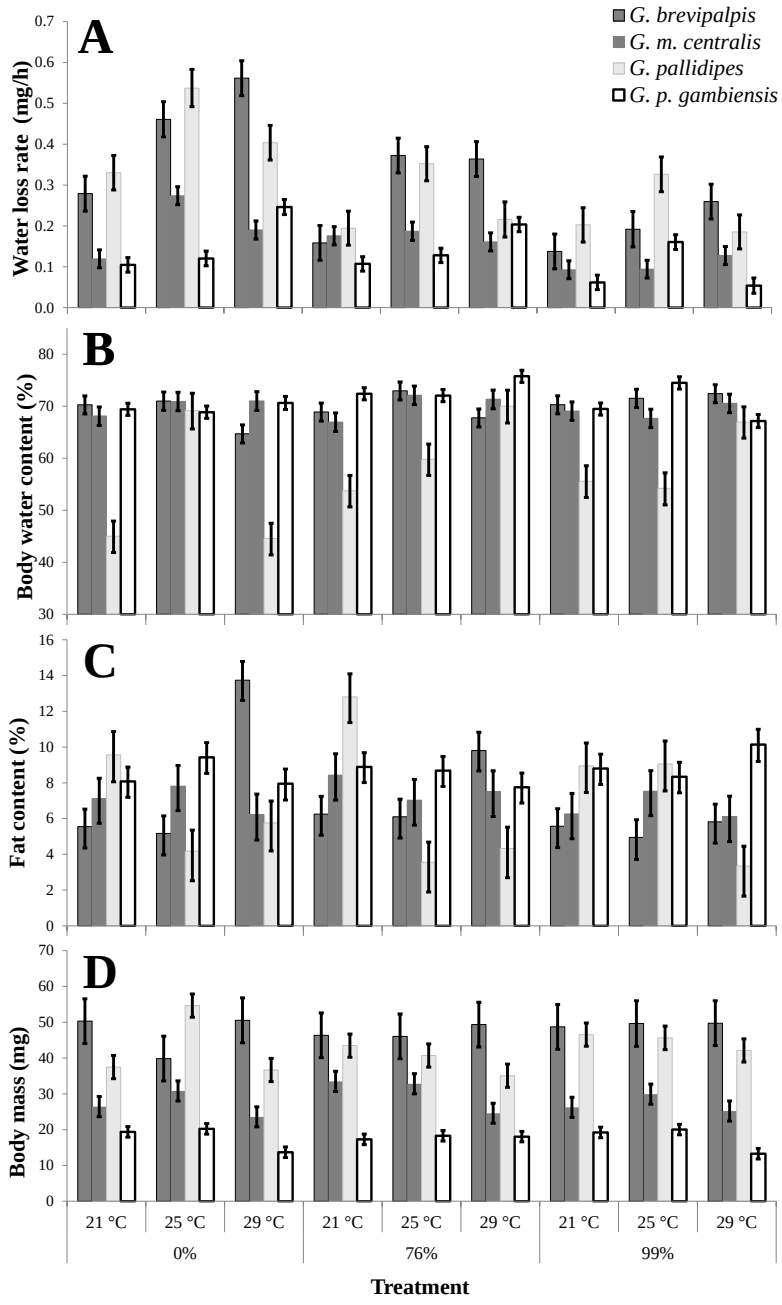

Figure 2.1. Mean (± 95 % confidence intervals) results of *Glossina brevipalpis* (mesic), *G. morsitans centralis* (xeric), *G. pallidipes* (xeric) and *G. palpalis gambiensis* (mesic) A) water loss rate (least squares means in mg/hour), covariate mean body mass = 33.99 mg, B) body water content (as a % of initial body mass), C) body lipid content (as a % of initial body mass) and D) initial body mass (in mg). Water balance traits were measured at three different relative humidities and three different temperatures.

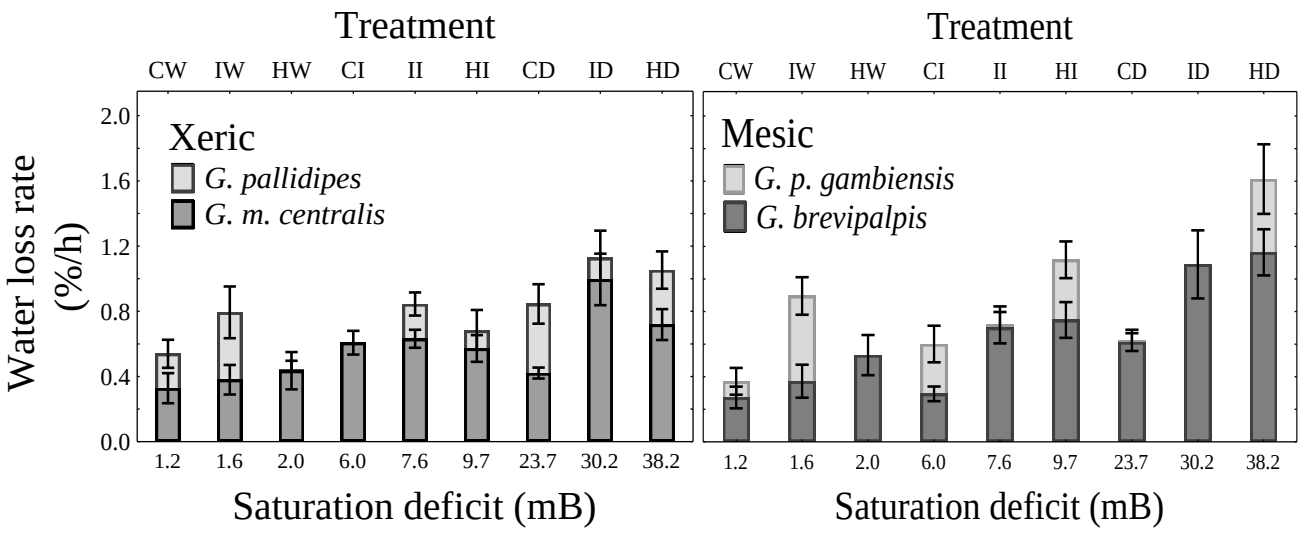

Figure 2.2. Means (± 95 % confidence intervals) water loss rates (% of initial body mass lost per hour) across a range of saturation deficits corresponding to the respective temperature and relative humidity treatments for xeric (left) *G. pallidipes* and *G. morsitans centralis* and mesic (right) *Glossina brevipalpis* and *G. palpalis gambiensis*. Treatments and corresponding saturation deficits include the following temperature (°C), relative humidity (%) combinations: 21,99 (CW); 25,99 (IW); 29,99 (HW); 21,76 (CI); 25,76 (II); 29,76 (HI); 21,0 (CD); 25,0 (ID) and 29,0 (HD) where C = cold, W = wet, I = intermediate, H = hot and D = dry.

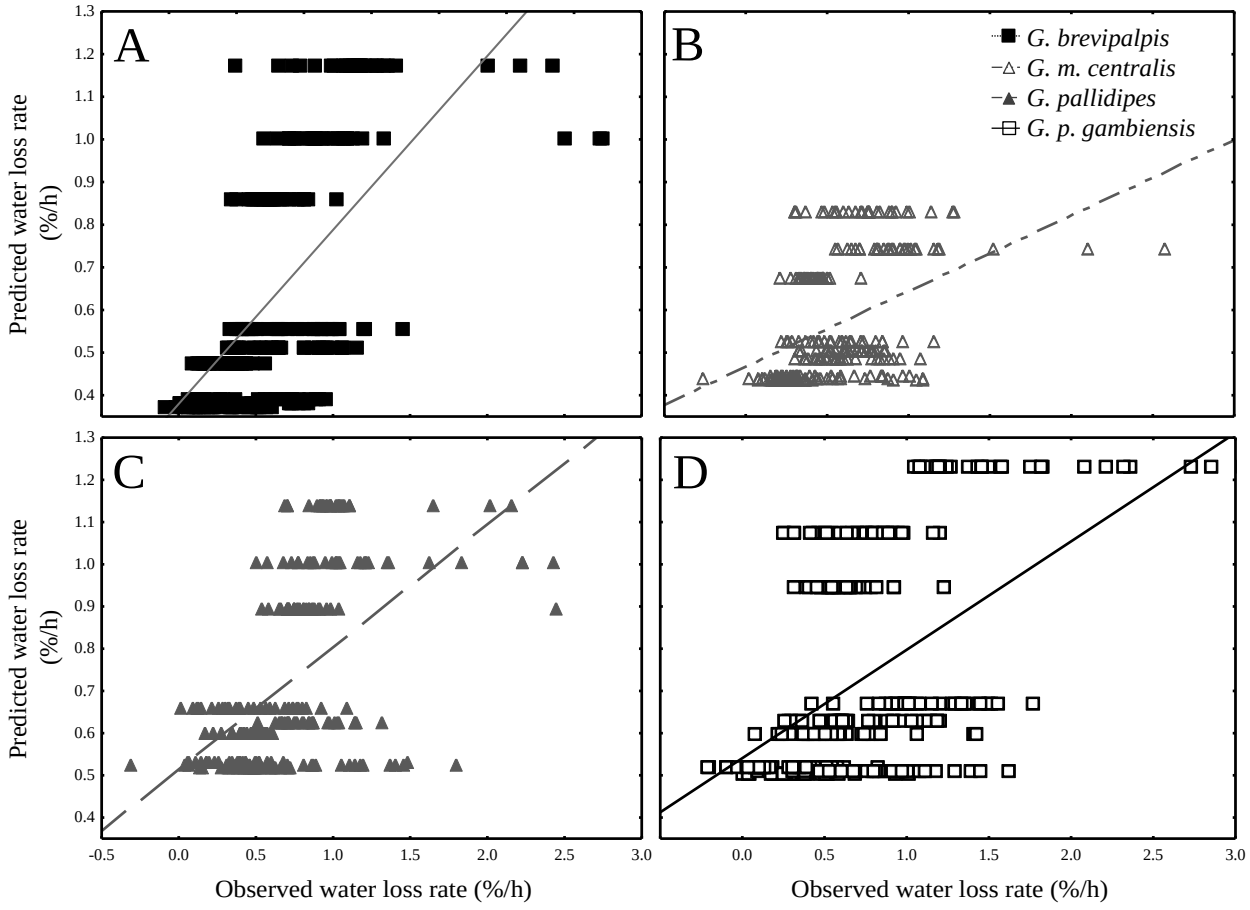

Figure 2.3. General regression model results of the least-squares means fit for the nine distinctive treatments. The observed vs. regression-predicted water loss rate (% of initial body mass per hour) per individual are plotted for A) *Glossina brevipalpis* (mesic), B) *G. morsitans centralis* (xeric), C) *G. pallidipes* (xeric) and D) *G. palpalis gambiensis* (mesic).

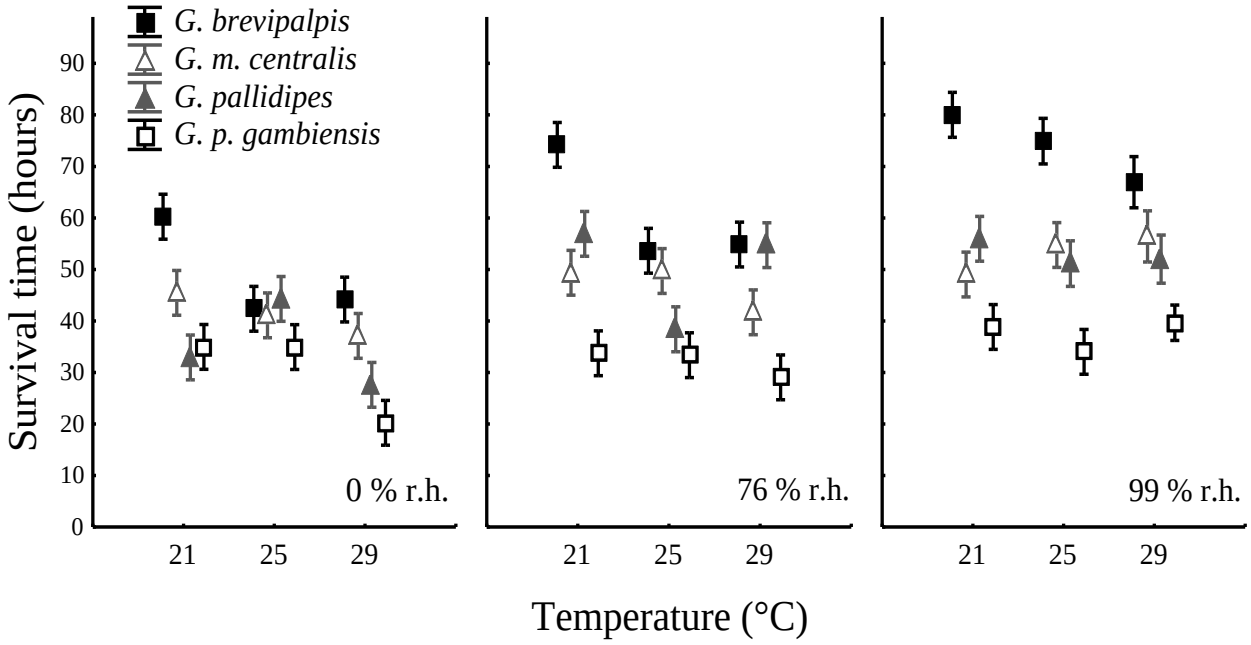

Figure 2.4. Means (± 95 % confidence intervals) for estimated survival time (in hours) for *Glossina brevipalpis* (mesic), *G. m. centralis* (xeric), *G. pallidipes* (xeric) and *G. p. gambiensis* (mesic) across the range of temperature and relative humidity treatments. Mesic species are presented by squares and xeric species by triangles. Survival time is calculated as metabolic water yield from lipids (in mg), given the critical lipid mass (see methods for details) and the initial body water content (in mg) (see Materials and Methods for details), given the critical body water content of each species. This is divided by the rate of water loss (in mg H_2O/hour) under resting conditions. All symbols are offset for clarity.

2.7 Tables

Table 2.1. Summary of generalized linear model results (degrees of freedom (d.f.), chi–square (χ^2) statistic and corresponding p–value (*P*)) for water loss rate (WLR in mg/h), body water content (BWC in mg) and body lipid content (BLC in mg) as dependent variables, initial body mass as a continuous predictor and relative humidity, temperature or temperature and relative humidity interaction effect (indicated with ×) as independent variables. Traits were measured for *G. brevipalpis* (mesic), *G. morsitans centralis* (xeric), *G. pallidipes* (xeric) and *G. palpalis gambiensis* (mesic). Non–significant effects are indicated in bold.

Species	Trait	Parameter	d.f.	χ2	*P*
G. brevipalpis	WLR	Body mass	1	349.51	< 0.0001
		Humidity	2	165.44	< 0.0001
		Temperature	2	133.94	< 0.0001
		Humidity × Temperature	4	22.32	0.0002
	BWC	Body mass	1	5857.33	< 0.0001
		Humidity	2	21.11	< 0.0001
		Temperature	2	20.44	< 0.0001
		Humidity × Temperature	4	23.66	< 0.0001
	BLC	Body mass	1	129.78	< 0.0001
		Humidity	2	38.37	< 0.0001
		Temperature	2	100.56	< 0.0001
		Humidity × Temperature	4	55.55	< 0.0001
G. m. centralis	WLR	Body mass	1	146.91	< 0.0001
		Humidity	2	77.31	< 0.0001
		Temperature	2	30.59	< 0.0001
		Humidity × Temperature	4	55.07	< 0.0001
	BWC	Body mass	1	5859.05	<.0001
		Humidity	**2**	**2.07**	**0.3551**

		Temperature	2	33.03	<.0001
		Humidity × Temperature	4	17.53	0.0015
	BLC	Body mass	1	75.62	<.0001
		Humidity	**2**	**5.8**	**0.055**
		Temperature	**2**	**4.33**	**0.1146**
		Humidity × Temperature	**4**	**8.36**	**0.0791**
G. pallidipes	WLR	Body mass	1	51.16	<.0001
		Humidity	2	127.17	<.0001
		Temperature	2	84.35	<.0001
		Humidity × Temperature	**4**	**4.56**	**0.3353**
	BWC	Body mass	1	760.58	<.0001
		Humidity	2	14.72	0.0006
		Temperature	2	75.75	<.0001
		Humidity × Temperature	4	217.32	<.0001
	BLC	Body mass	1	32.33	<.0001
		Humidity	2	13.7	0.0011
		Temperature	2	114.64	<.0001
		Humidity × Temperature	4	74.53	<.0001
G. palpalis gambiensis	WLR	Body mass	1	212.77	<.0001
		Humidity	2	90.43	<.0001
		Temperature	2	91.99	<.0001
		Humidity × Temperature	4	205.29	<.0001
	BWC	Body mass	1	13473.6	<.0001
		Humidity	2	41.64	<.0001
		Temperature	2	20.95	<.0001
		Humidity × Temperature	4	123.26	<.0001
	BLC	Body mass	1	38.01	<.0001
		Humidity	**2**	**0.89**	**0.642**
		Temperature	2	32.27	<.0001
		Humidity × Temperature	**4**	**7.54**	**0.1099**

Table 2.2. Summary of generalized linear model results (degrees of freedom (d.f.), chi–square (χ^2) statistic and corresponding p–value (*P*)) (normal distribution of errors and identity link function) for time to death (in hours) as dependent variable, initial body mass (in mg) as a continuous predictor and relative humidity (in %), temperature (in °C) and species main and interaction effects (indicated with ×) as model parameters.

Parameter	d.f.	$\chi 2$	*P*
Body mass	1	502.39	<.0001
Species	3	235.99	<.0001
Humidity	2	387.32	<.0001
Temperature	2	84.73	<.0001
Species × Humidity	6	125.94	<.0001
Species × Temperature	6	102.23	<.0001
Humidity × Temperature	4	35.19	<.0001
Species × Humidity × Temperature	12	64.66	<.0001

Chapter 3

Potential responses of a disease vector (*Glossina* spp.) to climate change: application of a bottom–up mechanistic model

3.1 Introduction

Unforeseen changes in disease burden due to the impacts of climate change are of global interest. Emerging new or neglected diseases, actual changes in vector distribution and the unanticipated impact of well-known diseases are among the most urgent and challenging issues facing health services and intervention planning (McMichael 1997; Githeko *et al*. 2000; Martens and Thomas 2005; Patz *et al*. 2005; Courtin *et al*. 2008; Paaijmans *et al*. 2009; Paaijmans *et al*. 2010; Eisen and Eisen 2011).

The use of species distribution models has grown tremendously in the past decade (Guisan and Zimmerman 2000; Thuiller *et al*. 2009). Correlative predictive modelling is increasingly used to predict climate change impacts on vector-borne disease dynamics (Peterson 2006; Elith *et al*. 2006; Ford *et al*. 2009). Present-day observed distributions of insect vectors are typically matched spatially and statistically to remotely-sensed climate variables (Rogers and Randolph 1993; Randolph and Rogers 2000; Randolph 2001; Rogers *et al.* 2002; González *et al*. 2010) to establish a multivariate climatic description of present distributions and, ultimately, health-related risk (e.g. Rogers and Packer 1993; Rogers and Randolph 2000; Kuhn *et al*. 2003; Peterson 2009). The best combination of climatic predictor variables, that describes the past or current distribution of a disease vector, is then typically correlated to one or more Global Circulation Models (GCMs) to predict the future vector distribution at large scales (Rogers and Randolph 2000; Hales *et al*. 2002; Kuhn *et al*. 2003; Peterson 2009). Moreover, infectious disease outbreak frequency has been accurately correlated with past climatic changes (Patz *et al*. 2005; Purse *et al.* 2005; McMichael *et al*. 2006; Soverow *et al*. 2009, but see Lafferty 2009, and see Eisen and Eisen 2011 for a review). However, it becomes clear that the impact and outcomes of climate change on the physiological state of insects needs to be considered carefully to make accurate future disease-risk predictions (Kearney *et al*. 2009; Lafferty 2009). And thus, future mechanistic, physiological approaches require consideration of the potential evolutionary responses of insects that may further modify or exacerbate the impacts of climate change on vector population or disease dynamics (Huey and Berrigan 1996; West-Eberhard 2003; Ghalambor *et al*. 2007; Hoffmann 2010a,b). Although much recent attention has focused on future vector distributions, remarkably few studies have integrated physiological responses or the result of evolutionary outcomes in predictive modelling (Huntley *et al*. 2010; but see Kearney *et al*. 2009; Kolbe *et al*. 2010; reviewed in Hoffmann and Sgrò 2011).

Correlative models (*sensu* Elith and Leathwick 2009) differ from mechanistic models in an important way (Kearney and Porter 2009). Despite these differences, the use of correlative, climate

envelope models (CEMs) have been questioned extensively on the basis of their underlying assumptions, applicability in terms of inherent simplifications and ecological relevance (Davis *et al*. 1998; Parmesan *et al*. 2000; Gaston 2003; Hulme 2003; Pearson and Dawson 2003; Araújo and Guisan 2006; Araújo and New 2006; Barry and Eith 2006; Elith *et al*. 2006; Heikkinen *et al*. 2006; Randin *et al*. 2006; Beale *et al*. 2008; Marmion *et al*. 2009; Huntley *et al*. 2010; Lobo *et al*. 2010). Regardless, support for the accurate prediction of species' distributions using this method cannot be ignored (e.g. Beerling *et al*. 1995; Hijmans and Graham 2006; Green *et al*. 2008; Duncan *et al*. 2009; Buckley *et al*. 2010). Moreover, observed presence/absence data, which are essential for CEM application and verification, are sparse, if not lacking for a vast majority of species (Elith *et al*. 2006). By contrast, mechanistic models, although often more data–intense and computationally perhaps more challenging, require no prior knowledge of the species' current distribution or abundance (Kearney and Porter 2009). Mechanistic models do not consult current abundance or occupancy data and, in addition, provide a measure of the physiological and behavioural properties that might influence an organism's fitness in the environment (Kearney and Porter 2004, 2009). Furthermore, biophysical ecology allows the determination of how future climatic change might influence species' distribution from a biophysical and physiological perspective (Kearney and Porter 2004; Kearney *et al*. 2009; Kearney *et al*. 2010a). In addition, it provides a useful tool for the estimation of energy, nutrient and water requirement as a function of climate and terrain (Porter *et al*. 2010) and may provide insights into several aspects of population dynamics.

It is well established that physiological mechanisms link the relationship between *Glossina* population dynamics and environmental variation (Rogers and Randolph 1986; Rogers 2000; Rogers and Robinson 2004; Hargrove 2004; Terblanche *et al*. 2006; Terblanche and Chown 2006). Trypanosomiasis, vectored solely by tsetse, is declared a neglected tropical disease (Cattand *et al*. 2006). However, the links between tsetse physiology, future climate change and abundance have not been studied in great depth (but see Rogers and Packer 1993; Rogers and Randolph 1993). Furthermore, to our knowledge the fundamental niche of tsetse has not yet been predicted spatially or temporally in the peer-reviewed literature. The most recent spatial predictions of tsetse distribution across Africa were compiled using correlative approaches by the Food and Agriculture Organization (FAO) of the United Nations and the International Atomic Energy Agency (Wint and Rogers 2000).

A range of ecophysiological traits likely relates population abundance and geographic distribution of tsetse. For example, desiccation resistance is a trait that is probably directly related to evolutionary fitness in tsetse (Bursell 1958, 1959; Hargrove 2004) although possibly to a greater

extent in pupae than in adults (Kleynhans and Terblanche 2009; Bursell 1959). Temperature, by contrast, has been argued to be the major role-player in adult tsetse development-, mortality- and birth-rates (Hargrove 2001, 2004) as in insects more generally (see e.g. Irlich *et al.* 2009). Furthermore, the co–relationship between saturation deficit, normalized difference vegetation index (NDVI) and temperature form the major components determining, or at least linked with, adult abundance and geographic distribution (Rogers and Randolph 1991; Robinson *et al*. 1997a; Rogers and Randolph 1986; Rogers and Robinson 2004). This, in addition to Chapter 2, suggests an important role for physiological tolerance to climate stress in the relationships between population abundance, geographic distribution and variation among *Glossina* species (Rogers and Randolph 1986; Hargrove 2004). Furthermore, marked variation exists among species and populations for some physiological traits related to climate (see Chapter 2), and support a link with evolved variation in tsetse physiology (Kleynhans and Terblanche 2009; Terblanche *et al*. 2009; but see Terblanche *et al.* 2006) which are likely critical traits in the context of predicted climate change impacts of insects in general (see Hill *et al*. 2011).

In this Chapter, I implemented a suite of mechanistic 'bottom-up' models (NicheMapper) collectively (Porter and Mitchell 2006; Kearney and Porter 2009). The tsetse *G. pallidipes* was used as a model for vectors of infectious zoonotic disease in Africa. The aim of this Chapter was to explore and develop a mechanistic understanding of the current and future geographic distribution of *G. pallidipes*. To reach this goal, energy- and mass-balance equations were solved for *G. pallidipes* (Porter and Gates 1969, Gates 1980, Porter *et al*. 2010). Model results were compared to the established, known *G. pallidipes* probability of presence (Gp_{PP}) data (Wint and Rogers 2000) to validate the model performance. This first step of model testing provides an index of model accuracy. I identified the single best physiological predictor-variable from the mechanistic model that describes the established *G. pallidipes* data. This physiological predictor variable is used to forecast climate change impacts on future *G. pallidipes* distribution. I considered the downscaled forecasts under the Hadley Centre for Climate Prediction and Research (HadCM3) extreme A2a emission scenario for 2080 according to the IPCC 3rd assessment report (IPCC 2001; Pope *et al*. 2002; see Sanderson *et al*. 2011). A scaled up application of the mechanistic approach is undertaken to determine the affect of climate change on species' distribution. Second, since tsetse generally have pronounced acclimation responses to changes in thermal and atmospheric moisture conditions (e.g. Terblanche *et al*. 2006; Terblanche and Kleynhans 2009), I aimed to explore the effects of phenotypic plasticity on future *G. pallidipes* distribution and abundance. Specifically, I explore water balance and thermal tolerance plasticity and apply this at the four range limits of the current

G. pallidipes distribution. Under four different climate change scenarios (hot/dry, hot/wet, cold/dry, cold/wet) I determine if plastic physiological responses can potentially 'rescue' tsetse in the face of climate change. Finally, I solve a mathematical model for the basic reproductive number of the disease, transmitted by tsetse, based on the expected relationship between metabolic rate and biting frequency in all the countries of current Gp_{PP} (fifteen African countries).

3.2 NicheMapper setup and validation

3.2.1 Model procedures and database setup

Numerical iterations were performed in NicheMapper to solve the steady-state heat balance equations (see Equation 3 and supplemental information in Porter and Kearney 2009). The basic equations include radiative (both solar and infrared), convective, evaporative and metabolic processes (Porter *et al*. 1994, 2000, 2002, 2006; Kearney *et al*. 2010b). Two main models are solved sequentially. First, a microclimate model that calculates microclimate parameters per landscape pixel. Second, an animal model that calculates animal–dependent physiological parameters, given the output from the available microclimate calculated in the first step, and based on morphological, behavioural and physiological properties of the animal that govern heat and mass exchange with the local microenvironment.

Estimates of air temperature were calculated from the maximum and minimum temperature data included initially per pixel. Here, the model fits a sinusoidal curve to the input parameters, and for the purpose of the simulations in this thesis, allowed daily air temperature to be lowest at sunrise and highest one hour after solar noon (similar to Natori and Porter 2006). This can be modified to allow for local known microclimate variation (e.g. a 3 pm maximum air temperature). However, since this is largely unknown for most of the sites modelled, I worked with this assumption although it can be easily altered accordingly in future. The amount of solar radiation reaching the earth's surface is modified by shade. Furthermore, shade provides thermal opportunities to the animal by trapping infrared radiation. I therefore allowed shade-seeking behaviour from the animal. Reflection from the animal, absorption of radiation by the animal and evaporative heat transfer profoundly affect the heat balance of the animal best described by an effective thermal conductivity expression for the geometry of a sphere (or ellipsoid) so that heat energy balance will be

$$Q_{in} + Q_{gen} = Q_{out} \quad \text{(Equation 3)}$$

where Q_{gen} is the total heat generated (metabolic rate), Q_{in} is the total heat entering at the inner boundary layer and Q_{out} is the total heat lost by convection, radiation and evaporation from the outer surface of the animal (see Porter and Kearney 2009 for additional details).

Calculations were done at hourly and monthly temporal scales for 366 simulation days with microclimate and animal models (Porter and Mitchell 2006). The resulting output tables were created in a MySQL (version 5.1) database by the open Perl code (using Active Perl (v. 2)) running the FORTRAN microclimate and animal calculation programs for each landscape pixel. NicheMapper model output variables included the daily amount of water available in the environment (in g/d), discretionary water in the environment (in g/d), activity hours available in the environment (in h/d), discretionary energy available in the environment (in J/d), water evaporated from *G. pallidipes* (in g/d), *G. pallidipes* food requirement (in g/d), *G. pallidipes* metabolic rate (in J/d) and environmentally available energy (in J/d) (see e.g. Natori and Porter 2007 for an example of NicheMapper application to estimate metabolic rate of a large mammal). Discretionary water and energy are calculated as the difference between available environmental water and evaporative water from the fly, and available environmental energy and the fly's predicted metabolic rate, respectively.

3.2.2 Physiological and biological input parameters

To investigate the effect of climate on the physiological response of tsetse, calculations were done from first principles, using the "animal" component of NicheMapper (Porter and Mitchell 2006). The behavioural and physiological data required for the calculation procedure were extracted from the peer–reviewed literature (Table 3.1). Measurement of solar reflectance from adult flies was undertaken across a spectral range from 350 – 2500 nm (0.1 nm resolution). Measurements were made in a photographic darkroom using an ASD (AZ Technology Model LPSR 300, USA) portable spectroreflectometer with a mass of fresh thawed tsetse flies completely covering and obscuring the source light emanating from the quartz window of the upward pointing hand held sensor. Computation of the average reflectance over the spectral range integrated the percentage reflectance for each 0.1 nm wavelength interval multiplied by the solar energy available in that interval (Porter 1967). The main substrates of tsetse metabolic energy are proteins and lipids (Bursell *et al*. 1974). Bursell and Taylor (1980) provides a comprehensive study of the energy budget of *G. morsitans morsitans* which shows that female energy requirements are higher than that of male flies due to the relatively high cost of reproduction in the field. About 35 % of *Glossina* dry body mass is sub-cuticular lipids (Bursell 1959).

Tsetse core body temperatures (T_c) vary between 26 – 32 ºC (Howe and Lehane 1986) and 40% of a blood meal is excreted in the form of excess water approximately 30 min post–feeding (Howe and Lehane 1986). More than 90 % of the dry weight of blood ingested by *Glossina* consists of protein, leaving very low lipid and carbohydrate contents (Bursell 1965). Tsetse usually fly for mating and foraging between 7 – 10 am and 5 – 8 pm (Leak 1999) for 15 – 42 min/d, at 3 – 6 m/s 1.5 – 2.5 min at a time (Rajagopal and Bursell 1966; Bursell and Taylor 1980) and about 4 flights per day (Bursell and Taylor 1980; Brady 1991). I ran the NicheMapper model under the assumption that host availability and vegetation structure is constant throughout the simulation sites. Vegetation structure included aspects of vegetation complexity, type and density of grasses, shrubs and riparian trees, and other surface parameters such as urban development and agricultural practice is also included to allow shade–seeking behaviour of the flies should the energy budget require this.

3.2.3 Current spatial distribution records

The current spatial records of Gp_{PP} were extracted from Wint and Rogers's FAO report (2000) on a 5 km (2.5 arc-minute) spatial resolution (Figure 3.1). This map was generated based on known presence-absence records established from drive-round and on-the-ground trapping by Ford and Katondo (1977), and then integrated with indices of climate and vegetation by Wint and Rogers (2000) to produce the latest, high-resolution map of *G. pallidipes* probability of presence in Africa. The map data described above are considered as a reference dataset against which I compare the mechanistic model's results spatially. The reference map of Wint and Rogers (2000) is the result of logistic regression of fly presence/absence against a wide range of predictor variables for a large number of regularly spaced sample points for each area. Predictor variables included remotely sensed surrogates of vegetation, temperature and moisture that were then subjected to Fourier processing to provide an additional set of seasonal and time related measures for each parameter (Wint and Rogers 2000). According to the current distribution map of Wint and Rogers (2000), the mean Gp_{PP} is > 1 % in fifteen African countries. I chose five, out of fifteen African, countries to test model performance spatially. In these five countries the mean Gp_{PP} is > 10 % per country, estimated as 16 ± 36 % (mean ± standard deviation) in Kenya, 16 ± 31 % in Rwanda, 19 ± 29 % in Tanzania, 23 ± 31 % in Mozambique and 24 ± 35 % in Uganda (Figure 3.1). I used the grouped area (total area of all five countries simulated: 2 591 705 km^2) as an additional 6^{th} area for model performance testing and thus also test the model across a range of spatial scales. This "grouped" area represented 59.4 % of the total *G. pallidipes* distribution across 15 African countries. Model simulations for this first part of the Chapter were run on a 2.5 arc–minute resolution (0.05 degrees ≈ 20 km^2 at the equator).

I used spatial weather records to calculate the microclimates across the sites for point simulations using mechanistic biophysical modelling software (McCullough and Porter 1971; Kearney and Porter 2004; Porter and Mitchell 2006; Natori and Porter 2007); the 'microclimate' component of NicheMapper (Porter and Mitchell 2006). This microclimate model component included elevation (m above sea level), mean monthly maximum temperature (°C), and mean monthly minimum temperature (°C) and mean monthly precipitation (mm) data extracted from WorldClim (Version 1.4, release 3 2004; Hijmans *et al.* 2005). The grid data were in a latitude/longitude coordinate reference system (WGS84) and extracted by points using ArcGIS (v. 9.3.1, ESRI 2005). The input climate data were described as interpolations of observed data representing the period 1950 – 2000 (referred to hereafter as 'current climate' or '2000'). Furthermore, Staub and Rosenzweig Zobler soil unit surface slope (in °), were obtained from the National Geophysical Data Centre of the U.S. National Oceanic and Atmospheric Administration (NOAA; ftp.ngdc.noaa.gov) database. Wind data (m/s) 10 meters above ground level, were extracted from a global–wind–database (New *et al.* 2000). The resolution of the wind data was extremely coarse (10 arc-minute resolution), however, for the NicheMapper microclimate input tables I only required an average wind speed per country per month.

3.2.4 Spatial model validation and statistical analyses

Capture and storage of NicheMapper predictor variable data were conducted in MySQL while preliminary preparation of digital spatial environmental data was conducted in ArcGIS (v. 9.3.1, ESRI 2005). All data analyses were performed using R (v. 2.12, R Development Core Team 2010). Analyses were undertaken systematically for the predicted average annual data to measure model performance across the countries. Thus, all spatial results considered the time–averaged solutions, and although this approach is weaker for determining local population trends, this is probably more informative for the large-scale, long-term patterns I was primarily interested in. I also investigated the possible co-linearity of predictor variables from the model in a correlation matrix (Murphy and Winkler 1992) using Pearson's product moment correlation coefficient (Table 3.2). The correlation matrix for the broad set of predictor variables resulting from the mechanistic model across the five countries assessed indicated that the amount of water that was available in the environment was strongly positively correlated with discretionary energy in the environment, available energy in the environment and predicted metabolic rate of *G. pallidipes* ($r > 0.95$, $p < 0.001$). The latter three variables were also strongly positively correlated ($r > 0.95$, $p < 0.001$) with each other and similar results were obtained in correlation tests undertaken for each country separately (results not shown).

I therefore consider the single best predictor variable identified during spatial validity of the model results for further analyses.

Two different approaches were used to evaluate the NicheMapper output variables' accuracy at predicting Gp_{PP} spatially: MapCurves and the area under the receiver operating curve. MapCurves (Hargrove *et al*. 2005) is a quantitative goodness-of-fit (GOF) method that gives an indication of model performance by estimating the degree of spatial overlap (positive spatial correlation) between two maps. I used R to calculate the degree of map overlap per category and Excel (Microsoft Office Professional Plus 2010) for GOF score estimation (see DeVisser and Messina 2009). This method is applied by categorizing the continuous Gp_{PP} data and explanatory mechanistic predictor variable data. The sum of the positive spatial correlation determined among categories of two maps, returned a GOF value between 0 and 1, with higher GOF scores indicating better agreement between the two maps. The GOF algorithm estimates the proportion of category overlap weighted by the fractional share of category area to prevent distortion of larger maps with little overlap (Hargrove *et al*. 2005; DeVisser and Messina 2009) and is comparable across models (Williams *et al*. 2008). Therefore, a single physiological predictor variable resulting from the model that describes Gp_{PP} best could be determined by comparing GOF scores across physiological predictors for 0.2 of the reference area (Williams *et al*. 2008). This would indicate that the maps agreed for at least 20 % of the area, with 10% bin ranges in the reference map (DeVisser and Messina 2009). The concordance between a predictor variable and the distribution map was judged to be good when the GOF score was larger than 50 %, which indicated the predictor variable explained the reference map better than random.

I assessed spatial performance of NicheMapper output variables by applying the area under the receiver operating characteristic curve (AUC) (Hanley and McNeil 1982; He *et al*. 2009; Tuszynski 2009). Typically, the AUC value is interpreted on an accuracy level where AUC < 0.5, 0.5 – 0.7, 0.7 – 0.9 and > 0.9 indicate a performance worse than a random guess, low-, useful- and high predictive ability, respectively (Hanley and McNeil 1982). AUC estimates were calculated with transformed binary outcomes of the reference map, where all the data < 10 % Gp_{PP} represented absence and > 90 % Gp_{PP} represented presence of *G. pallidipes*, unlike the multimodal structure of the reference data used for the GOF score estimation. One of the major concerns regarding the AUC method was that the intermediate Gp_{PP} data might have added some significant value to the GOF estimates. This intermediate slice of data (all Gp_{PP} records between 10 – 90 %) was thus ignored during AUC calculations. In consequence, the AUC value was only used to produce an estimate of the omission to commission error rates (see Lobo *et al*. 2008).

The NicheMapper model output variable MR_{PRED} performed well in its predictive ability (GOF > 83 % in Kenya, Rwanda and Uganda; GOF ≥ 75 % in Tanzania and Mozambique) in all five countries tested (Table 3.3; Figure 3.2). In addition, MR_{PRED} showed the highest concordance with the reference map (average GOF score of 83 %) across the five countries combined (Table 3.3). NicheMapper predicted evaporation of water from the fly was the second best predictor of current Gp_{PP}, explaining ≥ 80 % of the reference map variance in Kenya, Rwanda, Uganda and Tanzania, however, only 62 % in Mozambique (Table 3.3). The discretionary water available in the environment and the water evaporated from the animal were the most accurate predictors of Gp_{PP} (see AUCs in Table 3.3) according to the receiver operating characteristic curve plots (Figure 3.3), where sensitivity is the percentage correctly predicted presence and specificity is the percentage of correctly predicted absence.

The overall single best predictor variable of Gp_{PP} resulting from NicheMapper outputs, as identified by the highest GOF score, was predicted metabolic rate of the fly. I thus further examined this predictor to better understand the mechanistic link between fly metabolic rate and Gp_{PP}. I fitted a range of linear, simple and more complex polynomial models to the reference map to obtain an estimate of the nature of the relationship between the NicheMapper variable predicted metabolic rate and optimal or highest probability of *G. pallidipes* presence (see e.g. Kearney *et al*. 2010b, Figure 1b). The best fit model was determined based on the Bayesian Information Criteria (BIC) and calculation of akaike (or in this case, Bayes) weights (w_i) following the general approach for curve-fitting adopted in Angilletta (2006). I chose BIC because of the higher degree of penalizing for additional parameters in comparison to AIC. I then established a colour-ramp scale according to the results of the best-fit model from NicheMapper predicted metabolic rate (Kearney *et al*. 2010b) and present the simulation results spatially on this colour ramp using ArcGIS.

3.2.5 Temporal model validation and statistical analyses

To assess the validity of the large–scale current and future estimates of *G. pallidipes* distribution, I also sought to compare the hourly and monthly NicheMapper outputs from the model to field estimates of resting metabolic rate and seasonal variation in field trap catches. I obtained metabolic rate estimates from the NicheMapper model as predicted for October for a point simulation undertaken at the exact same location where flies were caught for laboratory trials done in Zambia in October 2006 (Mfuwe 13°1'47.99"S, 31°26' 59.99"E, see Terblanche *et al*. 2009 for site specifications and laboratory trial details). Major factors influencing tsetse metabolic rate include temperature and body size (e.g. Terblanche and Chown 2007). I therefore specifically considered

simulations for three fly body sizes held constant (39.1, 48.7 and 53.2 mg) which represented the largest male fly across all laboratory trials, the largest female for trials done at 20 and 24 °C and the largest female fly measured during the 28 and 32 °C trials. Temperatures for these model simulations were 20 ± 0.24 24 ± 0.26 28 ± 0.22 and 32 ± 0.30 °C respectively. The mean MR_{PRED} (± SD) from the NicheMapper model was compared to laboratory obtained estimates of resting metabolic rate (MR_{LAB}) for the range of fly sizes. Measurements of MR_{LAB} were made across a range of constant temperatures (20 ± 0.5; 24 ± 0.5; 28 ± 0.5 and 32 ± 0.5 °C) for the given masses of males and females respectively (Terblanche *et al*. 2009). Model performance was assessed by plotting MR_{PRED} as a function of MR_{LAB} and testing if the relationship differed significantly from a slope of 1 (following e.g. Piñeiro *et al*. 2008).

Field trap catch data for *G. pallidipes* were obtained from Table 1 in Hargrove (2003) although the data were originally reported by Vale *et al*. (1988). Here I used the data from the two sites where there were no insecticides applied (i.e. control sites in Vale *et al.* 1988). NicheMapper predictions of resting field metabolic rates at three points within the trapping sites (16°1'47.91"S 28°56'46.42"E; 16°5'24.08"S 29°2'0.36"E; 16°9'35.79"S 29°9'17.88"E) were compared to trap catch data to determine the relationship between model-based predictions of optimal metabolic rate and population size variation in the field. I estimated the relative number of flies caught per trap per day for each site, as a proportion of the highest number of flies caught during the trapping period. Specifically, I assessed if fly populations declined below optimal MR_{PRED} and increased when above MR_{PRED} as a test of NicheMapper model performance.

NicheMapper MR_{PRED} in Zambia in October closely agreed with laboratory estimates of MR for a range of sizes and at a range of temperatures (Figure 3.5). Comparison of slopes showed that the slope of the relationship between MR_{PRED} and MR_{LAB} did not differ significantly from 1 (t_7 = 1.58, p = 0.92). The exponential function describing the metabolic rate (J/d) – temperature (°C) relationship from the NicheMapper simulation in Zambia for a constant body size can be written as

$$MR_{PRED} = 3.85e^{0.078 \times T} \qquad \text{Equation 4}$$

where MR_{PRED} is in J/d and temperature (T) is in °C. Further temporal model validations on an annual scale showed that fly trap-catch data from the Zambezi Valley in Zimbabwe (mean ± SE) followed an offset, though correlated, pattern of monthly MR_{PRED} (mean ± SE) (Figure 3.5). Like MR_{PRED}, the number of flies caught per trap decreased from February 1984 to May 1984. These trap catches remained fairly constant while MR_{PRED} was low and declined even more during July to September as a result of the very low metabolic energy predicted in the previous month. However,

soon after MR_{PRED} rose above the optimum obtained from curve-fitting procedures (26–27 J/d) the trap catch data increased, suggesting adequate model performance at annual time–scales.

3.3 Incorporating plastic responses into NicheMapper

Between-population variation in climatic stress resistance in tsetse can be explained by phenotypic plasticity induced by thermal acclimation of adults (Terblanche *et al*. 2006, 2008) and I therefore undertook model simulations for three possible plastic responses. First, I assessed responses of tsetse to no-plasticity (NP) to serve as a null-model, second, using a beneficial acclimation (BA) type response and, finally, a deleterious acclimation (DA) type response in the adult life stage.

Plastic response simulations were conducted at the most northern (7°31′N, 34°23′E), eastern (8°54′S, 38°25′E), southern (20°30′S, 33°42′E) and western (5°30′S 26°00′E) known distribution range limits of Gp_{PP} (see Table 3.4) for elevation (m), mean monthly temperature (°C) and annual precipitation (mm) information). Here, I considered four sub-optimal climate change outcomes in terms of moisture availability and temperature to explore their impacts on *G. pallidipes*. These realistic ranges of climate combinations were referred to as hot/dry, hot/wet, cold/dry, cold/wet scenarios (Table 3.5).

I also considered a set of 'control' conditions, corresponding with optimal laboratory-rearing conditions as a benchmark against which to draw comparisons under altered climate scenarios. To explore these cases I set up microclimate data (consistent throughout the year) for each simulation, creating a full factorial design of environmental variation by changing relative humidity (%) and temperature (°C) in the microclimate input tables.

Response parameters were based on results by Terblanche *et al*. (2006) and the full range of input trait values is given in (Table 3.5). After a 10 day acclimation at 19, 24 and 29 °C the lower temperature acclimation groups showed a significantly lower critical thermal minimum (CT_{min}) than the other temperature acclimation groups (Terblanche *et al*. 2006). This suggested a significant plastic, beneficial response towards cooler conditions in *G. pallidipes*. Consequently, I consider a CT_{min} of 15 °C during BA simulations at colder scenarios (Table 3.6) while using a CT_{min} of 19 and 23 °C under NP and DA simulations respectively. Prior acclimation to warmer conditions significantly decreased water loss rates (WLR) at hotter test conditions (from 364.6 to 183.3 µg/h) (Terblanche *et al*. 2006). Furthermore, acclimation at cooler conditions led to a significant increase in body water content (BWC) (29.2 ± 0.7 mg; Terblanche *et al*. 2006). By contrast with all other

simulations, in these plasticity simulations I also allowed maximum body mass (Mb_{max}) to vary according to the plastic response shown in the BWC trait in the plasticity simulations so that Mb_{max} was 77.2 mg under BA and 59.4 mg under DA, cooler simulations. The critical thermal maximum (CT_{max}) did not show a significant plastic effect after acclimation (Terblanche *et al*. 2006) and was thus held constant across all simulations. The DA simulations followed the exact opposite patterns than BA simulations relative to NP. In this part of the Chapter I specifically concentrated on WLR (µg/h) and metabolic rate (J/d) to determine if phenotypic plasticity could compensate for climate change or perhaps 'rescue' populations under risk of extinction.

Water, rather than energy consumption, is more strongly influenced by plastic responses in *Glossina* (e.g. Terblanche *et al*. 2005, 2006). Drier scenarios in particular led to higher rates of water loss through evaporation (Figure 3.6. A) while hotter scenarios led to higher metabolic rates given that these flies are ectothermic (Figure 3.6. B). Ultimately, at the four edges of the current *G. pallidipes* geographic range, it seemed like water loss would be the main factor leading to population declines in the west and energy expenditure in the north under hotter/drier scenarios (Figure 3.6. A, B)

3.4 Vector distribution implications with climate change

3.4.1 Data presentation

Given the high GOF score for MR_{PRED} and Gp_{PP} (see Section 3.2.4), I attempted to find the best fit model describing the relationship between MR_{PRED} and the known Gp_{PP}. Upon comparing model fits of MR_{PRED} to the Gp_{PP} data (Figure 3.7), higher order polynomials consistently returned a higher AIC and BIC. I stopped model fitting at the fifth order polynomial because it was clear what values of MR_{PRED} explained Gp_{PP} best. The point where the derivative of each of the polynomial functions (Table 3.8) equalled zero (z_i) was estimated. The highest turning points (i.e. explaining the highest Gp_{PP}) of the third, fourth and fifth order polynomials z_i was 25.6 26.6 and 27.1 J/d, respectively (Table 3.8). I chose a range of 26 – 27 J/d as an indication of optimal MR_{PRED} for Gp_{PP} at a spatial and temporal scale. I present these values on a colour ramp based on the polynomial relationship (Figure 3.7).

3.4.2 Future distribution implications

I considered the five countries focussed on during model performance testing (Section 3.2.3 of this Chapter) at a 2.5 minute resolution, and all fifteen countries where *G. pallidipes* is currently present at a resolution of 5 arc-minutes (0.083 degrees ≈ 85 km^2 at the equator). The downscaled forecasts under the HadCM3 scenario of gas emissions is described by a high population growth, substantial increased energy use, fast land-use change and slow technological change (Collins *et al*. 2001; IPPC 2001; Pope *et al*. 2002). The climate variables that were included in NicheMapper to project the 2080 distribution included the mean monthly maximum temperature (in °C), mean monthly minimum temperature (in °C) and mean monthly rainfall (in mm) (see for e.g. Kearney and Porter 2004).

Spatial simulation results for MR_{PRED} in the five major countries of current Gp_{PP} at a fine spatial scale are shown in Figure 3.8 A-C with the optimum MR_{PRED} given in red. Here, MR_{PRED} under the current climatic conditions given from WorldClim (2000) (Figure 3.8 B) and future HadCRUT A2a global climate (2080) (Figure 3.8 C) shows a clear decrease in the optimal habitat as indicated by the reduction in area where MR_{PRED} is optimal. For these five countries the proportion of Gp_{PP} > 90 % was 8.3 % (Figure 3.8 A). In addition, the proportion of cells where MR_{PRED} fell between 26 and 27 J/d under current conditions was 8.2 % (Figure 3.8 B). Thus, no significant difference was detected between the areas where Gp_{PP} is currently present and the NicheMapper predictions under current (2000) scenarios (n = 81 412 cells, $p > 0.05$), further suggesting that MR_{PRED} worked well in capturing $Gp_{PP.}$ In consequence, I next scaled up to the whole area of all 15 countries where *G. pallidipes* can be found, and examined MR_{PRED} under the current and future climate scenarios (Figure 3.9). This analysis showed that MR_{PRED} values within the 26 – 27 J/d range were currently 631 (53 635 km^2) while in future (2080) it would be reduced to 469 cells (39 865 km^2) of optimal habitat. In sum, this indicates a 1.7 times smaller optimal area under future climate change scenarios than under the current climate scenario across all fifteen countries. Relative to the total number of cells (n = 12 440), the net decrease in optimal area was 162 ÷ 12 440 = 1.3 % of the total area. The mean results for the model predictions in the 15 countries where *G. pallidipes* can currently be observed indicate that there is an increased probability of encountering a higher average metabolic rate in those environments in the future (Figure 3.10). It appears that several countries that are currently sub–optimal for *G. pallidipes* will become optimal in the future based on MR_{PRED} (Figure 3.10), including Burundi, Malawi, South Africa, Tanzania, Uganda, Swaziland, Zambia and Zimbabwe. By contrast, only a few countries in Africa where *G. pallidipes* currently occur will

become sub–optimal in 2080 for MR_{PRED}. These include central Mozambique, the Democratic Republic of Congo, southern Sudan, northwest Kenya and Somalia (Figure 3.10).

3.5 Disease risk implications with climate change

In the final part of this Chapter, I estimated changes in the basic reproductive rate of the disease caused by *T. vivax* (R_0) calculated across all fifteen countries where *G. pallidipes* is currently present on a 5 arc–minute resolution. I solved a mathematical equation to calculate the days between blood meal feeding, termed feeding intervals (F_i), under the assumption that 'hunger' (i.e. energy depletion, and not simply water depletion) is the main reason for feeding. To predict the F_i, I considered the results from Hargrove (1999) who concluded that a lipid mass of approximately 2.5 mg becomes available to the fly after blood meal feeding. Given that the energetic equivalent of 1 mg lipids = 37 J (Cahill 1970 and see Custer 2005), I multiplied the total lipid reserves (mg) obtained during a feeding event by a constant of 37 to give an estimate of energy (in J) available. The equations describing F_i, estimating the time until the energy reserves have been depleted, as a function of lipid yield and energy consumption (predicted metabolic rate in J/d) can then be described by

$$F_i = (a \times b) \div MR \quad \text{Equation 5}$$

where F_i is the days between blood meal feeding, a is the amount of lipids provided by taking a blood meal (mg), b is the conversion constant of lipids to energy yield in J and MR is metabolic rate of *G. pallidipes* in J/d.

The basic reproductive rate, R_0, is commonly used to estimate the number of new cases of a disease that will arise from one current case (i.e. disease turnover), under the assumption that the entire host population is susceptible (Dietz 1993; see review in e.g. Heffernan *et al*. 2005). This rate of reproduction of *Trypanosoma vivax* as transmitted by a general tsetse vector can be described by the function

$$R_0 = (a^2 \times b \times c \times m \times exp\,[-uT]\,) \div (u \times r) \quad \text{Equation 6}$$

as given by Rogers and Packer (1993), where a is the biting rate of the vector on the host (calculated as $1 \div F_i$ obtained from Equation 5 above), b and c are the transmission coefficients from the fly to the animal and *vice versa*, m is the ratio of vector to host numbers, u is the mortality rate of the vector, T is the incubation period of the parasite in the vector and r is the rate of recovery

of the animal from infection. Keeping all parameters except *a* constant, I assume therefore that, from Equation 6,

$$R_0 = a^2 \qquad \text{Equation 7}$$

where *a* is squared because two feeding events are necessary for transmission of the parasite to a host. Note that these estimates of R_0 are likely conservative as incubation period of *T. vivax* may well decline as temperature increases (Rogers 1988). I used the Wilcoxon signed-rank statistic (W) to compare the results of R_0 between current and future climates (2000 and 2080) instead of a paired t-test as the normality and variance assumptions were not met for the latter and verified that all the assumptions for the Wilcoxon signed-rank test were met instead.

3.6 Disease transmission implications under climate change

When Equation 5, is solved, with average MR_{PRED} over the whole area of 15 African countries = 23.3 J/d, lipid yield (*a*) = 2.5 mg and *b* is a constant 37, the result is F_i = 3.97 days. This estimate is remarkably close to the value for F_i (4 days) used in Rogers' (1988) 4 days for calculation of *Ro*. When Equation 5 is solved using the average MR_{PRED} in the future, 31.5 J/d, keeping *a* and *b* constant, the resulting F_i = 2.94 days. These two estimates of the time between blood meals, reflect a daily biting rate (1 ÷ F_i) of 0.25 under current scenarios and 0.34 under future scenarios. Assuming that $R_0 \alpha a^2$ (Equation 6), where *a* is the daily biting rate, keeping all else constant, R_0 under current climate scenarios is 0.0625 and 0.1156 under future climate scenarios. If the R_0 value is comparable and the assumptions are reasonable, one obvious conclusion is that the number of new cases of disease that will arise from a single infection is 0.1156 ÷ 0.0625 = 1.85, which reflects an 85 % greater transmission risk in the future relative to the current estimation of transmission risk. The Wilcoxon matched pairs test showed that the median of MR_{PRED} was significantly higher under the future climate scenario compared to present day climate ($W = 885$, $p < 0.001$) (Figure 3.11). Furthermore the location of the median of F_i was significantly lower under the future climate scenario ($W = 96.5$, $p < 0.001$) (Figure 3.11). Subsequently, the daily biting rate was significantly higher ($W = 96.5$, $p < 0.001$) and the reproductive rate (*Ro*) significantly higher ($W = 96.5$, $p < 0.001$) under the future climate scenario. Ultimately, the estimation and analysis of R_0 suggests that, although there is a small but significant decrease in optimal area (1.3 % of the total area of the countries of current presence) for *G. pallidipes*, the R_0 of *T. vivax* increased by 85 % under the future climate scenario relative to the current scenario.

3.7 Discussion

In ectotherm insect vectors of disease there is generally a close agreement between core temperature (T_c) and the environment. Due to the small size of insects, T_c responds rapidly to changes in ambient temperature which affects a broad range of physiological and life-history traits, ultimately affecting incidence and seasonal variation of vector-borne disease (Rogers and Randolph 2006; Lambrechts *et al*. 2011). The energy budget of an animal in a steady state condition is a result of the difference of energy input and output of the organism (Porter and Gates 1969). Reflection is one of the parameters in the energy balance biophysics of an animal that cause an animal to lose heat (Porter and Gates 1969). An average reflection of ~ 30 % in tsetse fly *G. pallidipes* is considerably less than that of a dark black carabid beetle (80 %) and a light coloured lizard (~ 60% in Porter 1967; Porter and Gates 1969), however, slightly more than the average heliothermic lizard (~ 16.3 %) (Clusella-Trullas *et al*. 2008). The complex interactions of evaporation and heat energy fluxes influence the T_c of insects to a large extent. An increase in temperature generally increases biochemical reaction rates in insects and requires increased energy availability and consumption (either through increased foraging rates or amounts, or perhaps changes in fuel source) to maintain their energy balance (Chown and Nicolson 2004; Irlich *et al*. 2009; Knies and Kingsolver 2010). After steady–state energy balance calculations, NicheMapper estimates the amount of energy available, used and remaining for the animal (Porter and Mitchell 2006). Discretionary energy is the difference between available energy due to consumption above maintenance requirements and metabolic rate. Thus, one would expect a strong correlation between energy available to *G. pallidipes* in the field and discretionary energy. Increased metabolic rates permit increased activity, growth, development and reproduction (Bursell and Taylor 1980; Lafferty 2009), which drive population dynamics and also, potentially, species distribution. Colder temperatures typically decrease development rates of insect vectors or parasites within the vectors, leading to decreased disease risk (Lindsay and Martens 1998; Paaijmans *et al*. 2010). By contrast, increased temperatures lead to higher energetic demands, increased feeding rates and, in the case of insect disease vectors, a likely increase in disease transmission risk (e.g. Hargrove and Coates 1990; Githeko *et al*. 2000; Pascual *et al*. 2006; Paaijmans *et al*. 2009). Climate change science, together with sophisticated computer models of future global climate regime changes, enables an ensemble forecast system of disease dynamics (Elith *et al*. 2006; Cowie 2007, p. 357–359; Ford *et al*. 2009; Eisen and Eisen 2011) that can be applied to predict and prevent infections and assist vector control–efforts by identifying the direction of geographical shifts in populations (Martens *et al*. 1999; van Lieshout *et al*. 2004; Martens and Thomas 2005; Gething *et al*. 2010). The accurate prediction of species

distribution and abundance forms a central point in ecological applications and conservation science (Elith *et al*. 2006), in addition to playing a particularly significant role in disease intervention planning (Patz *et al*. 2005).

Vector control is arguably the best control-strategy for HAT and AAT in sub-Saharan Africa (McDermot and Coleman 2001; but see Bourne *et al*. 2001). While *Glossina* spp. are of crucial importance to health services and the socio-economic development of Africa, neither the current nor predicted future distribution of tsetse has been modelled following a mechanistic approach. Here I use *G. pallidipes* as a model organism for vectors of human and animal disease to explore such an approach. Exploration and validation of the model estimates were the first steps in the process to predict the distribution and abundance of *G. pallidipes* under current and future climate scenarios.

After investigating the co-linearity of the predicted NicheMapper estimates and finding a very high degree of parameter correlation, I decided upon a single parameter model for predicting future optimal sites of presence. I validated and chose the best predictor variable from the NicheMapper model using a known distribution map of *G. pallidipes* presence (Wint and Rogers 2000) as a reference map, before comparing the maps using two statistical methods that differed substantially in their practice. The AUC is a threshold-independent measure that compares a binary reference map (known distribution) to a continuous predicted map; however it did require that arbitrary set thresholds had to be used to form the binary presence/absence dataset. By contrast, the GOF score compared the reference map on a continuous scale to the predicted dataset, therefore using the whole set of data in both cases. The GOF compared the categories of each map to provide an estimate of how well the categories explained similar spatial resolutions (Hargrove *et al*. 2006; DeVisser and Messina 2009). Both approaches indicated a good model fit and I chose to use the GOF model predictions of the best estimate parameter, namely metabolic rate of *G. pallidipes* as predicted by NicheMapper. Comparisons of MR_{PRED} and MR_{LAB} showed good agreement under a range of temperatures and a range of body sizes (Figure 3.4). Furthermore, temporal model assessments with population dynamics and metabolic rate estimates made in the field also confirmed the model's applicability and accuracy (Figure 3.5).

The metabolic rate-temperature relationship explains a vast amount of inter population variability in *G. pallidipes* (Terblanche *et al*. 2009). Specifically, for the range of body sizes measured in Zambia, the temperature dependent relationship of the measured laboratory results of metabolic rate of a *G. pallidipes* population in Zambia can be written as

$$MR_{LAB} = 2.05e^{0.087 \times T} \qquad \text{Equation 8}$$

where *MR* is measured resting metabolic rate (in J/d) and temperature (*T)* is in °C, the mean body mass is 36.6 ± 6.40 SD mg (Figure 3.12). The MR_{PRED} is at most 6.39 J/d greater than the measured field metabolic rate at 24 °C. A possible explanation for this discrepancy is body size since the NicheMapper model used a fly body size of 59.4 mg (which is fairly large for *G. pallidipes* in general, however, represents a healthy female fly. The range of field fly body masses by Terblanche *et al*. (2009) was 37.31 ± 9.79 SD. Typically, metabolic rate in *Glossina* is positively correlated with body mass (Basson and Terblanche 2011).

The linear regression of the metabolic rate-body size relationship for a range of *G. pallidipes* body masses and metabolic rate records can be described by the equation:

$$MR_{LAB} = (279.71 \times Mb) + 5.9428 \qquad \text{Equation 9}$$

where MR_{LAB} is measured resting metabolic rate for wild caught individuals from four populations of *G. pallidipes* in East Africa (Terblanche *et al*. 2009) (in J/d) and Mb is body mass (in g). Measurements were made at a controlled temperature of 24 °C across a range of body sizes.

The offset pattern of approximately 30 days between trap catches and average model predictions per month (Figure 3.5) likely reflects the larval and pupal incubation period of *G. pallidipes* (approximately 32 days at 25 °C, Leak 1999). Specifically, approximately 1 month following the point where predicted metabolic rates of the flies exceeded ~ 26.5 J/d, the number of flies caught in the traps started rising. Due to the high cost of reproduction in this genus, this might reflect offspring production once the female flies' metabolic resources exceed a metabolic level high enough for larval development (Bursell and Taylor 1980). Furthermore, a metabolic rate lower than ~ 26.5 J/d seems to be a key determinant factor for extremely rapid population declines in the field.

Epstein (2010) noted that temperature affects the ranges of infectious disease outbreak, while weather affects the timing, intensity and location of these outbreaks. Extremes in weather might lead to the appearance of novel climate events which pose major challenges in a) predicting population-level responses to environmental variability, and b) long-term forecasting efforts on species distribution and abundance. Several CEMs have been implemented for *Glossina* distribution (Rogers 1991; Rogers and Randolph 1991; Rogers and Williams 1993, 1994; Williams *et al.* 1994; Hulme 1996; Rogers *et al.* 1996; Robinson *et al.* 1997b; Robinson 1998; Reid *et al*. 2000; Wint and Rogers 2000). However, most of these vector-distribution predictions are made on a local scale and very few of them consider future projections of climate change (but see Rogers and Packer 1993; Rogers and Randolph 1993 where a correlative approach was implemented). A critical assumption

from Wint and Rogers' (2000) probability of presence records was that the occurrence data, presence and absence from Ford and Katondo (1977), describe the current situation where distribution is limited by climate, and that species are in equilibrium with their environment (Duncan *et al*. 2009; Huntley *et al*. 2010). The latter assumes that species have expanded their realistic range in full with no potential to further expand in range.

Adult WLR significantly decreases after a 10 day exposure to hotter temperatures and BWC increases significantly after a 10 day cool temperature exposure (Terblanche *et al.* 2006). Although much recent attention has focused on disease vector distributions under climate change, remarkably few studies have integrated such plastic responses in predictions under changing temperature and moisture availability. However much attention has been centred on the application of mechanistic models (Buckley *et al*. 2010), to our knowledge, no mechanistic model that incorporates evolutionary life-history traits of tsetse has been published. Moreover, several CEMs have been implemented for *Glossina* distribution (Rogers 1991; Rogers and Randolph 1991; Rogers and Williams 1993, 1994; Williams *et al.* 1994; Hulme 1996; Rogers *et al.* 1996; Robinson *et al.* 1997b; Robinson 1998; Reid *et al*. (2000); Wint and Rogers 2000).

NicheMapper provided the perfect tool for exploring the potential of plastic physiological (and potentially life-history) responses of tsetse in Africa in a mechanistic manner. Here *G. pallidipes* served as a good model organism because its physiological and behavioural properties of this species have been studied intensively. The predicted evaporative water loss and metabolic rate of tsetse under different climate change scenarios were both influenced by beneficial plastic responses. In this Chapter I incorporated very little acclimation plasticity in terms of relative metabolic rate but much more plasticity in water balance (i.e. body water content captured in body mass and water loss rates). Furthermore, the differences between water loss rates under cooler/drier and cooler/wetter scenarios can be ascribed to body water content, while differences between hotter/drier and hotter/wetter is possibly owing to plastic or non-plastic responses (Figure 3.6 A). Moreover, CT_{min} was the only response affected under cooler scenarios; hence, a higher CT_{min} under DA responses could explain the greater thermal window under cooler scenarios, leading to an increased metabolic rate in general (Figure 3.6 B). Ultimately, water- and energy-balance estimates gave an indirect estimate of the probability of population declines from a physiological perspective under the different climate change scenarios. Specifically, the predictions supported water loss to be a limiting factor for *G. pallidipes* populations in the west and energy expenditure in the north under hotter/drier scenarios (Figure 3.6 A, B).

Large scale eradication efforts of tsetse seldom succeed, due to historical, ecological, logistical and financial restrictions (Rogers and Randolph 2002). However, there have been some successful tsetse population suppression schemes over short timescales at small scales (e.g. Vreysen *et al*. 2000). Furthermore, Bourne *et al*. (2001) concluded that anthropogenic environmental impacts such as an increase in the absolute human population size (i.e. settlements), road networks, agricultural practices and decreased number in wildlife and habitat function will add to autonomous control of tsetse in sub-Saharan Africa. The model results in this Chapter provide further support from a physiological perspective for a future decreased area of *G. pallidipes* presence, although coupled with an increased transmission risk. Reid *et al*. (2000) has projected a significant decrease of ~7 % in overall tsetse populations. In concordance with these findings, a significant net decrease (1.5–fold in comparison to current amounts) in habitats with a MR_{PRED} optimal value for abundance has been projected by 2080 in this Chapter. However, although a general decrease in optimality has been projected under future climate change scenarios, some countries that were sub–optimal under the 2000 scenario are likely to become more optimal in terms of average MR_{PRED} in the field under the 2080 scenario. These countries include Burundi, Swaziland, Zambia and Zimbabwe. Of greatest concern are countries such as Tanzania and Uganda that already have a high degree of *G. pallidipes* presence and are predicted to suffer significant increase in terms of optimum metabolism. The model estimates for metabolic rate used to calculate feeding intervals led to values (Figure 3.13) that overlap with previous estimates of feeding intervals in the field. Using Equation 8, I established that MR_{PRED} is 28 J/d when temperature is 25 °C, reflecting a feeding interval of 2.99 ± 0.33 SD days on average between the sensitivity analyses of 2 – 2.5 mg lipid yield from the blood meal. Bursell and Taylor (1980) reported a 2 – 4 day hunger cycle over a daily energetic cost range of 24 − 30 J/d. Randolph *et al*. (1991) also estimated that female *G. pallidipes* feed at intervals of approximately 3 days (see A in Figure 3.13). Moreover, Randolph *et al*. (1992) estimated a mean F_i of 3.5 – 4.5 days which corresponds with a fly with a slightly lower MR_{PRED} (25.1 J/d) at 24 °C, or higher lipid yield per feed, where model predictions of F_i is 3.82 ± 0.62 SD days (see B in Figure 3.13). My calculations of the basic reproductive number of the disease to livestock, caused by *T. vivax*, will increase up to three-fold relative to the current climate scenarios in some of the countries where *G. pallidipes* can be currently found. This supports the prediction of McDermott and Coleman (2001) of a net increase in disease risk (i.e. turnover from one infected individual to another) as a consequence of climate change, at least for AAT.

3.8 References

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3.9 Figures

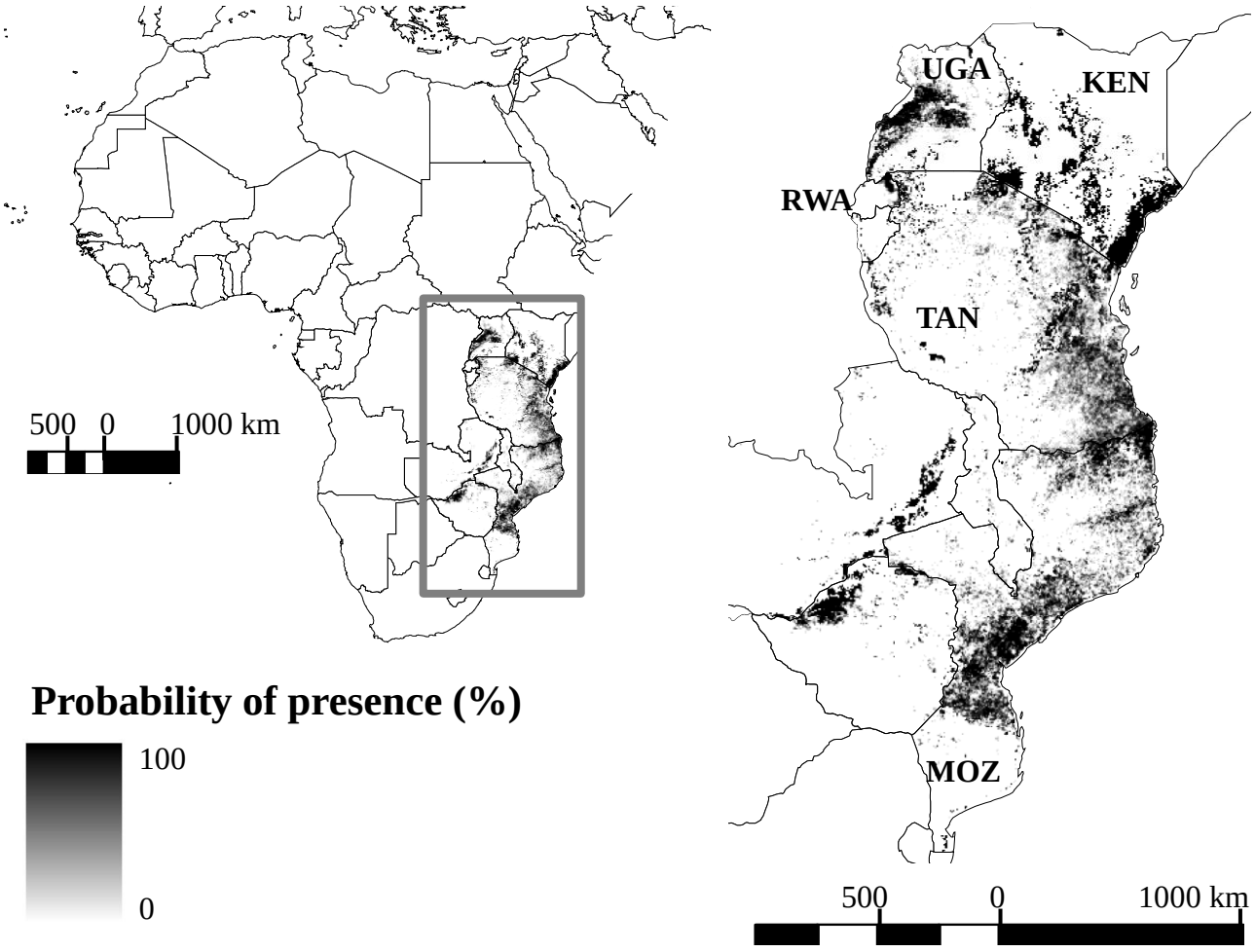

Figure 3.1. Map showing current *G. pallidipes* distribution from Wint and Rogers (2000) and inset shows the five countries in Africa used for model performance evaluation and spatial predictions under current and future climate scenarios. *G. pallidipes* probability of presence (Gp_{PP}) is presented as a percentage on a black to white gradient where black represents a 100 % Gp_{PP} and white a 0 % Gp_{PP}. The mean Gp_{PP} is > 10 % in each of these five countries (Wint and Rogers 2000) with an average ± SD GpPP of 16.1 ± 36.5 % in Kenya (KEN) 16.5 ± 31.4 % in Rwanda (RWA) 19.3 ± 29.0 % in Tanzania (TAN) 22.9 ± 31.4 % in Mozambique (MOZ) and 24.1 ± 35.1 % in Uganda (UGA). Together, these five countries represent 59.4 % of the total Gp_{PP}.

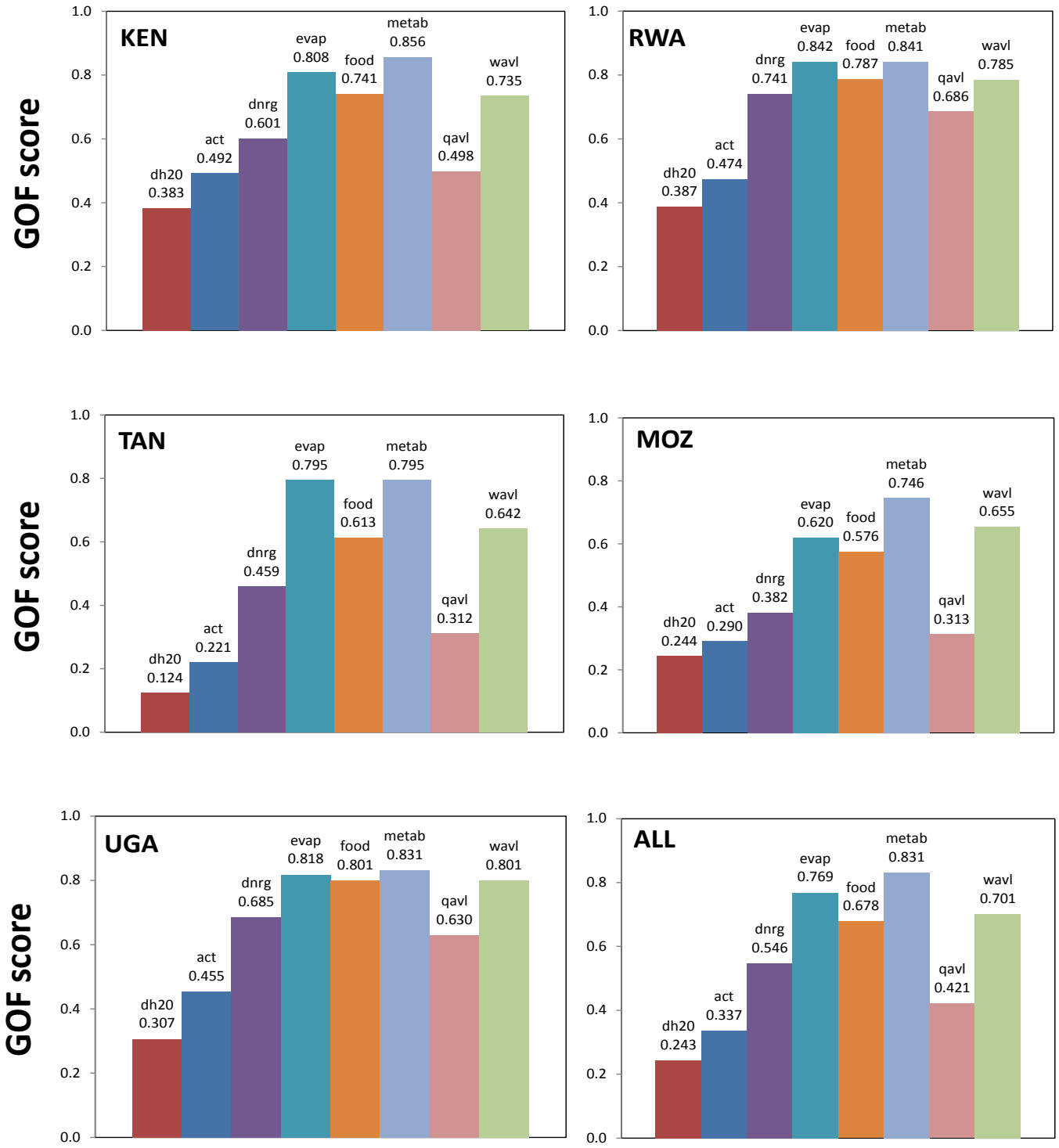

Figure 3.2. Goodness-of-fit (GOF) scores for NicheMapper variables in Kenya (KEN), Rwanda (RWA), Tanzania (TAN), Mozambique (MOZ), Uganda (UGA) and all five countries grouped (ALL). Predictor variables include the daily amount of water available in the environment (wavl in g/d), discretionary water in the environment (dh20 in g/d), activity hours available in the environment (act in h/d), discretionary energy available (dnrg in kJ/d), water evaporated from *G. pallidipes* (evap in g/d), *G. pallidipes* food requirement (food in g/d), *G. pallidipes* metabolic rate (metab in kJ/d) and available energy (qavl in kJ/d).

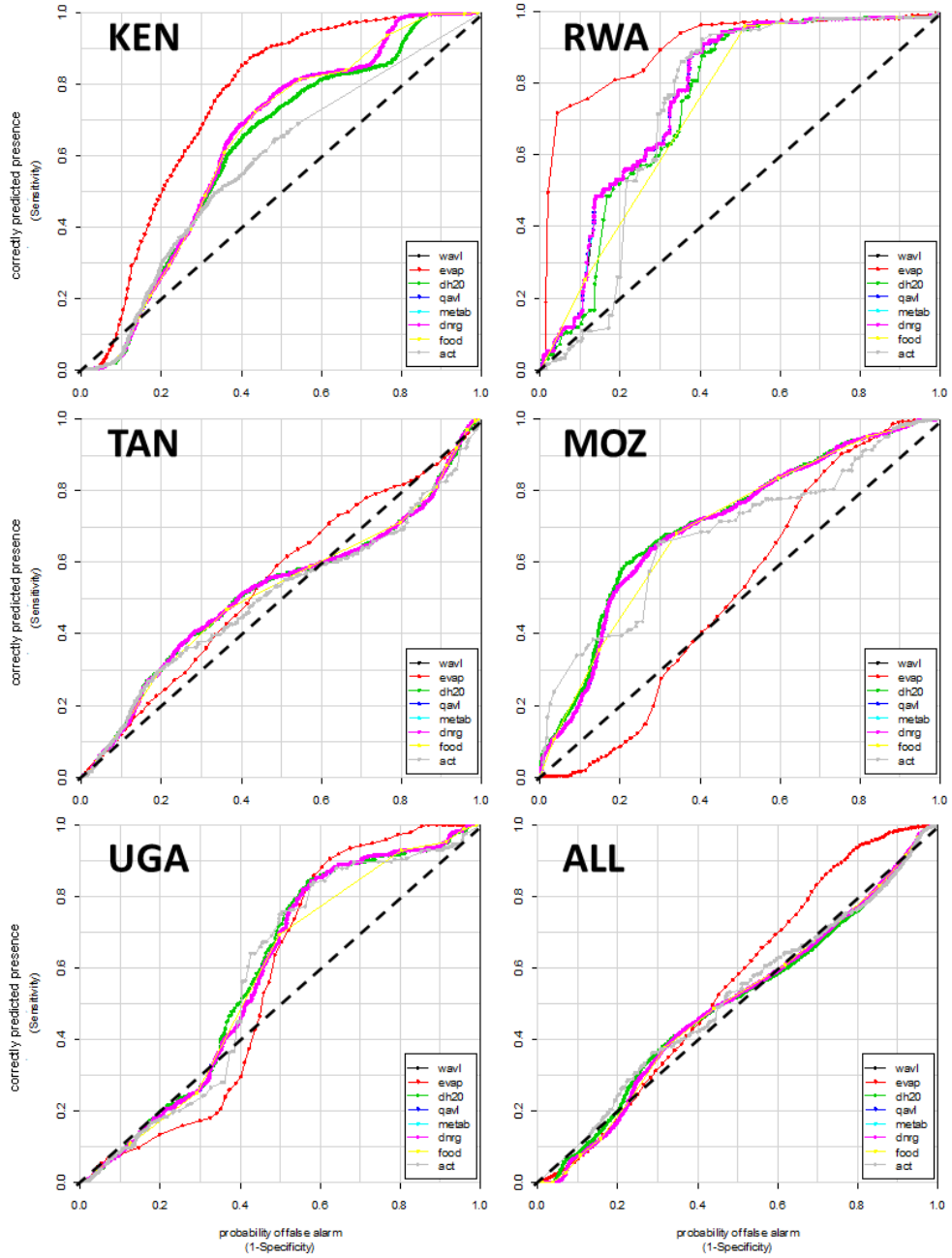

Figure 3.3. Receiver operating characteristic curves from which the AUC (area under curve) values were estimated independently for each predictor variable in Kenya (KEN), Rwanda (RWA), Tanzania (TAN), Mozambique (MOZ), Uganda (UGA) and all five countries grouped (ALL). The thick striped black line with a slope of 1 and intercept at 0 represents a chance equal to 50 % to accurately predict the current *G. pallidipes* presence. Predictor variables include the daily amount of water available (wavl in g/d), discretionary water (dh20 in g/d), activity hours available in the environment (act in h/d), discretionary energy available (dnrg in kJ/d), water evaporated from *G. pallidipes* (evap in g/d), *G. pallidipes* food requirement (food in g/d), *G. pallidipes* metabolic rate (metab in kJ/d) and available energy (qavl in kJ/d). Sensitivity is the % correctly predicted presence and specificity is the % of correctly predicted absence.

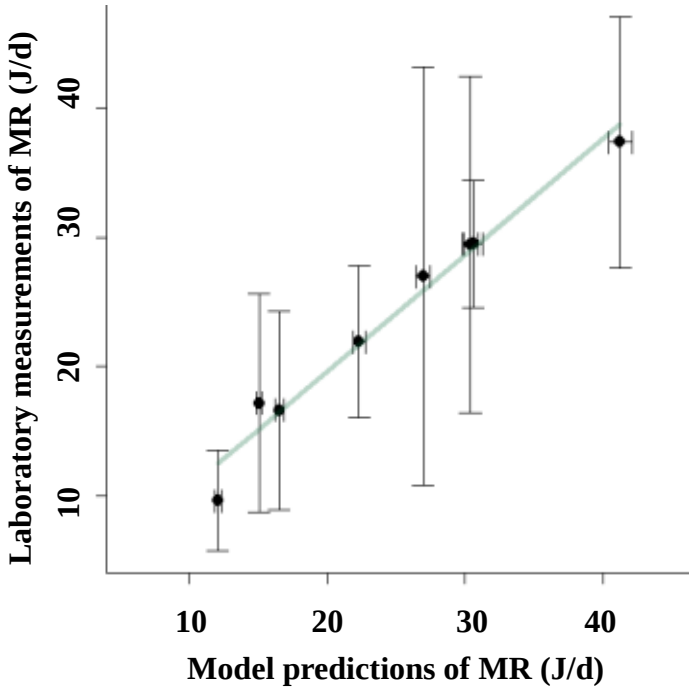

Figure 3.4. The linear relationship between NicheMapper predictions and laboratory measurements of resting field metabolic rate. The metabolic rate estimates from the NicheMapper model (mean ± SD) is those predicted for October from a point simulation done at the exact same spatial point where flies were caught for laboratory trials in Zambia in October 2006 (Mfuwe 13°1'47.99"S, 31°26' 59.99"E, see Terblanche *et al*. 2009). Model simulations were done for three constant body sizes (39.1, 48.7 and 53.2 mg) which represented the largest male fly across all laboratory temperatures, the largest female for measurements done at 20 and 24 °C and the largest female fly measured at 28 and 32 °C. Temperatures for the model simulations are 20 ± 0.24 24 ± 0.26 28 ± 0.22 and 32 ± 0.30 °C respectively. Metabolic rate measurements in the laboratory shows variation as a result of fly size variation. Fly sizes were 32.68 ± 4.35, 41.83 ± 5.51; 37.19 ± 9.15 and 46.27 ± 12.95 mg; 33.02 ± 4.03, 42.58 ± 6.11 , 32.83 ± 4.00 and 42.45 ± 6.34 mg for males and females across the range of constant temperatures 20 24 28 and 32 °C respectively. A comparison of slopes showed that the slope of the relationship between MR_{PRED} and MR_{LAB} did not differ significantly from 1 (t_7 = 1.58, p = 0.92), where the linear relationship can be described by the function y = (0.90 ± 0.06 SE) × x – (1.58 ± 1.64 SE) (R^2 = 0.97).

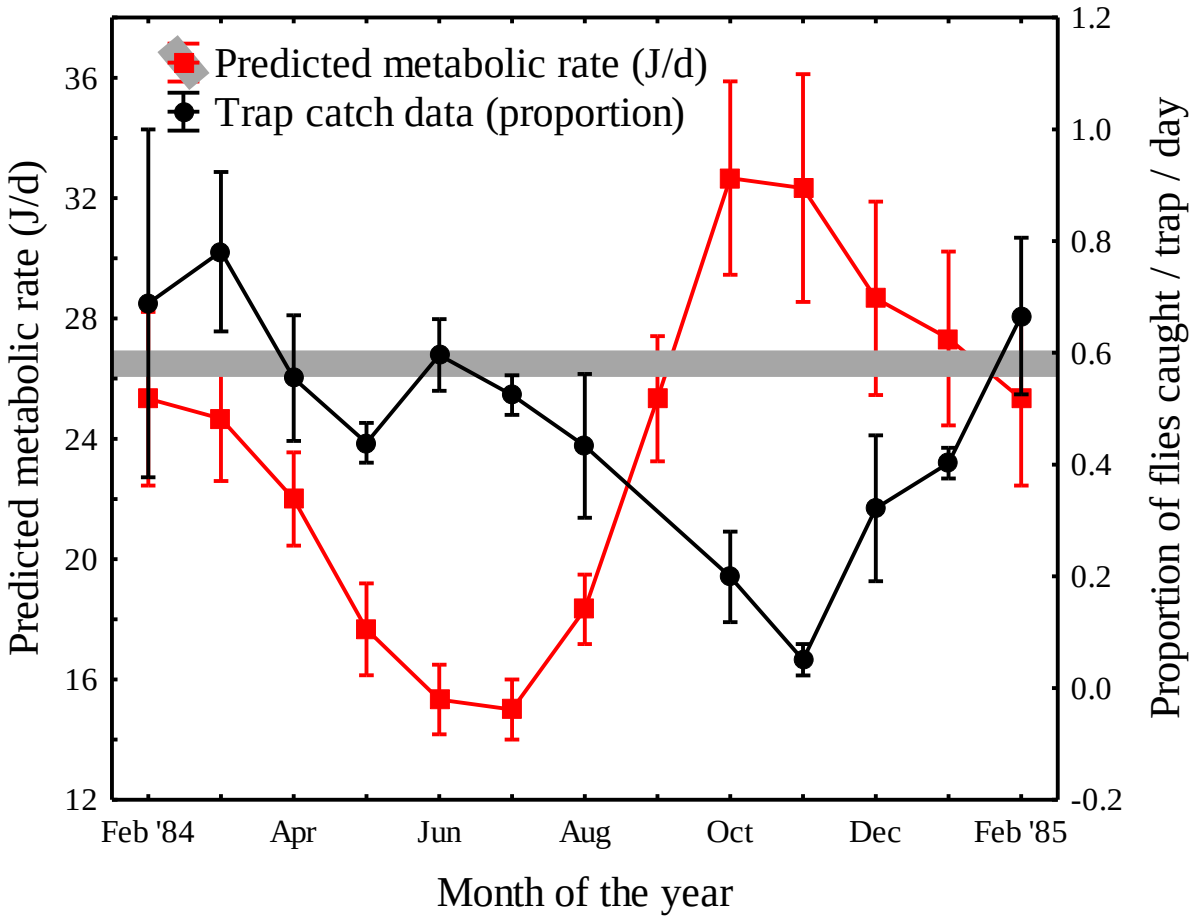

Figure 3.5. Mean ± SE for field trap catch data of *G. pallidipes* in the Zambezi valley, Zimbabwe (from Hargrove 2003) and temporal (monthly) model predictions (mean ± SE) of current resting field metabolic rate in J/d. The trap catches were conducted at two main sites during 1984 – 1985 and shows the mean proportional changes in *G. pallidipes* abundance of the trapping sites. Model simulations were run at three points within the trapping sites (–16°1'47.91"S 28°56'46.42"E; –16°5'24.08"S 29°2'0.36"E; –16°9'35.79"S 29°9'17.88"E). The horizontal grey line at 26 – 27 J/d indicates the optimal metabolic rate value to predict an optimal habitat of *G. pallidipes* in the field and, ultimately, a population size increase after a puparial incubation period of ± 30 days. Three NicheMapper point simulations were performed in the exact same area where the trap catches were obtained.

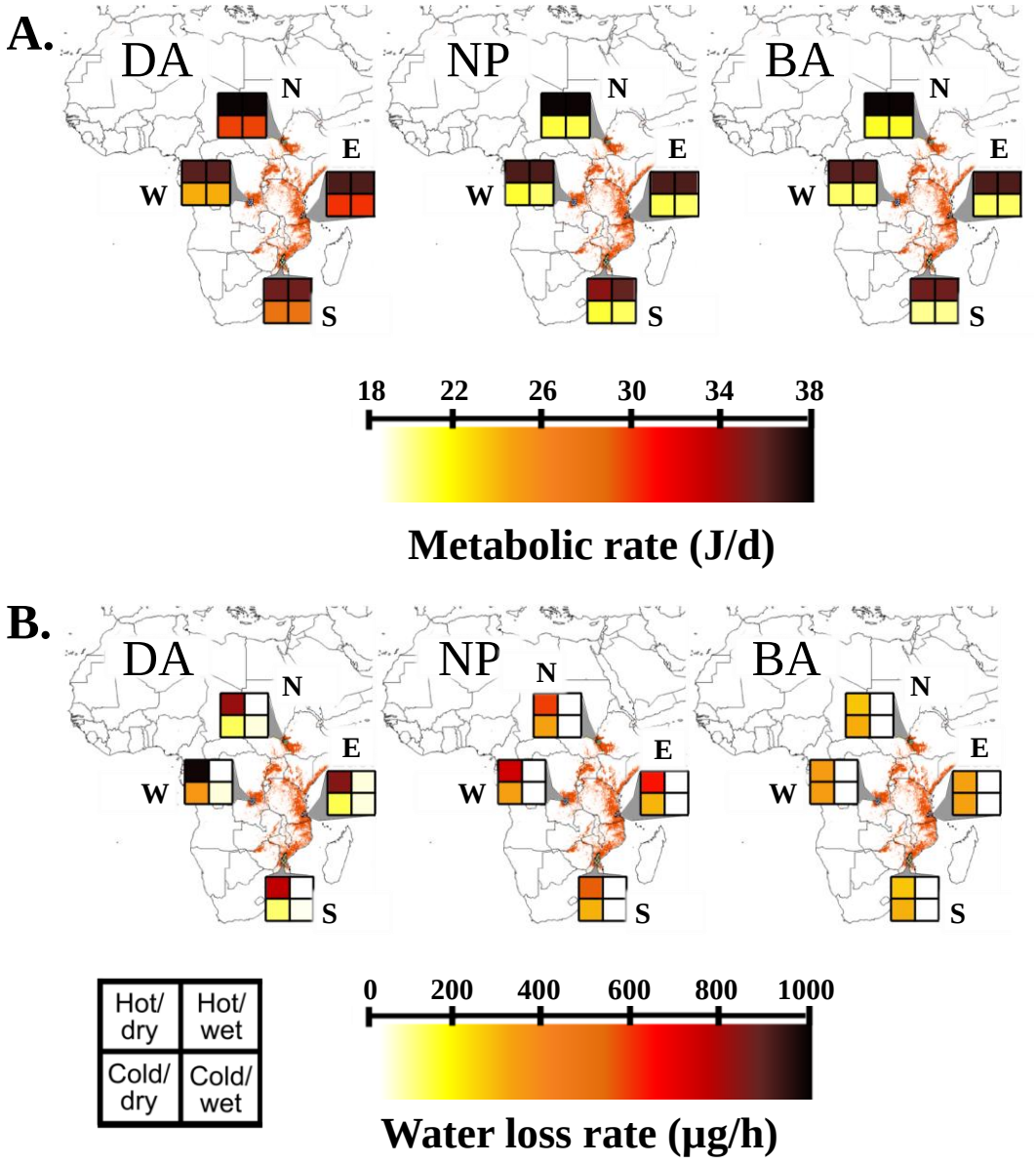

Figure 3.6. Mechanistic model simulation results for four climate change scenarios (cold/dry, cold/wet, hot/dry, hot/wet) at the northern, eastern, southern and western sites of G. pallidipes' range. Each block represents the results of a climate change scenario, shaded according to the (A) water loss rates (water loss rate in g/h) and (B) metabolic rate (metabolic rate in J/d) averaged from monthly data. These simulations were undertaken for adult flies by incorporating non-plastic physiology (NP, measured under static laboratory conditions), beneficial acclimatory (BA, obtained from Terblanche et al. 2006) and deleterious acclimatory (DA, opposite to BA) responses.

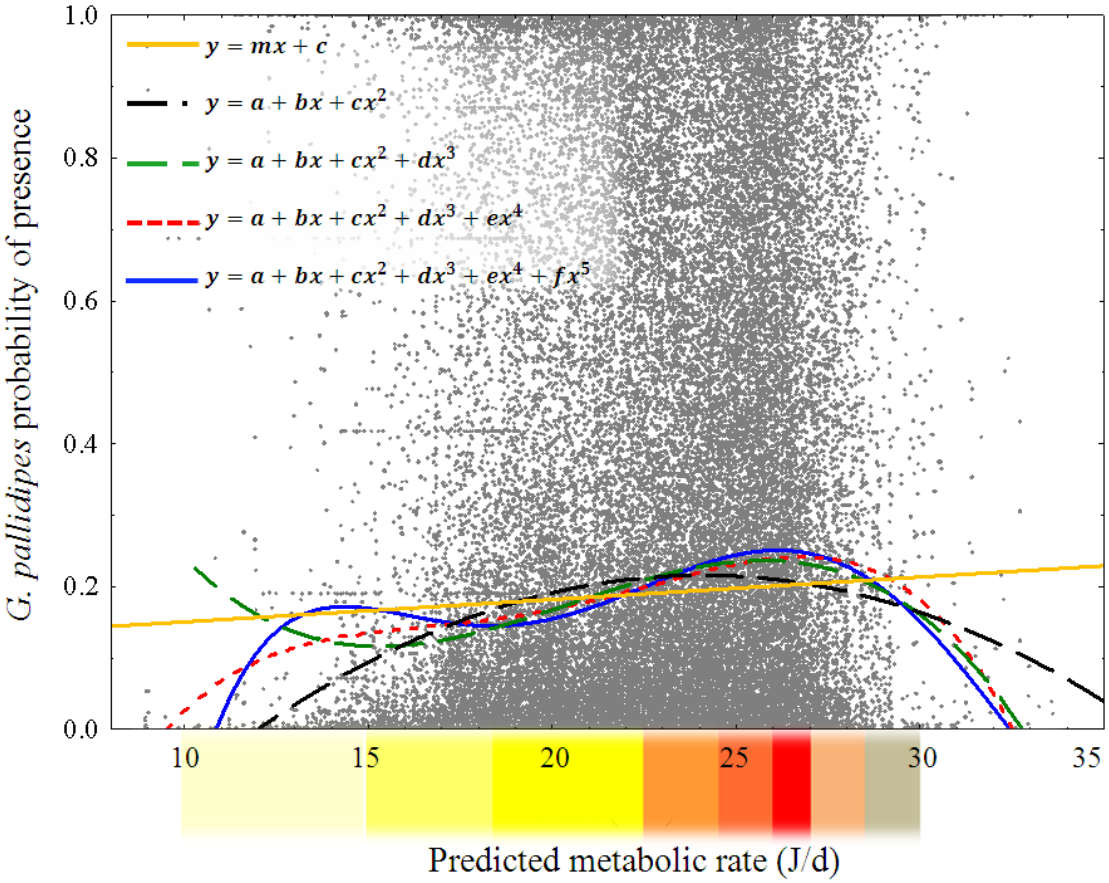

Figure 3.7. Scatterplot of spatial records of *G. pallidipes* probability of presence and NicheMapper predicted metabolic rate in J/d with the polynomial fits shown and the assigned colour ramp where 7 – 10 J/d is white 10 – 15 J/d is light yellow 15 – 18.5 J/h is darker yellow 18.5 – 22.5 J/d is bright yellow 22.5 – 24.5 J/d is orange 24.5 – 26 J/d is darker orange 26 – 27 J/d is red 27 – 28.5 J/d is light brown 28.5 – 30 J/d is grey–brown and 30 – 35 J/d is white again.

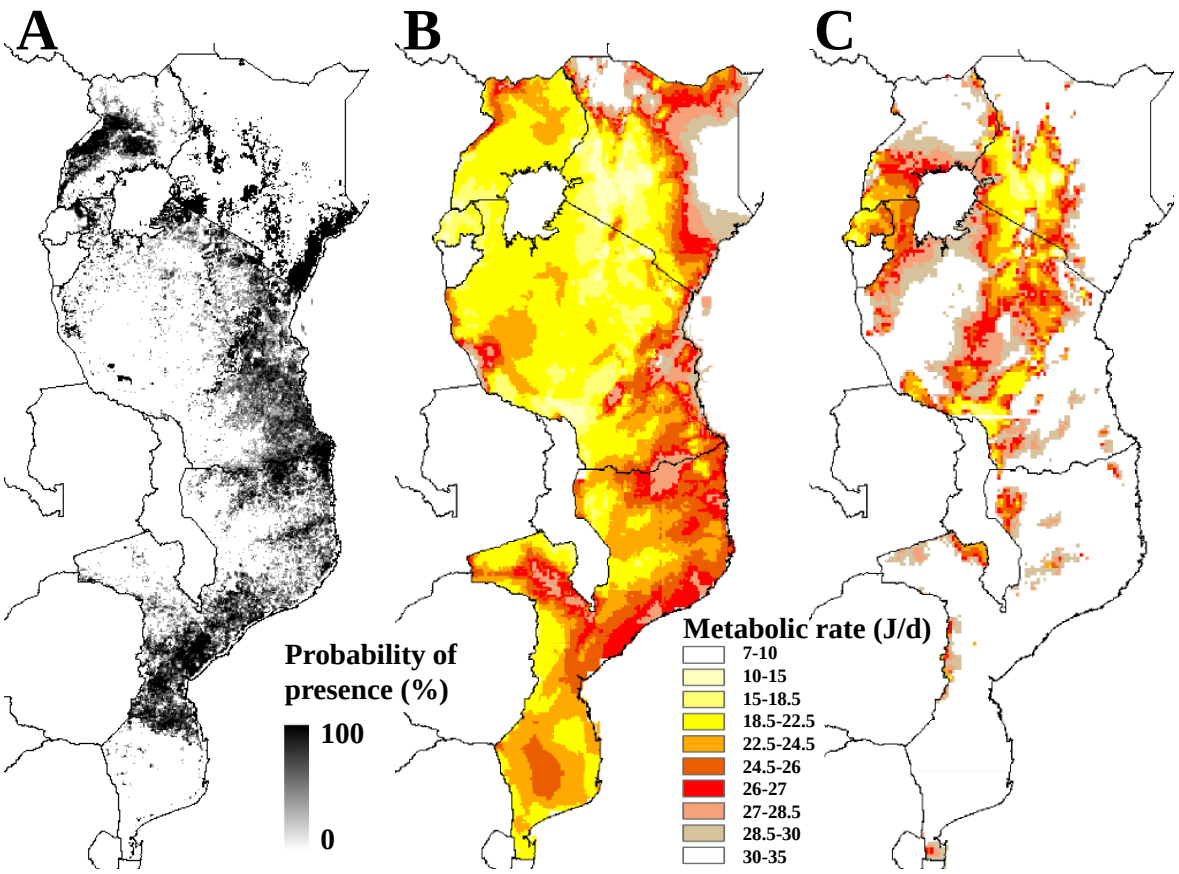

Figure 3.8. (A) Known current *G. pallidipes* probability of presence estimates by Wint and Rogers (2000) and the mechanistic model predictions (B–C) of metabolic rate (J/d) under current (B) and future (C) climate scenarios where the optimum metabolic rate is given in red. The NicheMapper simulation results represents a predicted metabolic rate of *G. pallidipes* under the current climatic conditions (B) given from WorldClim (1960 – 2000) and the HadCRUT A2a global climate change scenario for 2080 (C) at a spatial resolution of 2.5 arc–minutes.

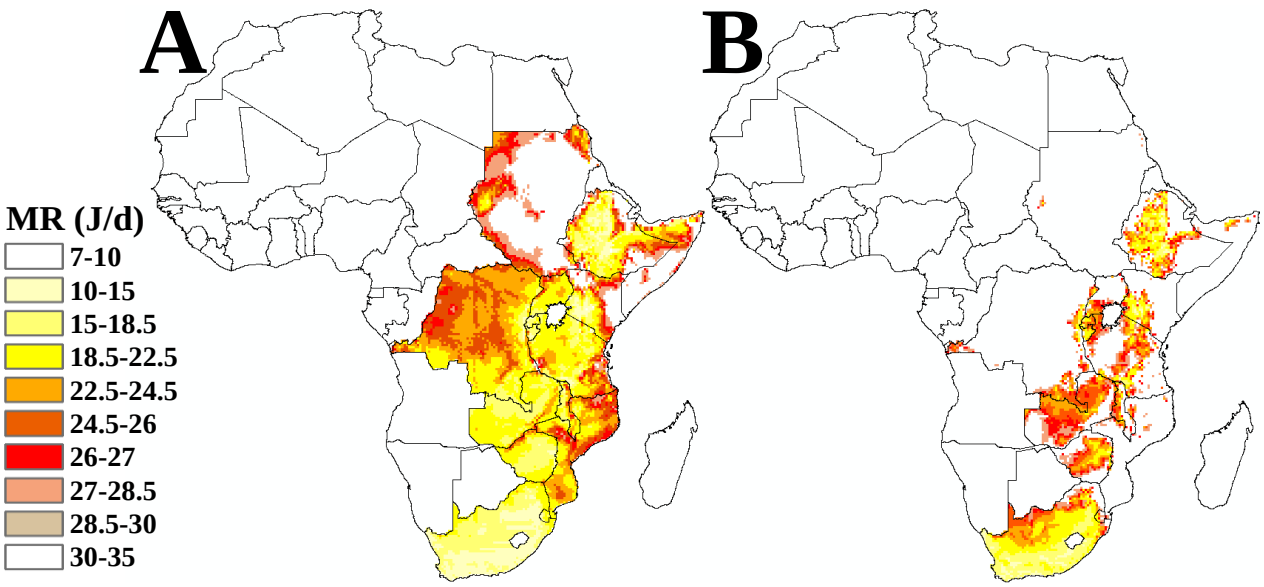

Figure 3.9. Mechanistic model predictions of metabolic rate (MR) in J/d under current (A) and future (B) climate scenarios. Optimal metabolic rate predictions is given in red and sub-optimal metabolic rate predictions both higher and lower than optimal shown lighter according to the colour ramp. The NicheMapper simulation results represents a non-plastic metabolic rate response under the current climatic conditions given from WorldClim (1960 – 2000) and under the extreme HadCRUT A2a global climate change scenario (2080) at a spatial resolution of 5 arc–minutes.

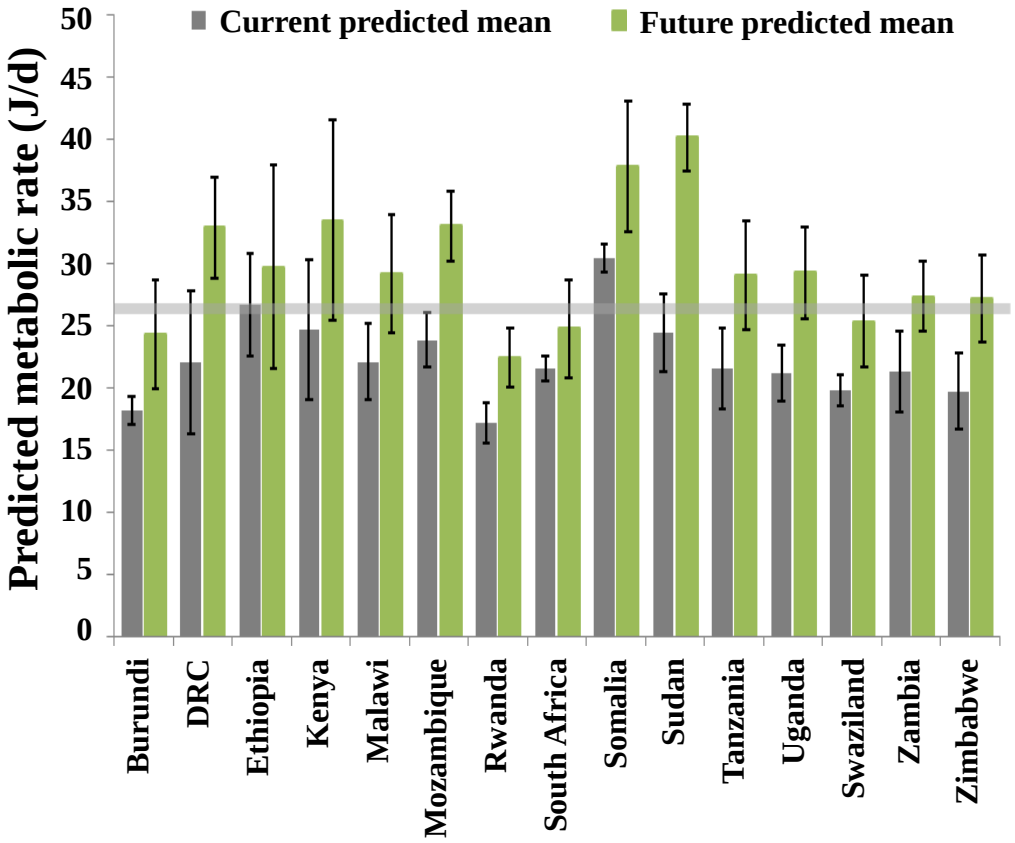

Figure 3.10. Summary results of predicted metabolic rate (mean ± SD) from NicheMapper under current (grey) and future (green) climate scenarios for 15 African countries (DRC = Democratic Republic of Congo) with known records of current (2000) *G. pallidipes* presence. The future climate scenario considered the extreme HadCRUT A2a scenario of 2080 climate projections. The horizontal grey line presents the predicted metabolic rate of ~ 26.5 J/d which suggested the highest probability of fly presence spatially.

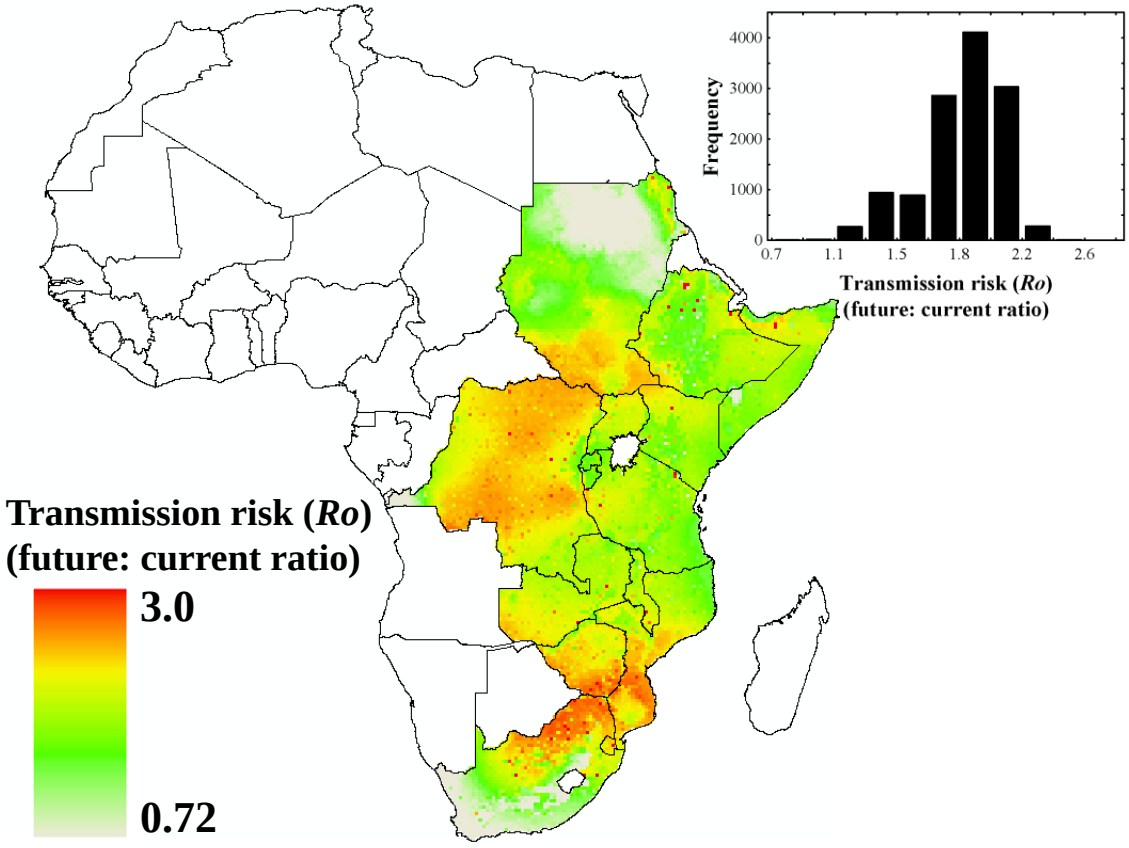

Figure 3.11. Estimated ratio of R_0 (future : current) for the fifteen countries, where *G. pallidipes* is currently present. Red presents an R_0 value up to 3 fold more than current model projections and green represents a smaller increase relative to current projections. The histogram shows the observed frequencies of the ratio between future (2080) and current (2000) predictions of R_0 from which the figure was drawn.

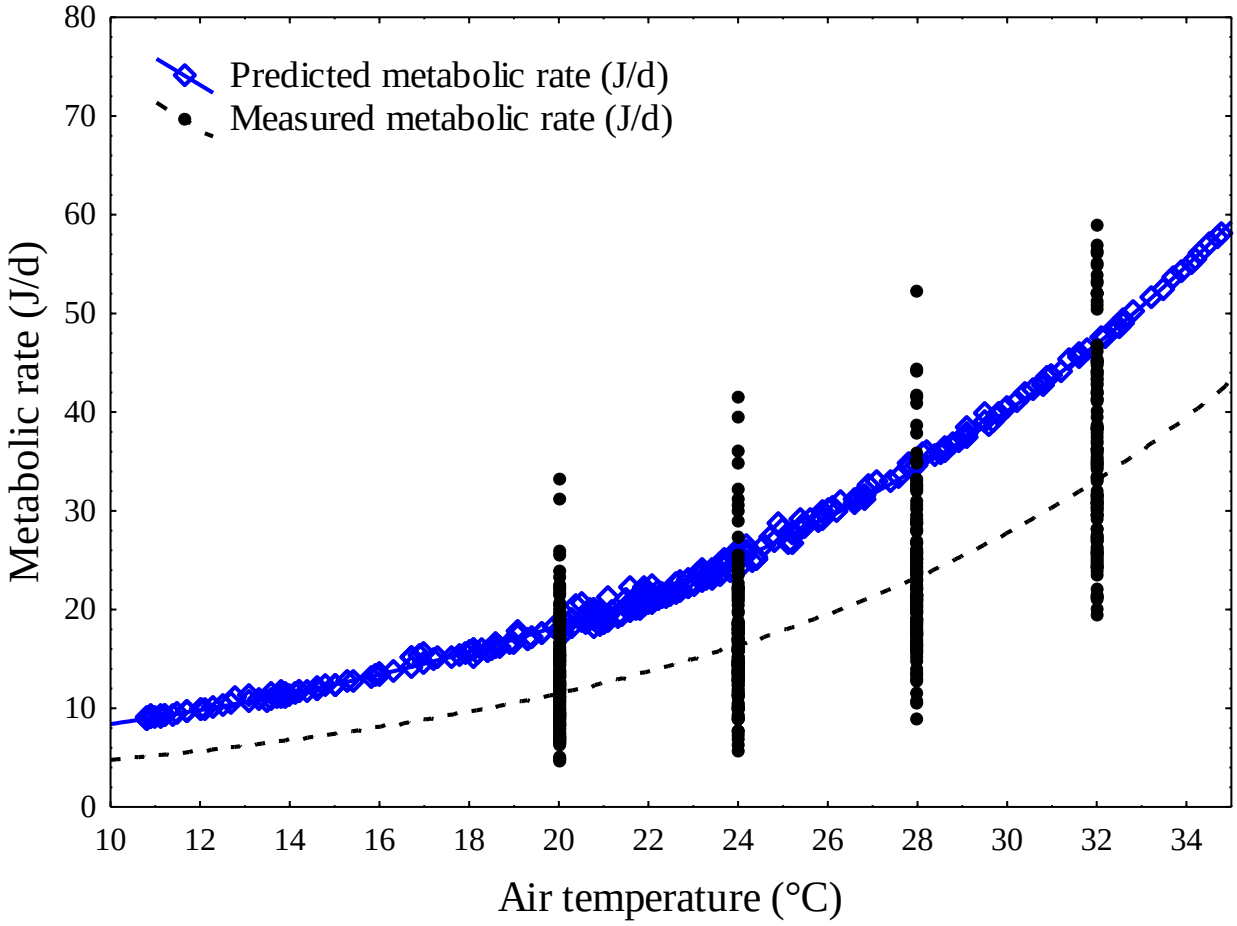

Figure 3.12. Scatterplot and regression of the relationship between predicted and measured metabolic rate (J/d) and air temperature (°C). Measurements were done for a variety of body masses from a wild population of *G. pallidipes* in Zambia at four controlled temperatures (Terblanche *et al*. 2009). The mechanistic model simulation results presented here is for a range of temperatures, but, only for an individual with body mass = 0.0549 g.

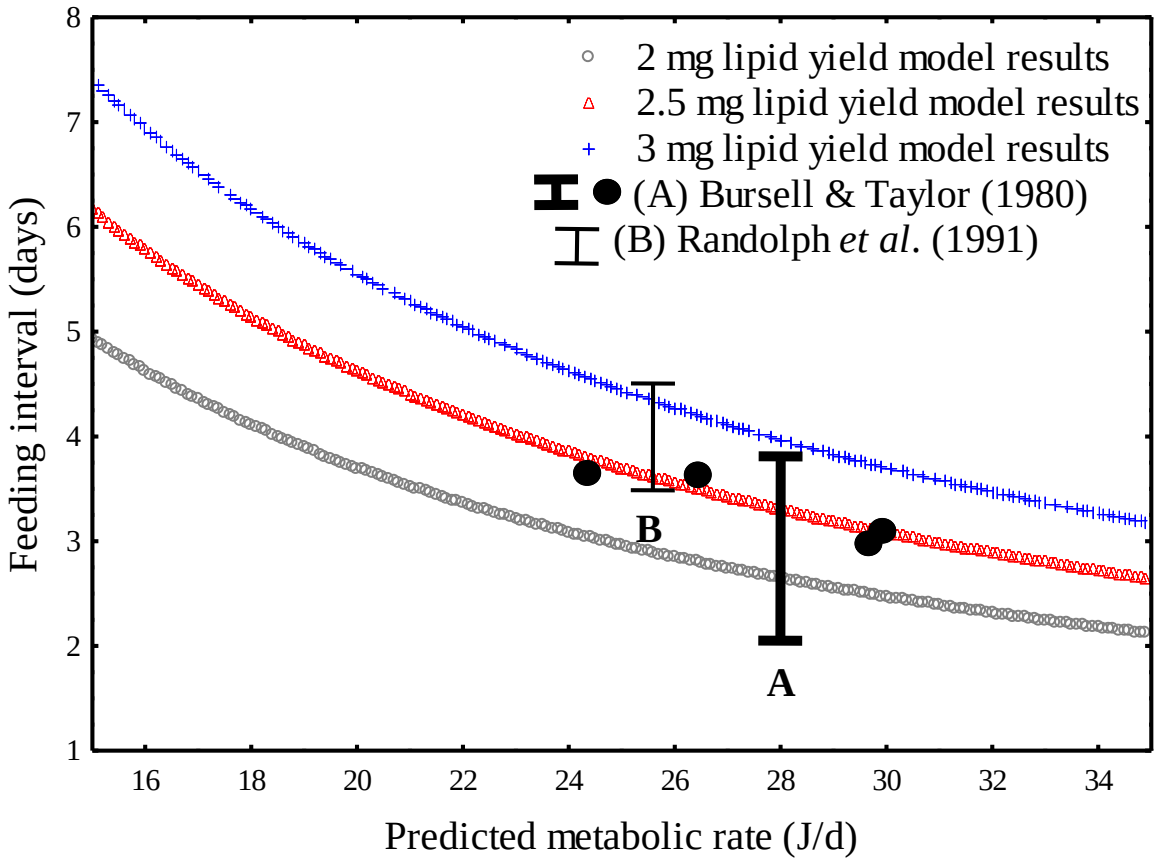

Figure 3.13. Scatterplots of the relationship between the estimated feed intervals (days) and the predicted metabolic rate (J/d) from the NicheMapper model. I solved for the time to lipid exhaustion from Equation 5 and added sensitivity analyses, where a line of small black open circles indicates a 2 mg lipid yield after a blood meal, red triangles indicates a 2.5 mg lipid yield and blue stars indicates a 3 mg of lipids from a single blood meal. Under the current climate scenario, estimates obtained in this Chapter showed good overlap with the A) Bursell and Taylor (1980) estimates of hunger cycles (2 – 3.8 days) for a range of temperatures (20 – 30 °C) and corresponding energy costs and B) Randolph *et al*.'s (1991) estimates of a 3.5 – 4.5 day hunger cycle at ~ 24°C (25.1 J/d). The Wilcoxon matched pairs test statistic showed that the location of the median of metabolic rate was significantly higher under the future climate scenario (W = 885, p < 0.001) and the location of the median of the feeding intervals was significantly lower under the future climate scenario (W = 96.5, p < 0.001).

3.10 Tables

Table 3.1. Source information used for the allometric, physiological and biological variables required by NicheMapper for adult *G. pallidipes* simulations. Each variable's empirically estimated value and the source reference are given. I also report the range of values found in the literature. DW = dry weight.

Variable	Value used in NicheMapper	Value range in source reference	Reference
Maximum adult weight (mg)	59.4	23.2 – 59.4	Terblanche *et al*. 2004
Adult sub–cuticular lipids (% of DW)	35.0	23.0 – 35.0	Bursell 1959
Adult core temperature (ºC)	27.3	24.1 – 31.4	Howe and Lehane 1986
Oxygen consumption (µl/h)	50.0	25.5 – 112.6	Terblanche *et al*. 2004
Flight speed (m/s)	4.8	Up to 5.5	Brady 1991
Flight duration (minutes per day)	30.0	12.0 – 42.9	Bursell and Taylor 1980
Critical thermal minimum (°C)	19.0	18.0 – 21.0	Terblanche *et al*. 2007
Critical thermal maximum (°C)	41.0	39.0 – 41.5	Terblanche *et al*. 2007
Excretion of H_2O after feeding (%)	40.0	20.0 – 60.0	Bursell 1960
Digestion of lipids (% of blood meal)	12.0	10.0 – 24.0	Custer 2005
Digestion of protein (% of blood meal)	78.0	72.0 – 85.0	Custer 2005
Water loss rate (µg/h)	600.0	474.0 – 789.0	Terblanche *et al*. 2006
Reflectance (%)	30.0	10.0 – 50.0	Laboratory measurement, this thesis

Table 3.2. Correlation matrix for the broad set of predictor variables resulting from the mechanistic model across the five countries with the highest current known presence of *G. pallidipes*. Predictor variables include the daily amount of water available in the environment (wavl in g/d), discretionary water in the environment (dh20 in g/d), activity hours available in the environment (act in h/d), discretionary energy available in the environment (dnrg in kJ/d), water evaporated from *G. pallidipes'* (evap in g/d), *G. pallidipes'* food requirement (food in g/d), *G. pallidipes'* metabolic rate (metab in kJ/d) and environmentally available energy (qavl in kJ/d). The values in the table is Pearson's correlation coefficient (r) and five different symbols for r ranging from 0 – 0.3 ( ), 0.3 – 0.6 (·), 0.6 – 0.8 (◦), 0.8 – 0.9 (•), 0.9 – 0.95 (••) and 0.95 – 1 (•••).

wavl	·	••	•••	•••	•••	••	◦
0.686	evap	·	·	·	·	·	
0.998	0.638	dh20	••	••	••	••	◦
0.100	0.686	0.998	qavl	•••	•••	••	◦
0.100	0.686	0.998	1	metab	•••	••	◦
0.100	0.686	0.998	1	1	dnrg	••	◦
0.995	0.683	0.993	0.995	0.995	0.995	food	◦
0.887	0.587	0.887	0.887	0.887	0.887	0.882	act

Table 3.3. Goodness–of–fit (GOF) and area under the receiver operating characteristic curve (AUC) results for the different predictor variables obtained from the mechanistic NicheMapper model estimated for Kenya (KEN), Rwanda (RWA), Tanzania (TAN), Mozambique (MOZ), Uganda (UGA) and all five countries grouped (ALL). Predictor variables include the daily discretionary water in the environment (dh20 in g/d), activity hours available in the environment (act in h), discretionary energy available in the environment (dnrg in J/d), water evaporated from *G. pallidipes* (evap in g/d), *G. pallidipes* food requirement (food in g/d), *G. pallidipes* metabolic rate (metab in J/d) and environmentally available energy (qavl in J/d). The best model parameter is shown in bold.

	KEN	*RWA*	*TAN*	*MOZ*	*UGA*	*ALL*
GOF scores						
act	0.492	0.481	0.221	0.290	0.455	0.337
dh20	0.383	0.404	0.124	0.244	0.307	0.243
dnrg	0.601	0.731	0.459	0.382	0.685	0.546
food	0.741	0.777	0.613	0.576	0.801	0.678
metab	**0.856**	**0.860**	**0.795**	**0.746**	**0.831**	**0.831**
qavl	0.498	0.686	0.312	0.313	0.630	0.421
wavl	0.735	0.807	0.642	0.655	0.801	0.701
evap	0.808	0.837	**0.795**	0.620	0.818	0.769
AUC scores						
act	0.581	0.736	0.505	0.680	0.577	0.513
dh20	0.622	0.749	0.522	**0.714**	**0.590**	0.503
dnrg	0.641	0.770	0.522	0.706	0.585	0.501
food	0.639	0.734	0.520	0.699	0.571	0.502
metab	0.641	0.770	0.522	0.706	0.585	0.501
qavl	0.641	0.770	0.522	0.706	0.585	0.501
wavl	0.641	0.770	0.522	0.706	0.585	0.501
evap	**0.752**	**0.901**	**0.544**	0.520	0.565	**0.554**

Table 3.4. Simulation site locations (coordinates in decimal degrees) and additional site information including the elevation (m above sea level); mean annual temperature (MAT in °C) and annual precipitation (AP in mm) per site (mean ± SD). Elevation and climate data (n = 100 points per site on a 2.5 arc-minute resolution) were extracted from WorldClim (Version 1.4, release 3 2004). The coordinates given were extracted on a WGS84 world projection in the centre of each simulation site.

Site	Coordinates	Elevation (m)	MAT (°C)	AP (mm)
North	7°31′N, 34°23′E	497.38 ± 57.83	26.01 ± 0.50	1240.85 ± 75.31
East	8°54′S, 38°25′E	241.86 ± 89.33	25.12 ± 0.58	1008.31 ± 76.36
South	20°30′S, 33°42′E	93.12 ± 50.52	24.24 ± 0.61	940.04 ± 90.38
West	5°30′S 26°00′E	647.93 ± 58.61	24.57 ± 0.34	1046.50 ± 38.59

Table 3.5. Climatic parameters changed during climate change simulations. Minimum and maximum relative humidity (RH in %) and temperature (T in °C) in pseudo microclimate input files for control (optimal), hot/dry, hot/wet, cold/dry and cold/wet simulation scenarios.

Scenario	RH (%)		T (°C)	
	Min	Max	Min	Max
Optimal	70	75	25	27
Cold/dry	0	10	19	21
Cold/wet	80	100	19	21
Hot/dry	0	10	27	31
Hot/wet	80	100	27	31

Table 3.6. Trait changes during model simulations of no-plasticity (NP), beneficial acclimation (BA) and deleterious acclimation (DA) of adult *G. pallidipes* to four different climate change scenarios. Traits include water loss rates (in µg/h), critical thermal minimum (CT_{min} in °C) and maximum body mass (Mb_{max} in mg). The BA response parameters were based on significant acclimation results from Terblanche *et al*. (2006) relative to non-plastic physiological traits and DA followed the exact opposite direction as compared to the BA response.

Trait	Scenario	NP	BA	DA
WLR (µg/h)	Cold/dry	295.6	295.6	295.6
	Cold/wet	218.8	218.8	218.8
	Hot/dry	364.6	183.3	488.5
	Hot/wet	177.0	183.3	488.5
CT_{min} (°C)	Cold/dry	19.0	15.0	23.0
	Cold/wet	19.0	15.0	23.0
	Hot/dry	19.0	19.0	19.0
	Hot/wet	19.0	19.0	19.0
Mb_{max} (mg)	Cold/dry	59.4	77.2	41.6
	Cold/wet	59.4	77.2	41.6
	Hot/dry	59.4	59.4	59.4
	Hot/wet	59.4	59.4	59.4

Table 3.7. Line–fitting comparisons of linear and polynomial functions to describe the relationship between NicheMapper predicted resting metabolic rate (J/d) and *G. pallidipes* probability of presence. For each model, I report K, the number of terms in the model, Akaike's Information Criteria (AIC) and the Bayesian Information Criteria (BIC) and the differential BIC (Δ_i), which is the difference between a given model's BIC and the lowest BIC across all models tested. I also report the Bayesian weight (w_i) calculated from BIC, adjusted goodness of fit value (r^2) and the maximum point where the derivative of the polynomial functions are equal to zero (z_i).

Model	K	AIC	BIC	Δ_i	w_i	r^2	z_i
Polynomial – quintic	6	1595950	1596041	25472	1	0.02669	27.10
Polynomial – quartic	5	1606339	1606417	35861	0	0.02341	26.60
Polynomial – cubic	4	1614316	1614381	43838	0	0.02088	25.65
Polynomial – quadratic	3	1639725	1639777	69247	0	0.01278	24.06
Linear model	2	1674778	1674817	10430	0	0.00149	

Table 3.8. A comparison of plausible models and their parameter estimates (mean ± std. error) in the functions to describe the relationship between metabolic rate and *G. pallidipes* probability of presence (Gp_{PP}). For each model I give the derivative which was used to calculate the optimal metabolic rate (*metab*) at the maximum polyroot (derivative = 0).

Model and function

Linear model

$y = mx + c$

$Gp_{\mathrm{PP}} = 3.137e^{-03} \pm 4.614e^{-05}\ \ metab\ + 1.193e^{-01} \pm 1.074e^{-03}$

Second order polynomial

$y = a + bx + cx^2$

$Gp_{\mathrm{PP}} = -6.408e^{-01} \pm 4.187e^{-03} + 7.118e^{-02} \pm 3.653e^{-04}\ \ metab\ - 1.479e^{-03} \pm 7.8574e^{-06}\ (metab)^2$

$Gp_{\mathrm{PP}}' = 7.118e^{-02} - 1.4479e^{-03}(2 * metab)$

Third order polynomial

$y = a + bx + cx^2 + dx^3$

$Gp_{\mathrm{PP}} = 1.446e^{00} \pm 1.371e^{-02} - 2.226e^{-01} \pm 1.875e^{-03}\ \ metab\ + 1.183e^{-02} \pm 8.371e^{-05}\ \ metab\ ^2 - 1.947e^{04} \pm 1.219e^{-06}\ \ metab\ ^3$

$Gp_{\mathrm{PP}}' = -2.226e^{-01} + 1.183e^{-02}\ \ 2 * metab\ - 1.947e^{-04}(3 * (metab)^2)$

Fourth order polynomial

$y = a + bx + cx^2 + dx^3 + \mathrm{e}x^4$

$Gp_{\mathrm{PP}} = -2.091^{00} \pm 4.187e^{-02} + 4.717e^{-01} \pm 7.991e^{-03}\ \ metab\ - 3.740e^{-02} \pm 5.571e^{-04}\ \ metab\ ^2 + 1.305e^{03} \pm 1.683e^{-05}\ \ metab\ ^3 - 1.663e^{-05} \pm 1.861e^{-07}\ \ metab\ ^4$

$Gp_{\mathrm{PP}}' = 0.4717 - 0.0374\ \ 2 * metab\ + 0.001305\ (3 *\ \ metab\ ^2) + 0.00001663\ (4 * (metab)^3)$

Fifth order polynomial

$y = a + bx + cx^2 + dx^3 + \mathrm{e}x^4 + \mathrm{f}x^5$

$Gp_{\mathrm{PP}} = -1.300e^{+01} \pm 1.148e^{-01} + 3.248e^{+00} \pm 2.836e^{-02}\ \ metab\ - 3.096e^{-01} \pm 2.726e^{-03}\ \ metab\ ^2 + 1.422e^{-02} \pm 1.277e^{-04}\ \ metab\ ^3 - 3.137e^{-04} \pm 2.918e^{-06}\ \ metab\ ^4 + 2.661e^{-06} \pm 2.608e^{-08}\ \ metab\ ^5$

$Gp_{\mathrm{PP}}' = 3.2495 - 0.3098\ \ 2 * metab\ + 0.0142\ \ 3 *\ \ metab\ ^2 - 0.0003\ \ 4 *\ \ metab\ ^3 + 0.00000426624\ \ 5 *\ \ metab\ ^4$

Chapter 4

Concluding remarks and future directions

The world is changing mainly as a consequence of anthropogenic intervention that results in loss of ecosystem services and changes in biodiversity (IPCC 2001; Schneider *et al*. 2007; IPCC 2007). These changes are projected to continue, or perhaps even accelerate, largely as result of habitat destruction and alteration, invasion, changes to climate, overexploitation and pollution (Millennium Ecosystem Assessment 2005). Average global surface temperature has increased by 0.7 °C since the early 20th century and the best estimates for predicted climatic warming expected by the year 2100 are in the region of 0.8 – 4.0 °C with likely ranges of 1.1 – 6.4 ° (IPCC 2007; Tebaldi and Sanso 2009).

There are emerging concerns that environmental change will exacerbate socio-economic impacts by increases in disease vector distribution (Chan *et al.* 1999; Githeko *et al.* 2000; Hunter 2003; Patz *et al.* 2005; and see Gale *et al*. 2010). Since 1973, 29 newly emerged pathogens have been listed which led to emerging infectious diseases becoming an increasing problem (Lambrecht 1964, 1985). Vector-borne disease is not easily managed through medical or prophylactic intervention (Welburn *et al.* 2001). Furthermore, range expansions of vector-borne diseases are a highly likely result of climate change and vector control may be the most reasonable way of achieving disease reductions (Martens *et al.* 1999; Kovats *et al.* 2001; Hoshen and Morse 2004, but see Rogers and Randolph 2000 where they argue the opposite). Indeed, the complexity of global climate change poses a significant and compounded challenge to epidemiologists (Martens and Thomas 2005; Moss *et al*. 2010).

For many African countries, disease outbreak is amongst the most important factors contributing to socio-economic vulnerability (IPCC 1997). Furthermore, recurrent droughts, poor economic status, and high population growth are likely to compound negative effects in Africa (IPCC 2001). Thus far, biological transmission models have predicted global climate change to result in an increase in malaria-transmission risk from *Plasmodium falciparum* and *P. vivax* (Martin and Lefebvre 1995; Martens *et al.* 1999). Climate change is also thought to be correlated with malaria outbreak frequency in Africa, especially after El Niño events like drought and flooding (Githeko *et al.* 2000). Furthermore, dengue-transmissions could potentially increase while schistosomiasis–transmission may decrease in geographic range as a result of climate change (Martens *et al.* 1997).

Tsetse (genus *Glossina*) consists of 22 species that occupy a wide range of habitats in sub-Saharan Africa. Given their critical importance as a major vector of tropical human and animal disease (trypanosomiases), understanding the physiological drivers of tsetse water balance is crucial for predicting disease dynamics under climate change scenarios. Tsetse are classified into three major

subgroups (ecotypes) on the basis of morphological differences in the structure of the male genitalia (Leak 1999; Rogers and Randolph 1986). The three subgroups can also be differentiated by variation in physiology, behaviour and ecological characteristics related to sensitivity to moisture availability (Bursell 1960).

Earlier work on the pupal lifestage showed that in puparia, WLRs were the most important trait for survival of dehydrating conditions and were positively related to mean annual precipitation even after phylogenetic-adjustment (Kleynhans and Terblanche 2009). Ancestral trait reconstructions suggested that a reduction in WLRs and increased body size likely evolved from an intermediate ancestral state and probably facilitated survival in xeric environments. The results suggest an important role for water balance physiology of puparia in determining inter-specific variation and strengthen conclusions reached by early studies of tsetse physiology that water balance is a critical component of tsetse population dynamics and geographic distribution.

Acclimation responses of desiccation resistance in tsetse puparia have not been assessed in full and were found to be complex and likely important in moderating climate effects over short time scales (see Terblanche and Kleynhans 2009). The results from work undertaken to explore these effects suggest an important role for phenotypic plasticity in moderating environmental variation within the tsetse life-time for traits of water balance, with possible dramatic results for evolutionary fitness and population dynamics. Furthermore, these results suggest that mesic and xeric species are not necessarily constrained to different acclimation responses under varying environmental moisture conditions which makes large-scale climate predictions difficult as species-specific information will be required for model input data.

The key aims of this thesis were to:

- Determine water balance physiology in adults of different ecotypes, body size and evolutionary origin under ecologically relevant conditions and assess the magnitude of water balance trait responses to altered environmental conditions;
- Determine likely responses of tsetse water balance physiology to simulated climate change scenarios;
- Explore the implications of physiological variation in water balance traits and the plasticity thereof on population demographics to fully examine predictions under future climate change scenarios.
- Establish and explore the implications of climate change on the basic reproductive number of the disease in the future.

First, I tested the effects of climate change on water balance traits of adult flies. Four species were exposed to a range of temperature (20 – 30 °C) and relative humidity (0 – 99 %) combinations (Chapter 2).

Second, work, in collaboration with Prof. Warren Porter at the University of Wisconsin, Madison in modelling the tsetse landscape from biophysical principles under altered rainfall and temperature scenarios were undertaken (Chapter 3). I combined the results of controlled laboratory simulations of climate change on physiological traits of *G. pallidipes* and demonstrate the use of a biophysical mechanistic model (NicheMapper^{TM}) to predict potential population responses to regional climate change.

Finally, I produced a disease transmission risk model for vector–borne trypanosomiasis in Africa under a future climate scenario (Chapter 3).

In Chapter 2, I measured aspects of water balance physiology in adults of four ecologically diverse species of tsetse under a range of environmentally-relevant combinations of temperature and relative humidity. I aimed to answer three main questions regarding water balance physiology of adult tsetse. I show support for a significant influence of temperature and relative humidity under static climate change conditions on tsetse water balance. Specifically, (1) species water balance physiology differs with changes in temperature and relative humidity. (2) The interaction between temperature and relative humidity do not elicit a consistent physiological response among individuals and among species in terms of magnitude and direction of change. Thus, water balance responses to temperature and relative humidity are not transferrable between species or ecotypes. (3) Extrapolating water balance responses to temperature and relative humidity among species or even among ecotypes should probably be undertaken with caution. Future efforts to model tsetse species responses to climate change may therefore require species-specific information if water balance physiology is to be incorporated into mechanistic models (see e.g. Kearney *et al.* 2009). This will likely increase the accuracy of predictions for the vector, thereby enabling better disease intervention planning in future.

It has been previously speculated that a limitation in critical thermal maximum might lead to decreases in *G. pallidipes* population sizes under hotter scenarios (Terblanche *et al*. 2008). The results from Chapter 3 support this idea in full, considering the limitation in plastic CT_{max} and results of the hotter scenario simulations on water balance. From an epidemiological perspective, vector habitat suitability is likely to decrease in the future, and R_0 will likely increase. The results of this comprehensive, integrative Chapter, well-grounded in empirical observations provide general

insights into processes determining population dynamics of *G. pallidipes* under climate change scenarios. In addition to the laboratory results (Chapter 2), the mechanistic model simulation results (Chapter 3) provide results that indicate negative effects of climate change, in particular hotter/drier scenarios, at the population-level.

I identified that a resting metabolic rate between 26 and 27 J/d provided a good estimate of optimal habitat range for *G. pallidipes* and also explained temporal patterns of population dynamics. I validated the mechanistic model results spatially using goodness-of-fit and area under the receiver operating curve approaches. I tested model predictions temporally using metabolic rate and trap catch data sampled from a field population of *G. pallidipes* in Zambia. Both tests of the model showed that values for resting metabolic rate predicted by NicheMapper have great potential to capture various aspects of population dynamics and biogeography in *G. pallidipes*.

The mechanistic model simulation of changing environments is capable of explaining much of the variation in species' distribution under different temperature and moisture availability (Pearson and Dawson 2003; Thomas *et al*. 2004; Porter and Mitchell 2006). The integration of plasticity with biophysical modelling provided a more complete picture of the possible responses to climate change, often resulting in an answer different from the model without the inclusion of phenotypic plasticity (see Kearney *et al*. 2009). Furthermore, behaviour, reproduction and survival of disease vectors are processes that depend on temperature and water availability and thus can lead to changes in vector abundances under climate change with increases in transmission risk of disease from an increased vector-range perspective (Hargrove 2004).

The biophysical model implemented here calculated the hourly local sun and available shade environments and animal temperature-dependent physiological and behavioural responses to them according to steady–state allometric, behavioural and physiological properties of the tsetse *G. pallidipes*. Metabolism in this environment links food- and respiration-mass balance to energy-balance, while evaporation links water- and energy-balance together. This creates a single mechanistic framework of energy balance given the environmental, physiological and behavioural condition of the study organism (Porter *et al.* 2010). A major assumption of this Chapter is that the distribution of *G. pallidipes* is constrained by complex spatial and temporal patterns of physiological thresholds (Helmuth *et al*. 2005). This model did not include the biotic interactions such as host availability, inter-species' competition or human control efforts. However, I recognise that these factors might have effects on the fine-scale distribution model results. Regardless, for the purpose of this Chapter and obtaining a general insight into the physiological limits of *G. pallidipes*

abundance and distribution the current approach was well validated and showed considerable potential. Nevertheless, future work could include adding these other parameters in future (Robays *et al.* 2004; Araújo and Guisan 2006; Soberón 2010). In addition, the mechanistic model might not predict current distributions perfectly, because, high specificity in terms of response variables (metabolic rate only) masks response effects (see Buckley *et al.* 2010). As a result, an over-specific calculation might miss the more general, sometimes obvious, ecological drivers of the distribution of species. Furthermore, differences in geographic distribution predictions between the fundamental niche (explored in Chapter 3) and the realized niche can be attributed to human intervention, including control efforts (Wint and Rogers 2000). I expected the model would thus over predict the current *G. pallidipes* distribution, such that the difference between the mechanistic model and the current distribution might indicate the role of biotic interactions that were not included in this model. It would be well worth including biotic aspects in the next step of distribution prediction under climate change and also potentially consider habitat transformation or other human intervention patterns. Chapter 3 assumed an even spread of hosts available for feeding as well as a uniform vegetation structure temporally. Given these limitations, NicheMapper predictions were still very accurate. Even an incredibly simplified spatial prediction based on a single model output variable (predicted metabolic rate) reported an accuracy of 83 % in five African countries where *G. pallidipes* is most prevalent. Second, temporal comparisons between model predictions and field measurements also reported remarkably good concordance. Third, plastic simulations are, however, more difficult to interpret due to the complexity in plastic traits, but were nevertheless easily implemented into NicheMapper. Finally, and perhaps most importantly, climate change scenario outcomes appear to be realistic for sub–Saharan Africa and infection probability calculations. A simplistic mathematical approach provided useful insight into future threats and population dynamics and suggested overall reductions in available habitat but increased disease transmission risk in the future. The latter emphasises that control efforts can be carried out more cost-effectively in smaller areas. In addition, these results provide a useful tool to accurately identify focal areas for local tsetse eradication schemes and areas that will be susceptible to invasion by tsetse due to climate-driven range-shifts.

4.1 References

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Addenda

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Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*)

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Abstract. 1. The invasion success of *Ceratitis capitata* probably stems from physiological, morphological, and behavioural adaptations that enable them to survive in different habitats. However, it is generally poorly understood if variation in acute thermal tolerance and its phenotypic plasticity might be important in facilitating survival of *C. capitata* upon introduction to novel environments.

2. Here, by comparison of widely distributed *C. capitata* with a narrowly distributed congener, *C. rosa*, we show that both species have similar levels of survival to acute high and low temperature exposures under common rearing conditions. However, these species differ dramatically in the time-course of plastic responses to acute low temperature treatments.

3. The range of temperatures that induce rapid cold hardening (RCH) are similar for both species. However, *C. capitata* has two distinct advantages over *C. rosa*. First, at 5 °C *C. capitata* develops RCH significantly faster than *C. rosa*. Second, *C. capitata* maintains a RCH response longer than *C. rosa* (8 vs. 0.5 h).

4. A simple population survival model, based on the estimated time-course of RCH responses determined for both species, was undertaken to simulate time to extinction for both species introduced into a similar thermally variable environment. The model showed that time to extinction is greater for *C. capitata* than for *C. rosa*, especially in habitats where temperatures frequently drop below 10 °C.

5. Thus, variation in RCH responses may translate into significant variation in survival upon introduction to novel thermal habitats for *C. capitata*, particularly in cooler and more thermally variable geographic regions, and may contribute to their ongoing invasion success relative to other, more geographically constrained *Ceratitis* species.

Key words. Acclimation, field fitness, invasiveness, phenotypic plasticity, population dynamics, rapid cold hardening.

Introduction

Overcoming environmental challenges is the first of several potential barriers determining whether a species becomes established, naturalised and, ultimately, invasive (Richardson & Pysek, 2006). Upon introduction to a novel environment, a species may be able to persist over short timescales either by having greater resistance to climate conditions, or by mounting a rapid response to these extremes and thereby avoiding potential detrimental effects. As such, short-term phenotypic plasticity could be an important mechanism enhancing survival of individuals upon a change in their environment (reviewed in Chown & Terblanche, 2007; Whitman & Ananthakrishnan, 2009). Indeed, acclimation responses to thermal extremes, which are a form of phenotypic plasticity, have been demonstrated to be a significant component of field fitness in insects (Kristensen *et al*., 2008) and may, in turn, be related to the habitat and the thermal variability a species experiences (Hazell *et al*., 2010). Phenotypic responses may also play a significant

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role in the immediate survival of alien species to novel environments (Lee *et al*., 2002; Chown *et al*., 2007; Slabber *et al*., 2007), without which it is unlikely that the species will become established (and see Hazell *et al*., 2010). Over longer timescales, rapid evolutionary adaptation (i.e. natural selection for novel phenotypes) may also aid in the naturalisation process (Huey *et al*., 2005; Lee *et al*., 2007), unless genetic constraints play a significant role (Gilchrist & Lee, 2007; Kellermann *et al*., 2009).

The Mediterranean fruit fly *Ceratitis capitata* has a detailed history of invasion success both in the New World and other parts of the world (e.g. Carey, 1991; Malacrida *et al*., 2007). The successful establishment of *C. capitata* outside its natural range probably stems from several physiological, morphological, and behavioural adaptations that enable them to survive in different habitats. Intrinsic factors that contribute to the species invasiveness probably include rapid generation times, polyphagy, and host-plant switching, while extrinsic factors likely include increased propagule pressure caused by repeated species introductions (Carey, 1991; Malacrida *et al*., 2007). *Ceratitis* species arose from East Africa (Baliraine *et al*., 2004) and *C. capitata* has become established in many countries worldwide while *C. rosa* has remained largely restricted to Africa (DeMeyer *et al*., 2008). In South Africa, *C. capitata* is widely distributed throughout agro-ecosystems, while *C. rosa* only occurs in cooler, wetter parts of the country (DeMeyer *et al*., 2008). These patterns suggest that variation in physiological tolerance to climatic stress may contribute to differences in present-day distributions. Moreover, climatic stress resistance is likely to be a significant factor during both active or passive dispersal (Parsons, 1991; Hazell *et al*., 2010). However, it is generally poorly understood if variation in acute thermal tolerance or its phenotypic plasticity might be important mechanisms facilitating survival of *C. capitata* upon introduction to novel environments. Indeed, to our knowledge, no studies of *Ceratitis* spp. to date have considered acute, rapid physiological responses to ecologically relevant temperature variation. Although sub-lethal effects may form an important component of the kinds of stress encountered by insects exposed to novel environments, and several processes are critical to sustained population growth (e.g. reproduction, resource availability and acquisition) during an invasion (discussed in Richardson & Pysek, 2006; and see, for example, Preisser *et al*., 2008), survival nevertheless serves as a good proxy for determining ecologically relevant variation among species (e.g. Addo-Bediako *et al*., 2000; Kimura, 2004). Indeed, survival responses probably form a major component of climatic stress resistance, and are used by insects to cope with temperature variation at daily timescales (Meats, 1973; Kelty & Lee, 2001; Kelty, 2007; Overgaard & Sørensen, 2008), but significantly, also upon introduction into new environments (Chown *et al*., 2007; Slabber *et al*., 2007; Kristensen *et al*., 2008; Preisser *et al*., 2008). Furthermore, survival is a critical first step in the invasion process, without which any further establishment through reproduction is impossible.

Here we test the hypothesis that the highly invasive Mediterranean fruit fly, *C. capitata*, is aided by phenotypic plasticity of thermal tolerance as opposed to improved basal thermal tolerance, relative to a narrowly distributed congener *C. rosa*. Furthermore, we ask if the time-course of the rapid responses to thermal extremes might provide further advantages to *C. capitata*'s invasion potential. First, we investigate survival of high and low temperature extremes, and the plasticity of survival to these extremes, in *C. capitata* and *C. rosa*. Essentially, we explore whether rapid cold hardening (RCH) or rapid heat hardening (RHH) occurs in each species. Second, we examine the range of temperatures that elicit RCH responses and the time-course of these responses in both species. Specifically, we investigate how long it takes to develop a maximal RCH response, and also the persistence of the enhanced survival effects. Finally, using a population extinction model in various thermal habitat scenarios, coupled with microclimate data from habitat in an area where *C. capitata* and *C. rosa* species' distributions overlap, we suggest that variation in RCH responses could translate into differential success in population establishment. Therefore, using a model based on the empirically determined time-courses of these responses, we estimate the potential effects on mortality that variation in RCH might afford *C. capitata* under natural conditions.

Materials and methods

Study animals and rearing conditions

Fruit flies (*C. rosa* and *C. capitata*) were reared in square Perspex™ cages (800 mm^3) in the laboratory on a L:D 12:12 h photoperiod, at room temperature ($25 \pm 1\ ^{\circ}C$) and $65 \pm 10\%$ relative humidity. Flies were provided with water, sugar, and hydrolysed yeast (MP Biomedicals, Aurora, Ohio) for food, and with bananas for oviposition. Cultures were first collected from Stellenbosch and Pniel in Western Cape Province of South Africa between December 2006 and March 2007 (summer months). Fruit fly colonies have been in culture for $\sim$2 years and once every month during summer, wild-caught flies were added to the colony to maintain genetic similarity to wild populations. The cultures of *Ceratitis* (10 000–12 000 individuals) were held in multiple cages and flies were randomised between cages to avoid inbreeding depression. All cages were held at similar low densities to avoid stressful crowding effects that might affect thermal tolerance estimates. All flies used in thermal tolerance experiments were of a similar age (24–48 h old) and had access to food and water *ad libitum*, but were of mixed genders since sex does not appear to affect thermal tolerance in either species (Nyamukondiwa & Terblanche, 2009).

Thermal tolerance and rapid thermal responses

To determine survival at an acute temperature, and whether *C. rosa* and *C. capitata* rapidly cold- or heat-harden (RCH or RHH respectively), standard protocols were followed (Fig. 1) following Terblanche *et al*. (2008). In preliminary trials, we first assayed for upper and lower lethal temperatures that cause 70–100% mortality in both species using 2 h exposures (termed the 'discriminating temperature'). For all hardening

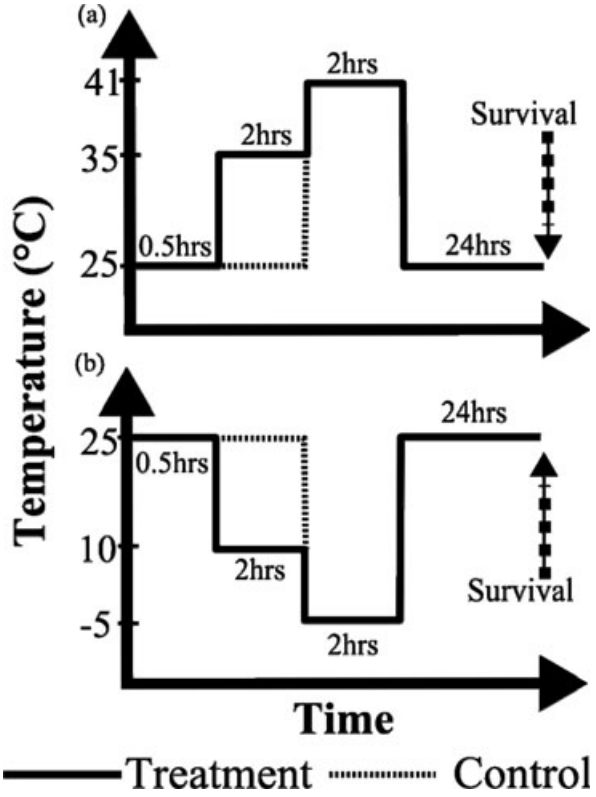

Fig. 1. Schematic diagram of experimental protocols used to induce rapid hardening responses in *Ceratitis* spp., following Terblanche *et al*. (2008) (see Materials and Methods for full description of treatments). (a) Rapid heat hardening, (b) rapid cold hardening.

assays, five replicate 60 ml vials of 10 insects each were placed in a growth chamber at 25 °C for 30 min, after which flies were exposed to a range of temperatures in programmable water baths (Grant GP200-R4, Grant Instruments Inc., Cambridge, U.K.) for 2 h before plunging vials containing flies directly into water baths set at lethal (discriminating) temperatures. After 2 h at the discriminating temperature (41 °C for high temperature responses, −5 °C for low temperature responses), flies were returned to 25 °C for 24 h before scoring survival. In all cases, five replicate vials of 10 flies per vial were used as handling controls. These control flies were sorted into vials, placed at normal rearing temperatures (25 °C) in a climate chamber for 30 min, then taken out of the climate chamber, handled for similar duration and with similar vigour to treatment flies, and placed back into climate chambers for 2 h. Next, control flies were exposed to discriminating temperatures for 2 h (at the same time as treatment flies) and then returned to climate chambers at 25 °C for 24 h before scoring survival. Treatment and control flies had access to food and water during the recovery period. Survival was defined as a coordinated response to mild stimulation (e.g. prodding) or normal activities (e.g. mating, walking and flying).

Since there were no pronounced RHH effects following a 35 °C pre-treatment for 2 h, we also investigated if a 36 °C pre-treatment for 1 h would induce a hardening response. RCH responses were more pronounced in both species than RHH. Consequently, we only further explored time-courses of RCH responses. A range of pre-treatment temperatures (0–35 °C at 5 °C increments) were explored for the potential to improve low temperature survival (2 h at −5 °C) (following Lee & Denlinger, 1991).

Time-course of rapid thermal responses

To determine how long it takes for *C. rosa* and *C. capitata* to develop a full hardening response (i.e. maximum RCH potential or maximum survival), flies were pre-conditioned at 5 °C for different durations (15, 30, 60, and 120 min) before subjecting them to a discriminating temperature of −5 °C for 2 h. In all cases, five replicate 60 ml vials of 10 insects each were used. A control batch of five replicate 60 ml vials of 10 flies each were taken directly to −5 °C for 2 h without pre-treatment and survival was scored after 24 h.

We then investigated how long the full RCH response lasted after pre-conditioning. Flies were cold-hardened by exposing them to 5 °C for 2 h and then returned to an environmental chamber at 25 °C. Subsequently, at various time intervals (0.5, 1, 2, 4, 8, and 16 h) after hardening treatment, survival was tested following the above protocol.

Microclimate data and population extinction model

Shaded microclimate temperatures were recorded from an orchard (Lakenvlei farm, Ceres, Western Cape, South Africa; 33.34°S, 19.57°E; 1046 m a.s.l.), where both *C. rosa* and *C. capitata* are typically found, using Thermocron iButtons (0.5 °C accuracy; 15 min sampling frequency). The iButtons were located in the centre of an apple tree at 1.25 m above the ground and a ca 3 month recording was obtained during late March to early June 2009.

Following this, we derived a theoretical model of the effects of RCH on the time to extinction for a finite population of non-reproductive, adult individuals of both species given the same variable, thermal opportunities. Simplified temperature variation for the population extinction model was created by generating sine waves of different amplitude and constant mean temperature (detailed below), and fitting the empirically derived survival curves of RCH for both species to these simulated temperature data. Thus, population size was simulated for at least 60 consecutive days to determine the influence that typical *C. capitata* or *C. rosa* RCH responses might have on the population survival over time. The best-fit non-linear (polynomial) equation was obtained by curve-fitting procedures in TableCurve2D software and comparison of Akaike weights to select the most likely model with the least number of model terms. These equations describing the proportion of survival after different durations of exposure to a RCH-inducing temperature (determined from the time-course experiments) were calculated and used for population extinction model simulations at three levels of temperature

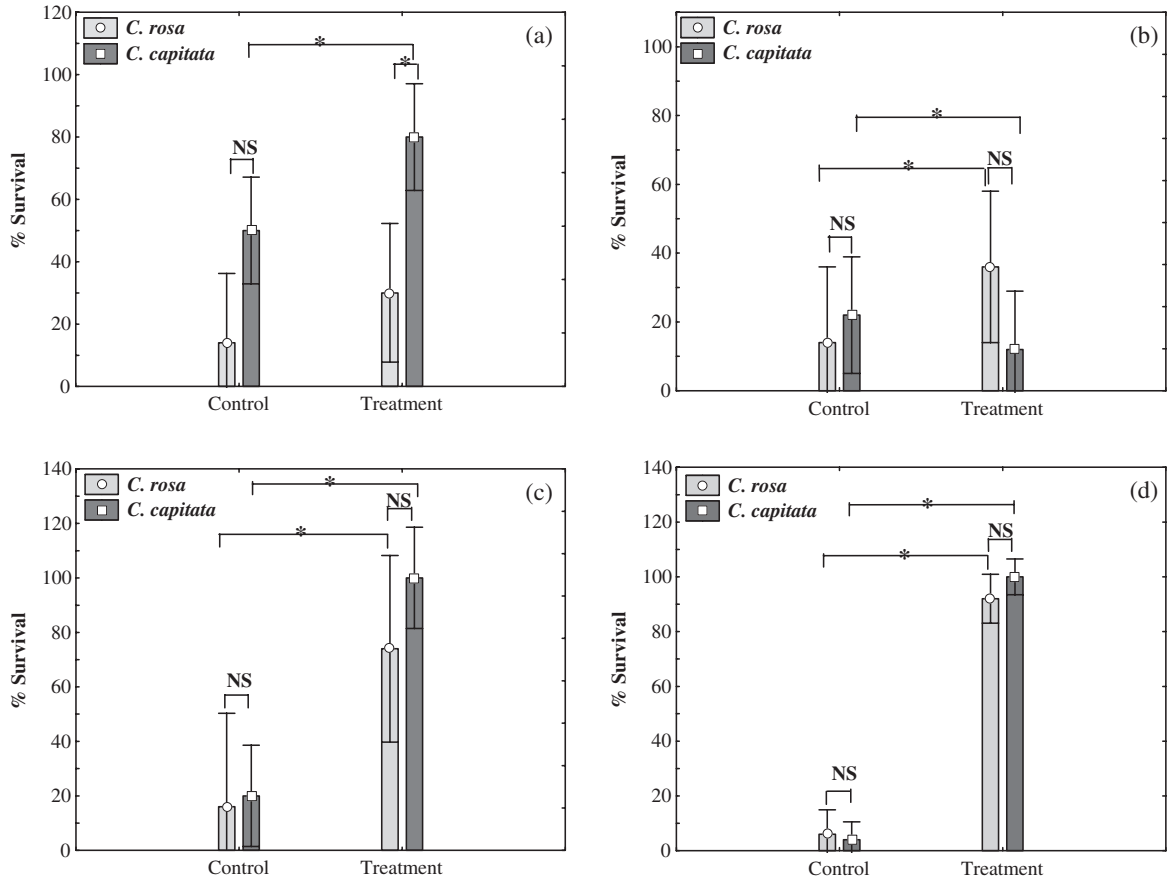

Fig. 2. Effects of (a) a 2-h pre-treatment at 35 °C (rapid heat hardening) on high temperature survival (2 h at 41 °C) of adult *Ceratitis rosa* and *C. capitata*; (b) a 1-h pre-treatment (rapid heat hardening) at 36 °C on high temperature survival (2 h at 41 °C) of *C. rosa* and *C. capitata*; (c) a 2-h pre-treatment at 10 °C (rapid cold hardening) on low temperature survival (2 h at −5 °C) of adult *C. rosa* and *C. capitata*; and (d) a 2-h pre-treatment (rapid cold hardening) at 5 °C on low temperature survival (2 h at −5 °C) of *C. rosa* and *C. capitata*. Values are means ± 95% CLs. $n = 50$ per group. NS, not significant. *Significant difference at $P < 0.05$.

variation (see eqns 1–4). For *C. capitata*, the time-course describing the initiation of RCH is

$$S = 0.7601 + 14.5314x - 15.3926x^2 + 5.1944x^3 \quad (1)$$

and the termination of RCH is given by

$$S = 9.6931 - 0.9792x + 0.1116x^2 - 0.0049x^3. \quad (2)$$

For *C. rosa*, the time-course of initiation of the RCH response is

$$S = 0.3125 - 1.1892x + 7.8546x^2 - 2.5196x^3 \quad (3)$$

and termination of the response is described by

$$S = 4.4408 - 0.8364x + 0.0406x^2 \quad (4)$$

where S is survival (percentage of population) and a function of x the time (in minutes) kept at temperatures that induce RCH, or time (in minutes) at temperatures that are likely to result in a loss in RCH. For simplicity, we model survival as a function of time below or above a given threshold (10 °C) for initiation and termination responses, respectively. Specifically, we assumed that RCH responses were triggered at temperatures <10 °C, as suggested by the experimental results, and that these responses proceed in a time-dependent manner thereafter. Similarly, we assume that RCH is terminated above 10 °C and thereafter proceeds in a time-dependent manner. A more realistic, although more complex, model could incorporate the non-linear relationship between time and temperature (e.g. Regniere & Bentz, 2007) across a wider range of ecologically relevant conditions and acclimation states, but data collection and model simulations would be logistically more challenging. Temperature simulations were undertaken for a total of 1440 points per day at a 1-min resolution. For the 5% simulation, 72 data points (= minutes) fall below 10 °C, 288 min for 20%, and 576 min for 40%. Each species started with a similar population size of 1000 individuals and the population was considered extinct when it reached <0.5% of its original size.

Statistical analyses

To examine the effects of RCH and RHH on fruit fly survival, treatment groups were compared using a generalised linear model (GLZ) assuming a binomial distribution and a logit link function in SAS statistical software (Proc Genmod), with corrections for overdispersion (following, for example, Marais *et al*., 2009). In all cases, treatments were compared with controls from the same experiment only. Similar GLZ analyses, but including the categorical, ordered effect of time, were carried out to determine the time-course of rapid thermal responses in these two species. Tukey–Kramer's *post hoc* tests were used to identify statistically homogeneous groups. Microclimate data were analysed for the number of potential RCH events following methods outlined in Sinclair (2001) with 10 °C as a threshold and 2 h duration as a minimum time for an event.

Results

Thermal tolerance and rapid thermal responses

A 2 h hardening at 35 °C did not significantly improve survival during a 2-h exposure at 41 °C in *C. rosa* ($\chi^2 = 8.78$, d.f. = 2, $P = 0.124$; Fig. 2a). However, this treatment (2 h at 35 °C) marginally improved survival in *C. capitata* at 41 °C ($\chi^2 = 7.90$, d.f. = 2, $P = 0.0192$; Fig. 2a). Pre-treatment of flies at 36 °C (1 h) significantly altered survival during a 2-h exposure to 41 °C in both *C. rosa* ($\chi^2 = 7.67$, d.f. = 2, $P = 0.022$) and *C. capitata* ($\chi^2 = 15.37$, d.f. = 2, $P < 0.001$). However, there was a marginal increase in *C. rosa* and a decrease in *C. capitata* survival (Fig. 2b).

Pre-treatment of flies for 2 h at 10 °C improved survival at −5 °C by ∼50% in *C. rosa* ($\chi^2 = 4.08$, d.f. = 2, $P = 0.013$; Fig. 2c) and by ∼80% in *C. capitata* ($\chi^2 = 6.89$, d.f. = 2, $P = 0.032$; Fig. 2c). Furthermore, following pre-treatment for 2 h at 5 °C, there was a significant increase (80–90%) in survival at −5 °C in both *C. rosa* ($\chi^2 = 31.34$, d.f. = 2, $P < 0.0001$; Fig. 2d) and *C. capitata* ($\chi^2 = 22.86$, d.f. = 2, $P < 0.0001$; Fig. 2d). However, the magnitude of the hardening effects were similar in both species and in both experiments (Fig. 2c,d).

Pre-treatment temperature significantly affected the development of RCH in both *C. rosa* ($\chi^2 = 50.67$, d.f. = 9, $P < 0.0001$) and *C. capitata* ($\chi^2 = 71.94$, d.f. = 9, $P < 0.0001$) (Temperature effect, Table 1). Two hours exposure at 0, 15, 20, 25, 30, and 35 °C did not improve survival at −5 °C in *C. rosa* (Fig. 3a). Only pre-treatment at 5 and 10 °C elicited a RCH response in this species (Fig. 3a). In *C. capitata*, maximum survival was achieved only following pre-treatment at 5 or 10 °C for 2 h (Fig. 3b). However, pre-treatment at 0, 15, and 35 °C also resulted in significant increases in survival relative to the control flies (Fig. 3b), although survival following these pre-treatments did not exceed 50%.

Time-course of rapid thermal responses

Duration of hardening at 5 °C significantly affected the development of a RCH response in both *C. rosa* ($\chi^2 = 35.70$, d.f. = 5, $P < 0.0001$) and *C. capitata* ($\chi^2 = 54.13$, d.f. = 5, $P < 0.0001$) (time effect, Table 1). Overall, *C. capitata* had significantly higher survival in this experiment [species effect, Table 1; *C. capitata*: 58.5 ± 25.2%, *C. rosa*: 38.5 ± 38.2% (mean ± SD)]. However, there was no time × species interaction effect (Table 1). In *C. rosa*, up to 30 min hardening at 5 °C did not significantly improve survival during 2 h exposure to −5 °C (Fig. 4a). In *C. capitata*, there was a significant increase in survival (40–50%) at −5 °C for 2 h after only 15 min hardening at 5 °C (Fig. 4b), which was significantly greater survival than in *C. rosa* at the same time point ($\chi^2 = 4.42$, d.f. = 1, $P = 0.0355$). However, in both species 80–100% survival was achieved following 2 h pre-treatment at 5 °C indicating RCH (Fig. 4a,b).

Time after a 2 h long 5 °C pre-treatment significantly affected survival at −5 °C in both *C. rosa* ($\chi^2 = 63.51$, d.f. = 8, $P < 0.0001$) and *C. capitata* ($\chi^2 = 48.43$, d.f. = 8, $P < 0.0001$). RCH was lost after only 30 min at 25 °C in *C. rosa* (Fig. 4c), but in *C. capitata*, survival at −5 °C remained significantly higher than controls for up to 8 h after 5 °C pre-treatment (Fig. 4d). Overall, average survival across all time treatments was higher in *C. capitata* (74.3 ± 28.6%) compared with *C. rosa* (26.6 ± 34.9%).

Table 1. Summary of results for species effects from three thermal tolerance experiments.

Experiment	Effect	d.f.	Wald χ^2	P
Temperature variation effects				
	Temperature	8	82.48	<0.0001
	Species	1	0.00	0.9997
	Temperature × species	8	9.73	0.2848
Duration to achieve RCH				
	Time	4	61.40	<0.0001
	Species	1	9.72	0.0018
	Time × species	4	6.87	0.1427
Duration RCH persists				
	Time	7	273.04	<0.0001
	Species	1	179.31	<0.0001
	Time × species	6	23.43	0.0007

In all cases, survival was compared among treatments using a generalised linear model (GLZ) assuming a binomial distribution and a logit link function in SAS (Proc Genmod) with corrections for overdispersion. d.f., degrees of freedom; χ^2, chi-square statistic. *Temperature variation effects* = the effect of different pre-treatment temperatures on survival at −5 °C; *Duration to achieve RCH* = effects of pre-treatment duration at 5 °C on survival at −5 °C; *Duration RCH persists* = time taken for increased survival (determined at −5 °C) after a 5 °C pre-treatment to return to control levels at 25 °C. For full details, see Materials and methods.

Microclimate data and model simulation

The number of temperature events that fell below 10 °C for at least 2 h and that therefore might result in RCH responses were determined from a field season in 2009 (Fig. 5a). In an 86-day recording period during the late austral summer, 63 potential RCH events occurred in the field setting.

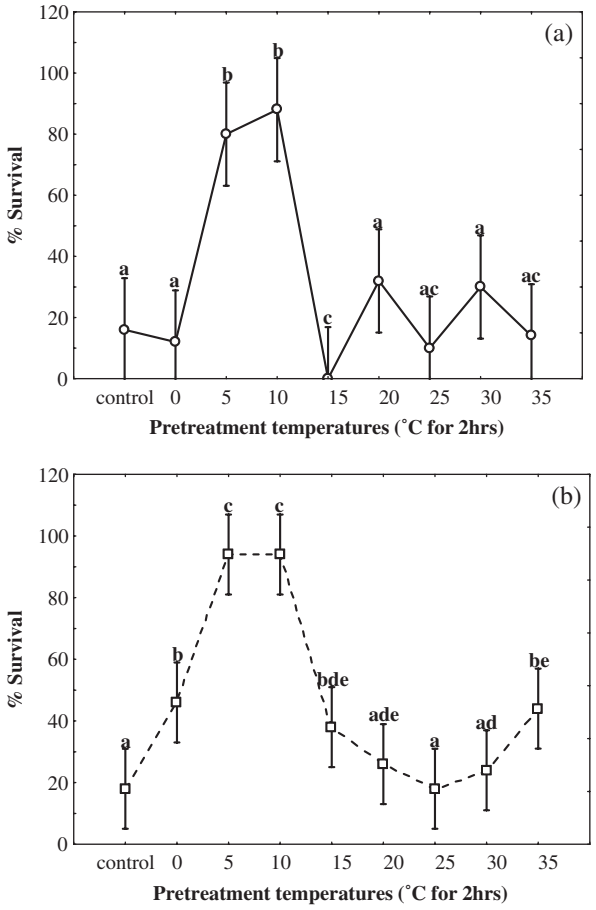

Fig. 3. Effects of pre-treatment temperature (2 h exposures) on the ability to survive 2 h at −5 °C in (a) *Ceratitis rosa* and (b) *C. capitata.* Control = pre-treatment for 2 h at 25 °C. Values are means ± 95% CLs. $n = 50$ per group. Tukey–Kramer's *post hoc* tests were used to separate statistically homogeneous groups at $P = 0.05$. Group means with the same letter are not significantly different.

The population extinction model showed that time to extinction for *C. capitata* is 7.5 days at a 40% temperature variability scenario, 4.5 days in the 20% scenario, and 3 days in the 5% scenario. By contrast, *C. rosa* population times to extinction were 2.5 days at 40% and 20% variability, and 2 days in the 5% simulation (Fig. 5b,c).

Discussion

The major physiological factors contributing to invasion success once an insect species is introduced into a novel environment, typically include either increased environmental tolerance or increased plasticity of environmental tolerance of the invasive species (Lee *et al*., 2002; Richardson & Pysek, 2006; Chown *et al*., 2007; Slabber *et al*., 2007). However, it is less well documented that the time-course of plastic responses of thermal tolerance can also have marked effects on differential survival among populations or species. When estimated as proportion surviving at acute high or low temperatures, both the widespread *C. capitata* and more restricted congener *C. rosa* have similar levels of basal thermal tolerance (although see Nyamukondiwa & Terblanche, 2009). In addition, these lethal temperatures are similar to those reported for other tropical–temperate insects (e.g. Addo-Bediako *et al*., 2000; Kimura, 2004; Hazell *et al*., 2010) suggesting that variation in basal tolerance may not be a major factor contributing to *C. capitata*'s invasion success.

At high temperatures there was significant variation in heat hardening effects between species, but the magnitude of plasticity was relatively low. However, this lack of hardening effect may be partly a consequence of the exact experimental protocol used, as it has been previously shown that including a recovery period of 30–60 min at an intermediate temperature (20–25 °C) can elicit stronger responses (Hoffmann *et al*., 2003; Chown & Nicolson, 2004). RHH has been observed in a number of *Drosophila* species (Chen *et al*., 1991; Loeschcke *et al*., 1997; reviewed in Hoffmann *et al*., 2003) and recently also in *C. capitata* (Kalosaka *et al*., 2009), but to our knowledge, such high temperature responses have not been documented in *C. rosa*. The physiological mechanisms underlying RHH involves the production of heat-shock proteins (HSPs) which can act as molecular chaperones by preventing accumulation of structurally damaged proteins (reviewed in Sørensen *et al*., 2003; Hoffmann *et al*., 2003). Consequently, HSPs are thought to be a significant component of field thermal tolerance in *Drosophila* (Feder *et al*., 2000; Overgaard *et al*., 2010) and also other insect species (e.g. Rinehart *et al*., 2006).

By contrast, substantial RCH responses were found in *C. capitata* and *C. rosa*, which were comparable in magnitude to those reported from other tropical fly species to date (e.g. Lee *et al*., 1987; Coulson & Bale, 1990; Kelty & Lee, 2001; Shreve *et al*., 2004 reviewed in Lee & Denlinger, 2010). However, RCH has not been demonstrated previously in any *Ceratitis* species [although see work undertaken on other Tephritids e.g. *Dacus tryoni* (Meats, 1973), *Bactrocera oleae* (Koveos, 2001), and *Eurosta solidaginis* (Bale *et al*., 1989)]. The results showed a significant improvement in low temperature survival following 2 h pre-treatment at 5 and 10 °C in both *C. rosa* and *C. capitata*. Indeed, survival at −5 °C for 2 h increased from ∼20% to 80–100% in both species. Experiments investigating which temperatures elicit RCH responses showed that only 5 and 10 °C pre-treatments (for 2 h) induce a full RCH response in both *C. rosa* and *C. capitata* (see Fig. 3). However, a pre-treatment at 0 and 35 °C also significantly improved survival in *C. capitata*, although a full hardening response was not achieved. High temperature exposures may confer low temperature tolerance (see Chen *et al*., 1991; Sinclair & Chown, 2003; Rajamohan & Sinclair, 2008). However, high and low temperature could be different stressors, and it has been argued that the mechanisms for high and low temperature tolerance may be fundamentally different (Hoffmann *et al*., 2003; but see discussions in Chown & Nicolson, 2004; Sinclair & Roberts, 2005). There may be some overlap in these mechanisms, for example through HSP production and membrane phospholipid composition changes (Murray *et al*., 2007; MacMillan *et al*., 2009a), and clearly inhibition of apoptosis occurs (Yi

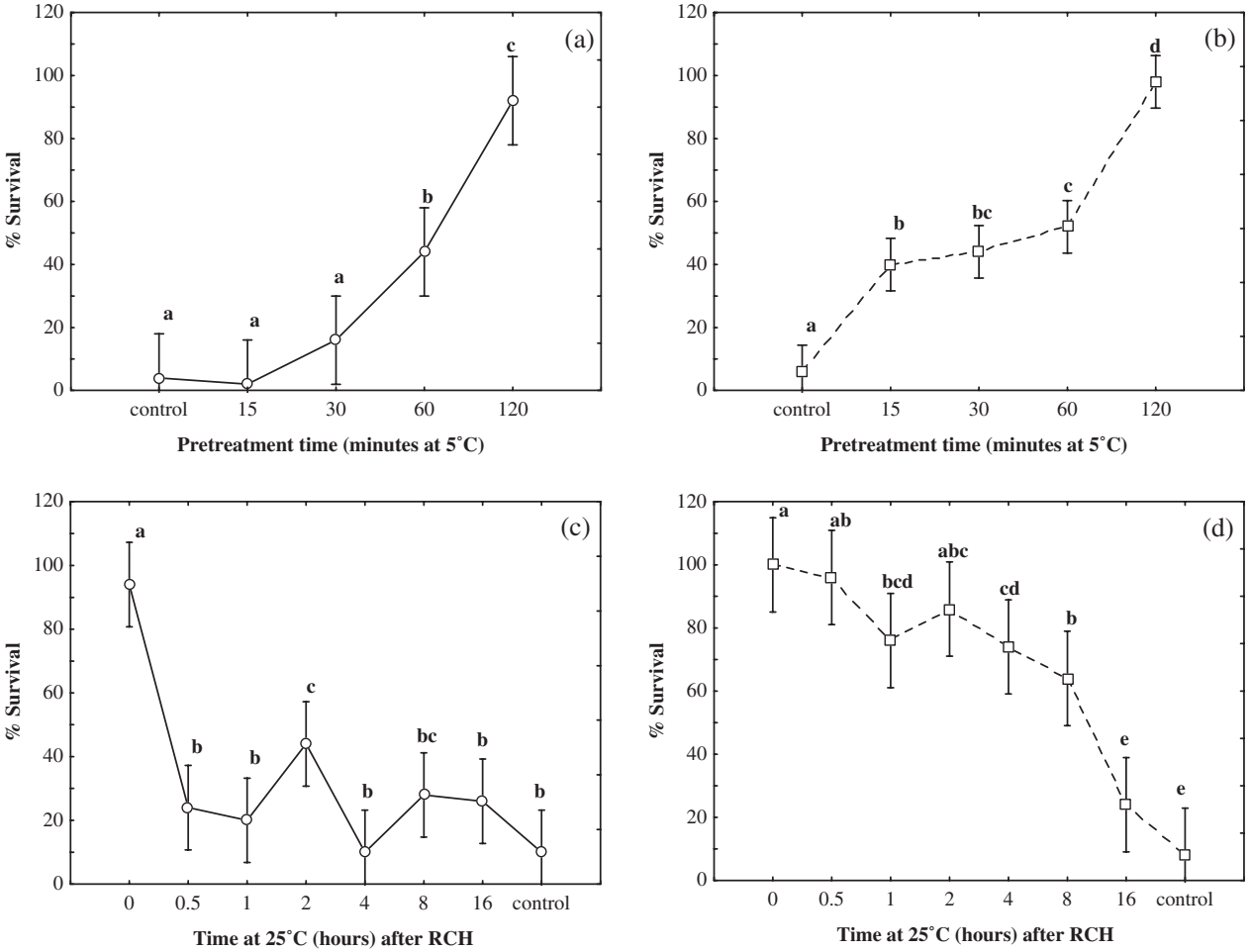

Fig. 4. Time course of rapid cold hardening (RCH) in *Ceratitis capitata* and *C. rosa*. Time to induce a full RCH response (100% survival) in (a) *C. rosa* and (b) *C. capitata* and duration that the RCH response lasts in (c) *C. rosa* and (d) *C. capitata*. Control = no pre-treatment. Values are means ± 95% CLs. $n = 50$ per group. Tukey–Kramer's *post hoc* tests were used to identify statistically homogeneous groups. Means with the same letter are not significantly different.

et al., 2007), although HSP production and membrane lipid remodelling may not explain the short-term survival increase conferred by RCH (MacMillan *et al*., 2009b). Regardless, the cross-tolerance observed in this study could translate to a survival advantage for *C. capitata* relative to *C. rosa*, as the former species can likely respond not only to low temperatures but also to increasing temperature variation.

The marked RCH responses documented here probably allow *C. rosa* and *C. capitata* to track diurnal changes in environmental temperature and may optimise feeding and mating during otherwise unfavourable temperature conditions (Kelty & Lee, 2001; Shreve *et al*., 2004; reviewed in Chown & Nicolson, 2004). The physiological mechanisms underlying RCH are unclear, but may include accumulation of glycerol, trehalose, polyhydric sugars, and alcohols (Denlinger & Lee, 1998), changes in whole body supercooling points (Czajka & Lee, 1990; Lee *et al*., 2006) and production of HSPs (reviewed in Hoffmann *et al*., 2003; Sørensen *et al*., 2003; Chown & Nicolson, 2004; although see discussion in Sinclair & Roberts, 2005). However, improved supercooling capacity may be of little significance in RCH if the cold shock temperature is much higher than the insect's freezing temperature unless they are correlated in some way (reviewed in Lee & Denlinger, 2010). Nevertheless, a range of species do not adjust thermal tolerance over short time periods. For example, little evidence exists for RCH in adults of the tsetse fly *Glossina pallidipes* (Terblanche *et al*., 2008), adults of the Antarctic midge *Belgica antarctica* (Lee *et al*., 2006), the sub-Antarctic kelp fly *Paractora dreuxi* (Marais *et al*., 2009), larvae of *Pringleophaga marioni* (Sinclair & Chown, 2003), or adult false codling moth *Thaumatotibia leucotreta* (Stotter & Terblanche, 2009).

The results of the present study are therefore novel since they show that the time-course of developing a RCH response may also provide a distinct advantage in the invasive *C. capitata*. *Ceratitis rosa* showed a significant increase in low temperature survival following a 1 h hardening at 5 °C. By contrast, survival of low temperature significantly increased after only 15 min of pre-treatment at 5 °C in *C. capitata*, indicating

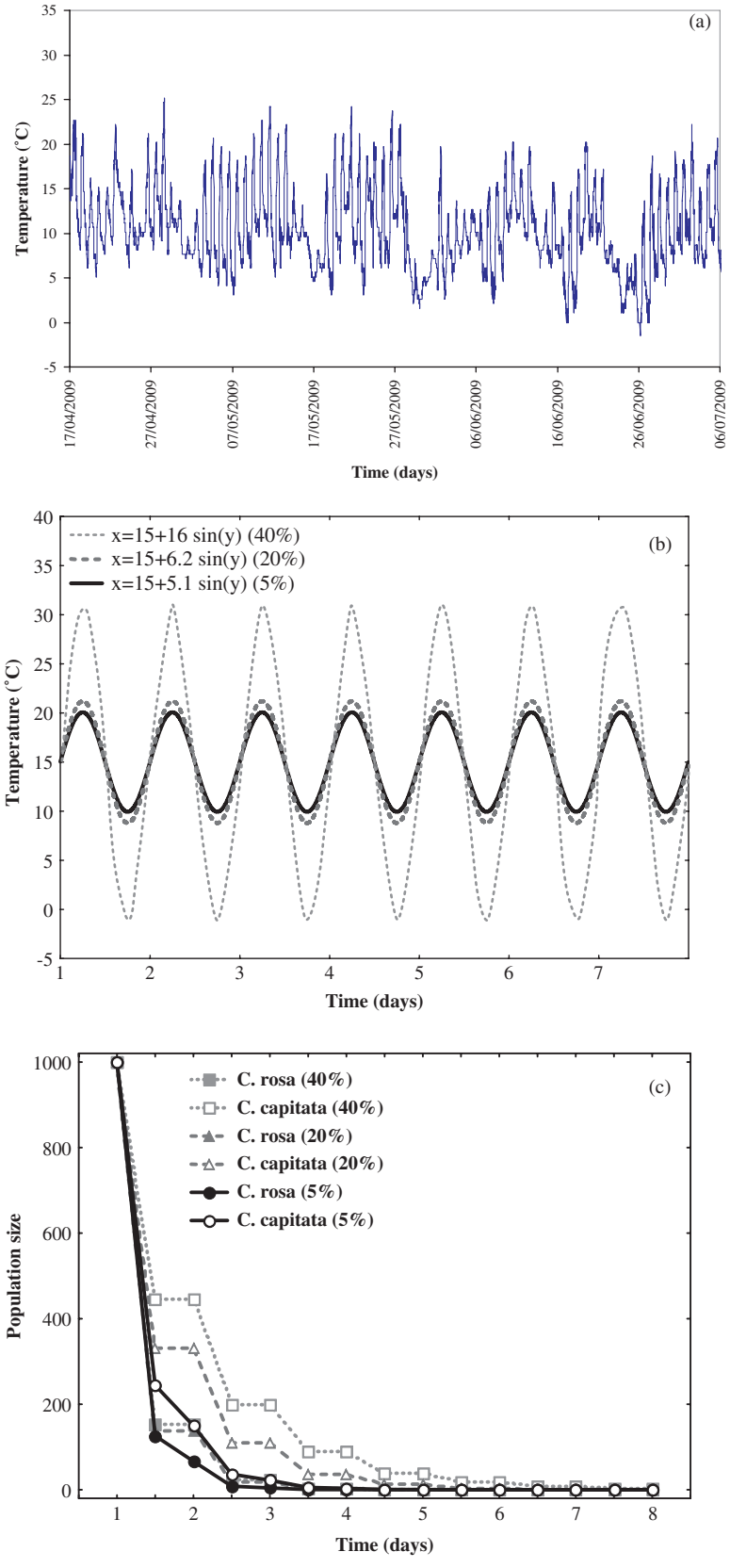

Fig. 5. (a) Microclimate temperature recording from an orchard (Lakenvlei farm, Ceres, Western Cape, South Africa) where both *Ceratitis rosa* and *C. capitata* are typically found. Shade temperature was recorded using Thermocron iButtons (0.5 °C accuracy; 15 min sampling frequency) in the centre of an apple tree at 1.25 m above the ground. From experimental results, temperature events that fall below 10 °C (stippled horizontal line) for at least 2 h can induce a rapid cold-hardening response. (b) Three simulated temperature scenarios used to model population extinction at different levels of temperature variability. The 40% model contained temperatures that spent 40% of their time <10 °C. The 20% and 5% models contained temperatures with 20% and 5% of the time <10 °C. (c) Results from the population extinction model simulation exploring differences in RCH responses between *C. rosa* and *C. capitata* at the three simulated temperature variation conditions.

a quicker RCH response in this species. However, a full RCH response was only realised following 2 h hardening at 5 °C in both *C. rosa* and *C. capitata* (Fig. 4a,b). Similar RCH responses have been observed in, for example, *Sarcophaga crassipalpis*, where only 30 min pre-treatment at 0 °C increased survival at −10 °C, although maximum survival was only achieved following 2 h pre-treatment (Lee *et al*., 1987).

After the RCH response has been acquired we found further differences in the duration of protection afforded between the two species (Fig. 4c,d). Survival at −5 °C for 2 h was significantly higher than control levels up to 8 h after RCH in *C. capitata* had been returned to 25 °C, although by 16 h post-RCH, all effects were reversed. By contrast, in *C. rosa* the RCH effects were rapidly lost. After only 30 min, improvements in survival were significantly lower and were indistinguishable from control values (Fig. 4c). Overall, this shows that in *C. capitata*, RCH occurs more quickly and lasts longer as compared with *C. rosa* indicating, on average, a survival advantage for *C. capitata* relative to *C. rosa* in these experiments. Previous studies have also found that RCH lasts for only a few hours, although this is dependent on the temperature post-hardening (e.g. Meats, 1973). Nevertheless, if *S. crassipalpis* was returned to 25 °C after low temperature pre-treatment, the effects of RCH disappeared rapidly (Chen *et al*., 1991; see also Meats, 1973; Coulson & Bale, 1990; Czajka & Lee, 1990; reviewed in Chown & Nicolson, 2004). In a broader context, however, asymmetric plastic responses to changes in stressful conditions have been found previously (e.g. Palumbi, 1984; Dekinga *et al*., 2002) and suggest that rates of acquisition of stress resistance might typically be faster than loss of stress resistance (but see also Murray *et al*., 2007), although this probably depends in part on the relative costs and benefits of acclimation responses (see discussions in Palumbi, 1984; Deere & Chown, 2006; Chown & Terblanche, 2007). However, such clear-cut differences in the time-course of plasticity in insect thermal tolerance are seldom demonstrated, even among closely related species.

Nevertheless, species comparisons make several simplifying assumptions regarding the evolution of thermal tolerance. For example, one of the species may have had significantly greater basal thermal tolerance in the past which was subsequently lost over evolutionary timescales. Alternatively, *C. capitata* may have evolved greater plasticity of low temperature tolerance as its range expanded with repeated introductions into novel habitats, rather than having a greater invasion success as a consequence of its present physiological responses. Thus, the direction of causality, and any co-relationship with biogeography, is not clear. Nevertheless, a major question posed by this study is whether or not extant variation in physiological responses may contribute to present-day differences in invasion success. To answer this question, we ran a population extinction model that predicts the time to extinction under various hypothetical temperature scenarios. Except for variation in the persistence of RCH responses, this model assumes that all else was equal among these two species (e.g. thermal requirements for development and growth rate), a simplifying assumption, but one which nevertheless gives an indication of the potential fitness benefits of variation in plastic time-courses of cold-hardening responses. Indeed, the model showed clear-cut differences in population persistence over time with the given variation in time-courses of RCH responses, and suggests that, upon introduction of both species to similar thermally variable environments, this may well constitute a fitness advantage for *C. capitata*. However, in the simulation in which both species are introduced to a similar thermally variable environment with a relatively high mean temperature (e.g. a tropical, equatorial environment), the variation in RCH time-courses is not likely to make a significant difference for population establishment. However, such a model does not consider the fitness costs of plasticity (see discussions in Kristensen *et al*., 2008), a major aspect of thermal tolerance plasticity that is poorly understood in insect hardening responses (Chown & Terblanche, 2007). Clearly further work is required to understand the costs and benefits of variation in plastic responses of thermal tolerance in Tephritid flies. Nevertheless, additional circumstantial evidence for this model is provided by microclimate temperature recordings from an area in South Africa where *C. capitata* and *C. rosa* distributions overlap. These climate data showed that temperatures eliciting these responses are frequently encountered under agro-ecosystem conditions in a cool, temperate environment. This suggests that RCH may be occurring frequently in the wild in this habitat, and possibly protecting flies against sudden temperature changes, as has been shown in *Drosophila* under semi-field (Kelty & Lee, 2001; Kelty, 2007) and field conditions (Shreve *et al*., 2004; Overgaard & Sørensen, 2008), assuming that opportunities for behavioural thermoregulation are similar for both species (e.g. Huey & Pascual, 2009). Therefore, RCH probably affords significant fitness advantages to *C. capitata* relative to *C. rosa* under natural conditions.

In conclusion, the results of the present study document significant variation in the time-course of phenotypic plasticity of acute low temperature tolerance in adult *C. rosa* and *C. capitata*. *Ceratitis capitata* rapidly cold-harden faster, have a wider range of temperatures that elicit RCH responses, and have RCH survival benefits that last longer than in *C. rosa*. These results suggest that plasticity in acute thermal tolerance might be an important mechanism facilitating survival of *C. capitata* upon introduction to novel environments, particularly in cool, temperate habitats. Broadly, these results suggest variation in physiological responses may aid *C. capitata*'s survival upon introduction to novel thermal habitats.

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Phenotypic plasticity of desiccation resistance in *Glossina* puparia: are there ecotype constraints on acclimation responses?

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Abstract

Phenotypic plasticity allows organisms to cope with environmental variation and may aid in the evolution of novel traits. However, whether phenotypic plasticity is beneficial, or if acclimation responses might be constrained to particular ecotypes is generally poorly explored. Here we test the beneficial acclimation hypothesis (BAH) and its alternatives for desiccation resistance to atmospheric moisture in mesic- and xeric-adapted *Glossina* species. Highly significant interactions among acclimation and test humidity were detected for water loss rates indicative of significant phenotypic plasticity. Ordered-factor ANOVA was unable to reject predictions of the 'drier is better' acclimation hypothesis in xeric *Glossina morsitans* and mesic *G. austeni*. Evidence for the 'deleterious acclimation hypothesis' was found for mesic *G. palpalis* as expected from the moist habitats it typically occupies. By contrast, support for the 'optimal acclimation hypothesis' was found in xeric *G. pallidipes*. Little support for BAH was obtained in the present study, although other hypotheses, which might enhance fitness within the environments these species are typically exposed to, were supported. However, acclimation responses were not necessarily constrained to xeric/mesic ecotypes which might be expected if adaptation to a particular environment arose as a trade-off between plastic responses and living in a particular habitat. These results highlight the complexity of acclimation responses and suggest an important role for phenotypic plasticity in moderating environmental effects on evolutionary fitness in *Glossina*.

Introduction

Phenotypic plasticity is an important mechanism allowing plants and animals to cope with environmental heterogeneity. However, the role of phenotypic plasticity, defined as 'the ability of an organism to react to an environmental input with a change in form, state, movement or rate of activity' (West-Eberhard, 2003), has come under close scrutiny in recent years and its role in adaptive evolution remains contentious (Chown & Terblanche, 2007; Ghalambor *et al.*, 2007). Although it is typically assumed that phenotypic plasticity can enhance survival under adverse conditions, whether phenotypic plasticity is a driver of novel traits, and potentially speciation, is more debatable (see recent reviews by West-Eberhard, 2005; Crispo, 2007; Ghalambor *et al.*, 2007). For plants, adaptive phenotypic plasticity is increasingly demonstrated (e.g. Caruso *et al.*, 2006) and demonstrations of the costs of plasticity are also becoming well elucidated (Van Kleunen *et al.*, 2000; Weinig *et al.*, 2006; reviewed in Pigliucci, 2005; Van Kleunen & Fischer, 2005). However, in animals these issues are far less clear despite their obvious importance (though see, e.g. Hoffmann & Hewa-Kapuge, 2000; Steiner & Van Buskirk, 2007; Gervasi & Foufopoulos, 2008; Kristensen *et al.*, 2008 and discussed in Hoffmann, 1995).

For many living organisms, it is considered that a prior experience of a novel environment can give rise to a performance advantage, with fitness benefits, when

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exposed to that environment on a subsequent occasion. Formally, this is known as the 'beneficial acclimation hypothesis' (BAH), defined as 'acclimation to a particular environment gives a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment' (Leroi *et al.*, 1994; p. 1917). The BAH is intuitively appealing, particularly to animal physiologists (e.g. Cossins & Bowler, 1987; Hochachka & Somero, 2002; see discussion in Deere & Chown, 2006), but has come under close scrutiny in recent years for several reasons of which three are perhaps most significant.

First, early explicit tests of the BAH in *Escherichia coli* refuted this hypothesis (Leroi *et al.*, 1994). However, the rejection of the BAH by Leroi *et al.* (1994) has subsequently been strongly criticized. Most notably, Wilson & Franklin (2002) argued that Leroi and colleagues examined developmental plasticity, and that this is a different type of phenotypic plasticity from the traditional physiologist's perspective of reversible acclimation (Wilson & Franklin, 2002; and see Piersma & Drent, 2003). Further research examining whether developmental plasticity and adult acclimation responses might confound interpretations of adaptive plasticity suggests that these concerns are indeed warranted (e.g. Terblanche & Chown, 2006). Many recent tests of the BAH have not found support, partly because at least one of the alternative hypotheses could not be rejected (Zamudio *et al.*, 1995; Huey *et al.*, 1999; Gibert *et al.*, 2001; Stillwell & Fox, 2005; Deere *et al.*, 2006). By contrast, several recent studies across a range of taxa have either demonstrated or claimed strong support for the BAH in animal physiology (e.g. Seebacher & Wilson, 2006; Gvozdik *et al.*, 2007; Rogers *et al.*, 2007; Wilson *et al.*, 2007; Frazier *et al.*, 2008; Marais & Chown, 2008), although support for environment-specific advantages (e.g. colder is better or hotter is better hypotheses) has been documented for some species (Deere & Chown, 2006; Frazier *et al.*, 2006; Santos, 2007).

Second, the environments which are used to induce the acclimation response might differ from that used to test the potential benefits of plastic trait changes. This may be an issue because the animal is sometimes tested under deleterious conditions which are not necessarily the same as, or as ecologically relevant as, the kinds of changes expected in the wild (see Huey *et al.*, 1999). Thus, the animal is exposed to an unnatural stressor which results in poorer performance on a subsequent exposure, hence lower fitness. This raises the question of whether the experimental conditions give sufficient insight into the actual environment in which the performance advantage might occur (Hoffmann, 1995; Loeschcke & Hoffmann, 2002; Chown & Terblanche, 2007) although the extent of such an issue is partly dependent on the degree of correlation in trait performance among various environments. For several traits of acute thermal tolerance in insects, which are likely under different genetic control (Anderson *et al.*, 2005; Rako *et al.*, 2007), the likelihood of similarity among trait responses and thus correlated performance is low (Rako & Hoffmann, 2006; Folk *et al.*, 2007; Kristensen *et al.*, 2008). Nevertheless, the extent of this problem is not well established for many traits and taxa.

Finally, it has been argued that assessing fitness responses is perhaps not the best way to test adaptive phenotypic plasticity. Woods & Harrison (2001, 2002) have argued that trait-specific acclimation responses should be considered rather than fitness responses *per se* (Woods & Harrison, 2001, 2002; Chown & Terblanche, 2007). Therefore, additional research is necessary to test for adaptive phenotypic plasticity using a strong inference approach (Huey *et al.*, 1999) while controlling for the types of plasticity (e.g. developmental vs. adult), considering the ecological relevance of the experimental conditions used, and across non-model organisms to determine the extent to which beneficial acclimation might exist.

Tsetse flies (Diptera: Glossinidae) serve as an excellent model system for studying acclimation hypotheses of water loss rates (WLR) with direct evolutionary significance. *Glossina* spp. are the vectors of human and animal trypanosomes which are invariably fatal for the infected organism. Among Diptera, tsetse are unusual because they reproduce using adenotrophic viviparity (i.e. *in utero* live birth) and have a K-strategy which requires high energetic investment by the mother per individual offspring (Leak, 1999). A third instar larva is deposited in the soil by its mother, which pupates to continue development underground without obtaining any additional resources (either water or food) (Leak, 1999, p. 17–24). In consequence, body water content and lipid reserves of puparia are largely a function of maternal investment in *Glossina* (see, e.g. Bursell, 1958; Langley & Clutton-Brock, 1998; reviewed in Langley, 1977; Leak, 1999). Hence, desiccation resistance is an important evolutionary strategy for maintaining body water and lipid stores. Careful regulation of whole-animal WLR would also ensure that newly emerged adult flies have more time available in which to obtain their first bloodmeal. This is likely under strong directional selection in the wild as the teneral flies experience high mortality under natural conditions (Bursell, 1959; Hargrove, 2001; reviewed in Hargrove, 2004). It is widely suspected that these flies are forced to take greater risks and actively seek hosts under adverse environmental conditions (e.g. hot and dry) when it is least efficient to forage (see Terblanche & Chown, 2007). Indeed, it has been well demonstrated that *Glossina* species have evolved desiccation resistance to match local climate conditions in the pupal stage (Bursell, 1958; Kleynhans & Terblanche, 2009) and desiccation resistance is a trait which is probably directly related to evolutionary fitness in this life stage (Bursell, 1958, 1959; Hargrove, 2004).

In addition, an important link exists between temperature, moisture availability and tsetse population dynamics. Local population abundances are generally higher in years when temperatures are nearer to optimal development temperatures and in years that are wetter (Rogers & Randolph, 1986, 1991; Torr & Hargrove, 1999; reviewed in Hargrove, 2004). Physiological and population dynamics studies support the view that temperature is a major factor determining the survival of adult tsetse (Hargrove, 2001; Terblanche *et al.*, 2006, 2007) and population dynamics suggests that moisture availability is important for pupal life stages residing in the soil (Hargrove, 2004). However, physiological assessments of intra- and inter-specific variation are generally limited in tsetse (though see Bursell, 1958, 1959; Terblanche *et al.*, 2006, 2008) despite the fact that much inter-specific variation exists (see, e.g. Rogers & Randolph, 1986, 1991; Hargrove, 2004). In particular, no studies of *Glossina* species have directly assessed alternative acclimation hypotheses for any physiological traits. Furthermore, to our knowledge, no traits have been investigated for acclimation responses in the pupal life-stage although some work on adult acclimation responses of *G. pallidipes* has been undertaken. These studies of adult physiology suggest that water balance and thermal tolerance are readily adjusted in response to different thermal regimes and that physiological responses might be stage- or age-specific (Terblanche & Chown, 2006; Terblanche *et al.*, 2006; reviewed in Bowler & Terblanche, 2008). Moreover, this study has shown that, at least for one of the most well-studied species, *G. pallidipes*, adaptive physiological variation of populations to local climates is equal to the magnitude of phenotypic plasticity thereby suggesting limited local adaptation (Terblanche *et al.*, 2006). This suggests that further research into intra- and inter-specific physiological variation is an important avenue for understanding the evolution of *Glossina*. However, the latter studies were not designed to fully explore acclimation hypotheses (Terblanche & Chown, 2006) and the present study therefore addresses this major lacuna.

As stated previously, exposure to a particular environment can give animals a performance advantage on a subsequent exposure in that environment and is termed the 'beneficial acclimation hypothesis' (BAH) (Leroi *et al.*, 1994; Huey & Berrigan, 1996; Deere & Chown, 2006). However, the conditions experienced may also result in a performance decline if the previous exposure is somehow detrimental [the 'deleterious acclimation hypothesis' (DAH)] (see Loeschke & Hoffmann, 2002). Moreover, some animals may simply be at an advantage under selected extreme conditions because of evolutionary adaptation and secondary constraint(s) and, thus, do poorly under all other conditions. In terms of moisture availability, these can be considered as 'wetter is better' (WIB) or 'drier is better' (DIB) hypotheses (for similar discussion with respect to temperature on life-history see, e.g. Frazier *et al.*, 2006) or, alternatively, optimal acclimation hypothesis (OAH) whereby an intermediate acclimation and test humidity is always best. Alternatively, animals may have no response to a particular range of conditions and therefore show no phenotypic plasticity (NP). The latter also represents a null model for phenotypic plasticity (see discussions in Seebacher, 2005; Angilletta *et al.*, 2006; Deere & Chown, 2006). Therefore, a range of responses to environmental variability might be achieved and represent evolutionarily distinct options with dramatically different implications for a species' fitness under natural climatic variations (Fig. 1). Typically, studies of alternative acclimation hypotheses are limited to temperature responses across a range of survival- or performance-related traits (e.g. Huey *et al.*, 1999; Deere & Chown, 2006; Kristensen *et al.*, 2008; Marais & Chown, 2008). Remarkably few studies have assessed these responses to water availability and changing atmospheric moisture availability (though see Woods & Harrison, 2001). However, climate change predictions emphasize that both temperature and moisture availability will change dramatically in the future (Easterling *et al.*, 2000; Tebaldi & Sanso, 2009) and that predictions of animal responses require a physiological approach (Helmuth *et al.*, 2005; Kearney & Porter, 2006; Chown & Terblanche, 2007). Here, we therefore test six alternative acclimation hypotheses to assess phenotypic plasticity of desiccation resistance for two xeric and two mesic species of *Glossina* during the pupal life stage in order to better comprehend evolutionary responses to climatic variation, and in addition, potential ecotype constraints.

Materials and methods

Study animals

Five- to seven-day-old puparia of *Glossina pallidipes*, *G. palpalis*, *G. austeni* and *G. morsitans* (subspecies: *centralis*) (Diptera: Glossinidae) were obtained by shipping from screened, infection-free laboratory colonies (*G. pallidipes*, *G. palpalis*, *G. morsitans*: Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria; *G. austeni*: Onderstepoort Veterinary Institute, Pretoria, South Africa). Puparia of all species were sent via air cargo in well-insulated non-airtight containers and kept under controlled temperature conditions during transportation (typically <2 days of transport, temperature range: 18–24 °C). Upon arrival, puparia were exposed to 25 °C, 76% relative humidity (r.h.) for 3–5 days to standardize conditions at the start of the experiments. Subsequently, puparia were randomly divided into three r.h. groups (0% r.h.: range 0–5%; 50% r.h.: range 50–58%; 99% r.h.: range 95–100%) at controlled developmental optimal temperature in a climate chamber (25.0 ± 1.0 °C). These species were selected for experimental use based on availability of established colonies and to represent two approximately

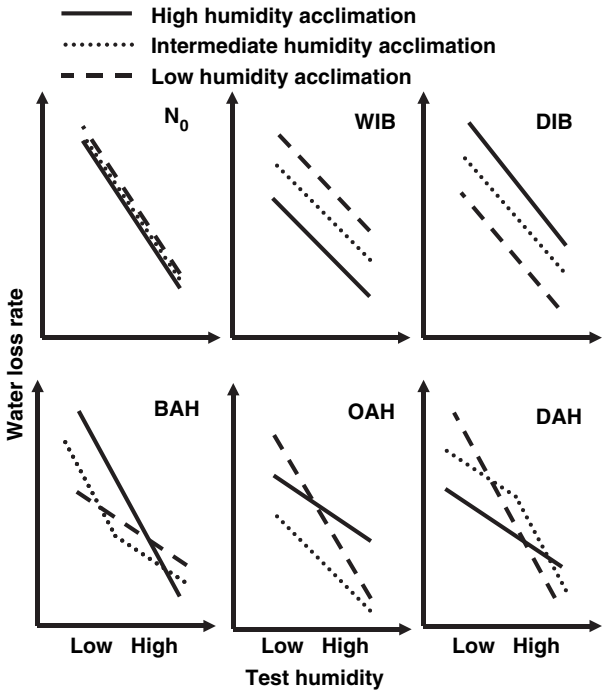

Fig. 1 Schematic diagram of potential acclimation responses of water loss rate (WLR) examined in the present study. Because of physical principles of atmospheric saturation deficit and cuticular permeability a negative reaction norm is always expected for WLR (see Hadley, 1994). N_0 represents the null hypothesis for plasticity of WLR and predicts that for different rearing humidity conditions WLR is similar under high or low test humidity conditions regardless of acclimation humidity. The wetter is better (WIB) hypothesis predicts that individuals exposed to low humidity acclimation will always lose water faster than individuals exposed to drier conditions, although no change in the shape of the reaction norm is expected. The drier is better (DIB) hypothesis predicts the exact opposite of the WIB hypothesis with the rank order simply reversed but no change in the shape of the reaction norm. The beneficial acclimation hypothesis (BAH) predicts that prior experience of low humidity will result in a reduction of WLR during a subsequent exposure relative to individuals which have not had the opportunity to acclimate to these conditions. Specifically, the BAH predicts a change in the shape of the reaction norm (i.e. that slopes are not parallel). The optimal acclimation hypothesis (OAH) predicts that WLR is lowest at a particular intermediate set of conditions and exposure to another set of conditions will change the rank order of low or high acclimation individuals. The deleterious acclimation hypothesis (DAH) predicts the opposite of the BAH, such that animals previously exposed to desiccating conditions were harmed during this exposure and simply perform worse (i.e. lose more water) during testing under these conditions.

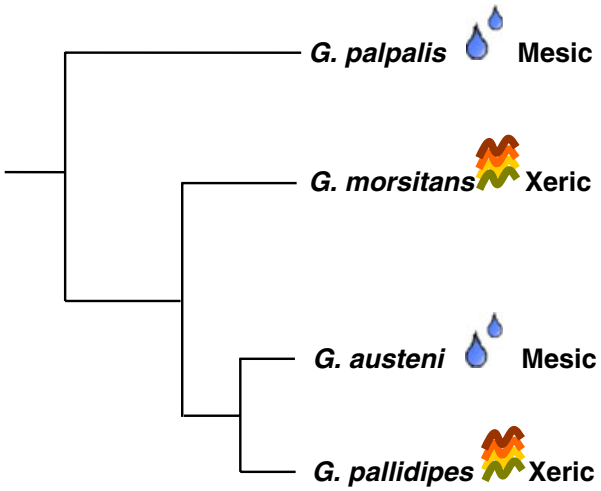

Fig. 2 Hypothetical phylogenetic relationships of the *Glossina* (Diptera: Glossinidae) species investigated with habitat type (mesic/xeric; assigned according to Bursell, 1958, 1959; and see Kleynhans & Terblanche, 2009) following the molecular consensus phylogeny derived in Peterson *et al.* (2007). *Glossina palpalis* mean pupal body mass: 24.80 mg, *G. morsitans*: 24.13 mg, *G. austeni*: 18.73 mg and *G. pallidipes*: 34.49 mg.

size-matched sets of species with a xeric and a mesic species (according to Bursell, 1958) in each size class and of known phylogenetic association (Fig. 2). Xeric or mesic classification was assigned according to the combined environmental mean annual precipitation and mean annual temperature (Bursell, 1959; Kleynhans & Terblanche, 2009). This experimental design was employed to assess possible constraints on acclimation responses based on phylogenetic or ecotype (xeric/mesic) association.

The oldest colony used in the present study (*G. pallidipes*) was established *c.* 1975 and has similar genetic variation to wild populations (Krafsur & Wohlford, 1999). All colonies of the four species studied are maintained in high numbers to avoid deleterious inbreeding effects. The youngest colony (*G. austeni*) has probably been in culture for *c.* 10 years. However, in adult *G. pallidipes* physiological variation occurs between field and laboratory populations with differences most prominent in a trait of low temperature tolerance (critical thermal minima, Terblanche *et al.*, 2006). By contrast, resting WLR of adult *G. pallidipes* appears broadly comparable between laboratory and field populations (though see Discussion). Here, we therefore assume that laboratory puparia are physiologically representative of wild flies and that inter-specific variation is greater than intra-specific (i.e. inter-population) physiological variation.

Experimental design

Individuals of *G. pallidipes*, *G. morsitans*, *G. austeni* and *G. palpalis* puparia were acclimated at three different rearing humidities at 25 (±1.5) °C for 10 days and then divided into three treatment groups and randomized immediately between the same three humidities for an additional 4 days during which time we determined the

rates of water loss (Fig. 3a). Thus, we undertook a fully crossed three-by-three experimental design under three humidity conditions (see, e.g. Stillwell & Fox, 2005) to assess multiple potential acclimation hypotheses (Fig. 1, Table 1). All treatment groups were handled for similar duration and with similar vigour to ensure that stress or physical disturbance was similar among treatments. It is unlikely that development or age-related changes are a confounding factor in these experiments as Bursell (1958) has demonstrated previously that WLR of puparia from a range of species are low and stable for the majority of this life stage (days 5–25) and all experimental work took place during this stable period. All experiments were undertaken in the dark, except during weighing. Pilot trials showed that brief exposures (<1 week) to either high- or low-humidity conditions did not significantly affect the proportion of emerging flies at eclosion relative to the intermediate (optimal) treatment group in these species ($P > 0.12$ in all cases, though see Bursell, 1958, p. 201 for data after rearing for the entire pupal life-stage).

Table 1 The predicted significance and direction (– or +) of the linear (L) and quadratic (Q) effects of humidity acclimation and humidity treatment and their interactions on water loss rates for each of the acclimation hypotheses (adapted from Huey *et al.*, 1999 and Marais & Chown, 2008).

Hypothesis	Acclimation	Treatment	Interaction
Drier is better (DIB)	L+		n.s.
Wetter is better (WIB)	L–		n.s.
Optimal acclimation (OAH)			Q+
Beneficial acclimation (BAH)			L+/–, Q+
Deleterious acclimation (DAH)			L+/–, Q–
No plasticity (NP)	n.s.		n.s.

n.s. = not significant.

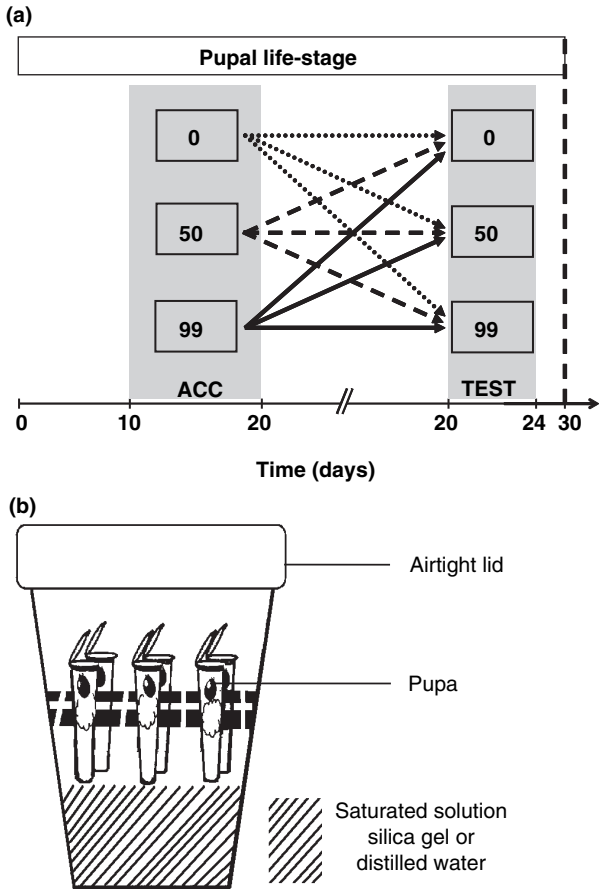

Fig. 3 Schematic illustration of (a) the experimental design and (b) the desiccation chambers used to investigate acclimation hypotheses of water loss rates in *Glossina* spp. The acclimation humidity (ACC, as relative humidity in %) are those at which puparia were held for ten days. The treatment humidity (TEST, as relative humidity in %) are those at which puparia were held for 4 days. Acclimation groups started with $n > 100$ individuals per acclimation humidity per species. During water loss rate estimation at the test humidity, each acclimation group was randomly subdivided into three and crossed to each of the possible humidity conditions to yield $n > 33$ per test humidity per acclimation per species. All experiments were undertaken at 25 °C in constant dark.

For each species, 300 individual puparia were placed separately into numbered, unsealed 0.6-mL plastic Eppendorf tubes which were randomly located within airtight plastic 100-mL vials (Fig. 3b). Each 100-mL vial held six individual puparia, replicated at least five times to give a total sample size of >30 puparia per treatment. The humidity within the outer 100-mL vial was manipulated for experiments. Care was taken to ensure that insects of all treatment groups were handled for the same duration during transfer from the climate chamber to the vials, and spent a similar amount of time outside the vials during weighing (~7–10 min per group). Relative humidity (in %) and temperature (T in °C) were recorded during all experiments using Thermocron iButtons (DS1402D-DR8, Dallas Semiconductors, Dallas, Texas, USA; sampling rate = 10 min) to ensure that desired humidity levels were achieved. Silica gel, saturated magnesium nitrate [$Mg(NO_3)_2{\cdot}6H_2O$] and Millipore-filtered, double-distilled water were used to produce low (0% r.h.), intermediate (50% r.h.) and high (99% r.h.) humidity treatments, respectively (see Winston & Bates, 1960). These r.h. treatments were chosen to maximize the possible effects of the experimental treatments while ensuring ecological relevance for *Glossina* puparia in the wild (Bursell, 1958, 1964). Preliminary trials demonstrated that equilibration times for salt solutions in these treatments were typically <3 h and remained stable thereafter for up to 2 weeks.

To determine individual WLR (expressed as μg H_2O h^{-1}), puparia were weighed separately on an electronic microbalance to 0.1 mg (BOECO, BBL 31, Hamburg, Germany) to determine body mass at the start of the experiment and final body mass after exposure to the experimental humidity treatment. The water loss rate was

then calculated as the difference in body mass divided by the duration of the experiment (4 days). As a result of the steady rate of water loss after 30 min in puparia (Bursell, 1957), and verified in our pilot trials, we consider that water lost after 96 h is a reliable and practical indication of the WLR under the specific experimental conditions. Pilot experiments also verified that significant loss of water in puparia could be detected in these experiments.

Statistical analyses

Initially, WLR and body mass (M_b) data were inspected using frequency distributions and box plots for extreme outliers, indicating individuals which died during the experiment, and thus, lost water rapidly. These outliers were discarded from the WLR data set. Subsequently, ordered-factor orthogonal polynomial contrasts were undertaken in SAS (as in Stillwell & Fox, 2005; Marais & Chown, 2008; for statistical background see Crawley, 2007). The six alternative acclimation hypotheses can be distinguished based on the interaction effects of the linear and quadratic terms from the orthogonal polynomial contrasts (Table 1) while expecting the null model of a negative relationship between WLR and test humidity (Fig. 1).

Because WLR data was not normally distributed and residuals of the preliminary analyses suggested violation of assumptions of general linear models, WLR was normalized by $\log_{10}$-transformation prior to final orthogonal polynomial contrast analyses. Orthogonal polynomial contrast analyses were implemented in SAS following Stillwell & Fox (2005) for a three-by-three experimental study design for each species separately. For all species, orthogonal polynomial contrasts met the assumptions of balanced design, homogeneity of variances within cells and normally distributed data residuals (Huey *et al.*, 1999).

Results

For all four species investigated, effects of acclimation and test humidity on WLR were highly significant (Table 2). Higher acclimation humidity typically resulted in a faster WLR compared with lower humidity rearing conditions, as indicated by the positive coefficient of the acclimation response (Table 2, Fig. 4). In accordance with physical principles, lower test humidity conditions typically resulted in higher WLR and were reflected by negative coefficients and significant treatment effects (Table 2, Fig. 4).

In each species, either linear, quadratic or both interaction effects were detected and are indicative of phenotypic plasticity (Table 3). However, the interaction effects differed in subtle but distinct ways, with significant implications for the acclimation hypotheses. Significant linear interaction effects were detected between acclimation and test humidity and these were negative in sign for *G. palpalis*, *G. morsitans* and *G. austeni*, but non-

Table 2 Results of the ordered-factor ANOVA with orthogonal polynomial contrasts for the effects of acclimation and treatment humidity on $\log_{10}$ water loss rate in *Glossina* species from mesic and xeric environments (following Marais & Chown, 2008).

	d.f.	SS	MS	*F*	*P*	Coefficient
G. palpalis – mesic						
Acclimation	1	3.345	3.345	267.99	<0.0001	0.277
Treatment	1	9.727	9.727	779.21	<0.0001	–0.473
Interaction (L)	2	0.329	0.164	13.17	<0.0001	–0.204
Interaction (Q)	2	0.102	0.051	4.10	0.018	–0.193
Hypothesis	DAH					
G. morsitans – xeric						
Acclimation	1	3.625	3.625	142.14	<0.0001	0.289
Treatment	1	12.660	12.660	496.38	<0.0001	–0.539
Interaction (L)	2	1.015	0.507	19.89	<0.0001	–0.200
Interaction (Q)	2	0.007	0.004	0.14	0.866	–0.017
Hypothesis	DIB					
G. austeni – mesic						
Acclimation	1	3.320	3.320	158.45	<0.0001	0.267
Treatment	1	11.236	11.236	536.23	<0.0001	–0.492
Interaction (L)	2	0.250	0.125	5.97	0.0029	–0.180
Interaction (Q)	2	0.037	0.018	0.88	0.4169	0.100
Hypothesis	DIB					
G. pallidipes – xeric						
Acclimation	1	3.273	3.273	183.61	<0.0001	0.274
Treatment	1	15.882	15.882	891.13	<0.0001	–0.604
Interaction (L)	2	0.058	0.029	1.64	0.1965	0.083
Interaction (Q)	2	0.118	0.059	3.32	0.0377	0.042
Hypothesis	OAH (BAH)					

L, linear; Q, quadratic; DAH, deleterious acclimation hypothesis; DIB, drier is better; OAH, optimal acclimation hypothesis; BAH, beneficial acclimation hypothesis.

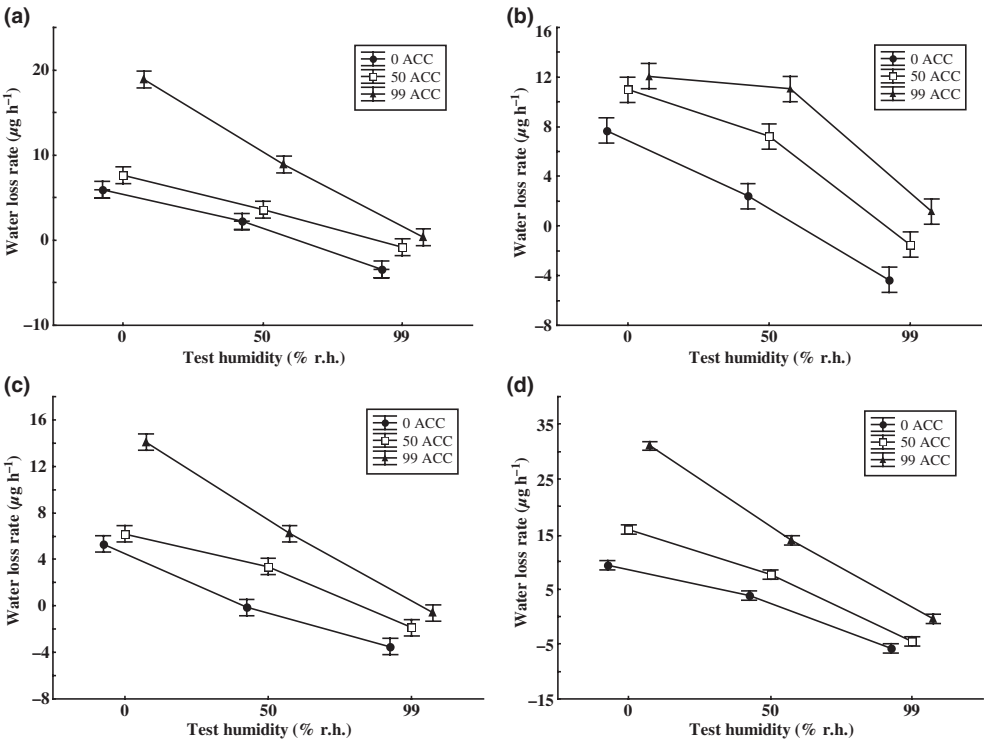

Fig. 4 Mean water loss rate (in μg h^{-1}) (±95% confidence limits) of (a) *G. palpalis* (mesic), (b) *G. morsitans* (xeric), (c) *G. austeni* (mesic) and (d) *G. pallidipes* (xeric) after 10 days acclimation at 0%, 50% and 99% relative humidity. Water loss rate was estimated under test humidities of 0%, 50% and 99% relative humidity over four days, given on the *x*-axis, for each acclimation group. Different acclimation humidity treatments are indicated by different symbols in the legend. Note the *y*-axes differ among species plots for clarity.

significant for *G. pallidipes* (Table 2). In all species, WLR were lowest for exposures of humidity that were the same as, or higher than, the acclimation humidity. However, puparia did not necessarily have the lowest WLR when the acclimation humidity and test humidity were the same, as is required by the BAH (e.g. Fig. 4a). For example, puparia acclimated to 99% should have the lowest WLR at 99% test humidity for the BAH to be the most parsimonious explanation. By contrast, for mesic *G. palpalis* the results were unable to falsify the predictions of the deleterious acclimation hypothesis for WLR (Fig. 4a). On the other hand, the other mesic species, *G. austeni*, showed support for the drier is better hypothesis as it generally had lower WLR across all treatment conditions when acclimated under low humidity (Fig. 4c).

Acclimation responses of xeric *G. morsitans* showed support for the drier is better hypothesis because the negative linear interaction term is not supportive of any alternative hypotheses and thus, the positive acclimation effect is assumed to represent the next most reasonable hypothesis (Table 2, Fig. 4b). In xeric *G. pallidipes* there was no significant linear interaction effect, while a significant positive quadratic interaction effect was detected (Table 2). This result therefore directly supports the optimal acclimation hypothesis. However, the results for *G. pallidipes* might support the beneficial acclimation hypothesis if the linear interaction effect became significant with increasing sample sizes.

Discussion

Among the major recent criticisms of tests of acclimation hypotheses or adaptive phenotypic plasticity in animal physiology are the lack of alternative hypotheses formulated and tested (Huey & Berrigan, 1996; Huey *et al.*, 1999), insufficient distinction between developmental and non-developmental plasticity (Wilson & Franklin, 2002; Terblanche & Chown, 2006) and extreme test conditions outside ecologically realistic environmental conditions resulting in deleterious responses (Loeschke & Hoffmann, 2002). Our study does not suffer from these problems since we were able to address at least six distinct acclimation hypotheses (Table 1, Fig. 1), the life-stage under investigation was constant and it is well established that the trait scored is stable across the period investigated (Bursell, 1958), and finally, the range of humidity conditions used in these experiments are typically found in *Glossina* environments at daily and seasonal timescales (Bursell, 1958, 1964; and see Hargrove, 2004; Terblanche *et al.*, 2006) and even in laboratory colonies which strive to maintain optimal rearing

Table 3 Results of the full-factorial ANOVA investigating the effects of acclimation and treatment humidity on $\log_{10}$ water loss rate in *Glossina* species from mesic and xeric environments.

	d.f.	SS	MS	*F*	*P*
G. palpalis – mesic					
Acclimation	2	3.3646	1.6823	134.77	<0.0001
Treatment	2	10.0606	5.0303	402.97	<0.0001
Interaction	4	0.4312	0.1078	8.63	<0.0001
Error	252	3.1457	0.0125		
G. morsitans – xeric					
Acclimation	2	3.3862	1.8431	72.27	<0.0001
Treatment	2	14.2664	7.1332	279.68	<0.0001
Interaction	4	1.0219	0.2555	10.02	<0.0001
Error	252	6.4271	0.0255		
G. austeni – mesic					
Acclimation	2	3.3200	1.6600	79.23	<0.0001
Treatment	2	11.5327	5.7664	275.23	<0.0001
Interaction	4	0.2870	0.0718	3.42	<0.0001
Error	270	5.6568	0.0210		
G. pallidipes – xeric					
Acclimation	2	3.4518	1.7259	96.84	<0.0001
Treatment	2	16.8543	8.4271	472.84	<0.0001
Interaction	4	0.1767	0.0442	2.48	0.0446
Error	252	4.4913	0.0178		

humidity (with the obvious exception of the results for mesic *G. palpalis*, see Discussion below).

Remarkably few studies have focused on the acclimation responses and alternative hypotheses for fitness-related traits of water balance physiology in insects (though see Woods & Harrison, 2001) and, to our knowledge, none have addressed *Glossina* physiology. Previous studies of terrestrial arthropod physiology have focused on running speed and its temperature dependence as a performance trait (Deere & Chown, 2006), mobility under cool, intermediate and warm conditions (Kristensen *et al.*, 2008) and thermal limits to survival (Deere *et al.*, 2006; Kristensen *et al.*, 2008; see also Marais & Chown, 2008). Similarly, much work has been undertaken on reproductive performance and other life-history traits (e.g. Stillwell & Fox, 2005; Frazier *et al.*, 2006; reviewed in Kingsolver & Huey, 2008). In addition, a strong-inference approach is now increasingly being adopted in studies of thermal biology (e.g. Deere & Chown, 2006; Kristensen *et al.*, 2008; Marais & Chown, 2008). However, the present study is particularly novel because it addresses a trait of water balance physiology in a non-model insect. Here, using six mutually exclusive hypotheses, we show that a range of two, or perhaps three, different acclimation hypotheses were supported although little support was found for the BAH. Predictions of the deleterious acclimation hypothesis could not be rejected for mesic *G. palpalis*. However, the drier is better hypothesis could not be rejected in mesic *G. austeni* or xeric *G. morsitans*. By contrast, xeric *G. pallidipes* showed support for the optimal acclimation hypothesis, or possibly also the BAH if increasing sample sizes result in linear interaction terms becoming significant. These results therefore demonstrate novel support for alternative acclimation hypotheses in animal evolutionary physiology.

Two potential confounding factors might complicate the interpretation of these results. The first, and probably less significant, is the production of body water through catabolism. A potential strategy for coping with desiccation which differs from starvation is to switch metabolic fuel source during desiccation from lipids to carbohydrates as the latter provide more metabolic water (Gibbs *et al.*, 1997; Marron *et al.*, 2003). For example, *Drosophila* from different mesic and xeric environments utilize carbohydrate metabolism during desiccation whereas during starvation some species tend to use a mixture of fuel sources (Marron *et al.*, 2003). However, no evidence of this has been found in *Glossina* which are thought to exclusively metabolize lipids via a proline pathway because of their haematophagous nature (Bursell *et al.*, 1974; Bursell & Slack, 1976; Bursell & Taylor, 1980). However, this is unlikely to be a significant confounding factor unless puparia in each group alter their preferred metabolic substrate in a consistent fashion. To our knowledge, no work has demonstrated a systematic shift in preferred fuel source under different humidity conditions in any insect.

Second, the experimental protocol might influence the results and constrain the acclimation response. For example, in some insects WLR declines as individuals become dehydrated and the pool of body water is reduced (Hadley, 1994). Thus, one could argue that acclimation to low humidity conditions has in fact simply made individuals appear more desiccation-resistant (i.e. apparent support for the BAH) as they now have a reduced body water content and hence a lower rate of water loss. However, this scenario makes at least two testable predictions which we investigated in a separate set of experiments in *G. pallidipes*. First, puparia should have high WLR during the early stages of desiccation but this should decline over time yielding a nonlinear decline in WLR over time within individuals. Such a prediction was not supported, at least over the experimental timescales employed here (Fig. 5a). Second, whereas it is well established that body water content and body mass are positively related (Bursell, 1958; Fig. 5b), body water content and water loss rate should be positively correlated, which was clearly not the case (Fig. 5c). These additional analyses therefore show that there is no reason to doubt the strength of the support for the acclimation hypotheses based on some sort of consistent methodological bias.

The magnitude of intraspecific variation in desiccation resistance induced by the acclimation treatments in these laboratory-reared species was substantial (a minimum of 15× range of variation within *G. pallidipes* and a maximum of 25× variation in *G. austeni*). Indeed, comparison with our previous study of inter-specific variation in

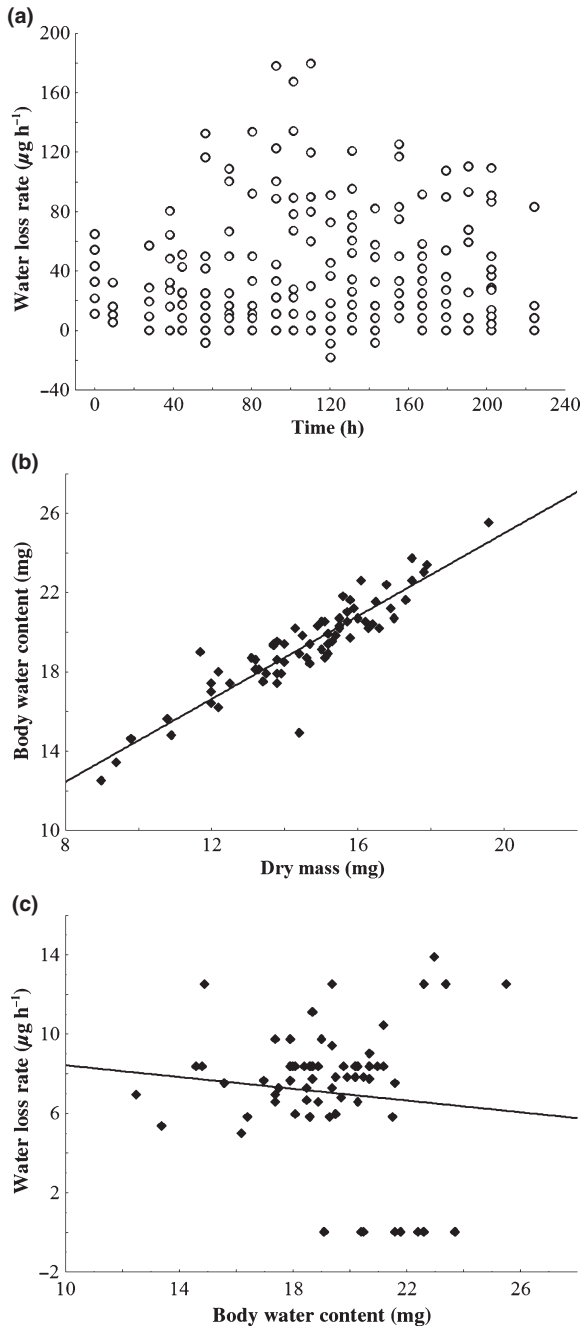

Fig. 5 The variation in (a) water loss rates (in μg h^{-1}) over time (in hours) within individuals (n = 30) subjected to 0% r.h. (at 25 °C), (b) body water content (in mg) with dry body mass (in mg) and (c) water loss rates (in μg h^{-1}) as a function of body water content (in mg) of *Glossina pallidipes* puparia at different stages of dehydration determined at 0% r.h. and 25 °C.

pupal WLR (Kleynhans & Terblanche, 2009) suggests that variation in desiccation resistance among species may be obscured by the magnitude of phenotypic plasticity, particularly for similar-sized species. This could be a significant problem when undertaking species comparisons (e.g. between mesic and xeric species) as the species assessed might not differ significantly if measured after rearing under uncontrolled, variable humidity conditions. Similarly, species compared immediately after collection in the wild would probably also be confounded as their recent hygric history would probably differ. These results therefore suggest that studies comparing species from different environments should control the state of phenotypic plasticity in water balance traits carefully in order to make meaningful physiological interpretations.

Another factor which might be argued to confound the present study is the use of laboratory-reared species as a comparator for wild *Glossina* (Chown & Terblanche, 2007). Results from previous studies of adult *G. pallidipes* suggest that the traits of lower thermal tolerance have diverged strongly in laboratory culture relative to wild populations; laboratory populations had critical thermal minima (the lower limit to normal activity) >6 °C lower than wild populations (Terblanche *et al.*, 2006; see also Gaston & Randolph, 1993). However, other traits such as critical thermal maxima and WLR in these laboratory-reared adult flies were in the range of wild flies' physiology (Terblanche *et al.*, 2006), which suggests that these changes are highly trait-specific. Research into other Diptera also shows rapid adaptation to laboratory conditions (Sgrò & Partridge, 2000), suggesting that this is a problem not simply restricted to *Glossina*. However, undertaking this work in the field for these species would be extremely difficult from a logistic perspective (collecting puparia directly or capturing and rearing wild flies for production of puparia). Presently, the degree of variation among wild and laboratory-reared puparia's WLR is unclear. To assess this potential problem we undertook a *post hoc* comparison of WLR data from our experiments with those of Bursell's (1958) for these same species. For these comparisons we only selected data for puparia reared at high humidity (99% r.h.) and transferred to low humidity (<5% r.h.) for WLR estimation which were probably the most similarly treated to those in Bursell's (1958) study. This comparison suggests that our values of WLR (in μg H_2O h^{-1} mg^{-1} body mass) for *G. palpalis* and *G. morsitans* were similar to Bursell's estimates. For *G. pallidipes* and *G. austeni*, however, the values of WLR we recorded are both higher and lower respectively than Bursell's (1958) values, suggesting no consistent trend in a particular direction.

Finally, the results of the present study might be confounded if the magnitude of phenotypic plasticity changes systematically in laboratory culture (Brakefield & Mazzotta, 1995; St Juliana & Janzen, 2007; Kingsolver *et al.*, 2009; and see discussions in e.g. Sgrò & Hoffmann, 1998; Chown & Terblanche, 2007). However, previous attempts to experimentally determine if this could be an issue for *Glossina* were unsuccessful because of a strong learned behavioural feeding response which prevents

wild-caught flies, which are used to feeding on wild animals, from being fed on a laboratory artificial feeding (membrane-tray) system (Terblanche *et al.*, 2008; see Bouyer *et al.*, 2007). For the species in laboratory culture for the longest period, *G. pallidipes*, seasonal variation of several physiological traits (including WLR) for wild populations was similar in magnitude to the temperature-induced variation in the laboratory population, which suggests that the magnitude of plasticity might be relatively well conserved in this species during culture (Terblanche *et al.*, 2006). If this was a confounding factor for our study, one might expect that species which have been in culture for longer will have lower levels of phenotypic plasticity of WLR. To assess the likelihood of this bias we investigated the relationship between duration in culture relative to the magnitude of the WLR acclimation × treatment response (F-value) as a measure of phenotypic plasticity for each species. These results showed no correlation [d.f. = 3; $r = 0.0053$, $P = 0.995$, y (magnitude of interaction response) = 0017 × years in culture + 6.0941]. Moreover, one of the few studies which has directly assessed this question suggests that this might not be a major problem as phenotypic plasticity was well conserved for various life-history traits under altered laboratory and field thermal conditions in a vertebrate ectotherm (St Juliana & Janzen, 2007). However, we are of the opinion that too few studies have been undertaken to generalize the extent of such a problem, particularly for insect physiological traits of water balance in response to altered moisture conditions.

Assuming therefore that these results are robust to the confounding factors discussed above, support for DAH in a mesic species (*G. palpalis*) and DIB in a xeric species (*G. morsitans*) is perhaps unsurprising, given the environments in which these species are typically found. By contrast, support for the DIB hypothesis in mesic *G. austeni* is perhaps more unusual, but not entirely unexpected given that these ecotype classifications can be somewhat arbitrary (see discussions in Bursell, 1958, 1959; Kleynhans & Terblanche, 2009). Moreover, *G. austeni* inhabits a fairly wide moisture gradient which may in fact be dry for the majority of the time, particularly with respect to soil moisture conditions. That xeric *G. pallidipes* support the OAH (or possibly the BAH) is also not surprising considering the wide support this hypothesis typically receives for life-history traits (Huey *et al.*, 1999; Stillwell & Fox, 2005; see also Kingsolver & Huey, 2008). However, the fact that various acclimation hypotheses are supported across xeric and mesic ecotypes (DAH and DIB in mesic species, DIB and OAH in xeric species), suggests that the types of phenotypic plasticity responses are not necessarily constrained to a particular ecotype which might be expected if adaptation to a particular environment arose as a trade-off between plastic and specialized phenotypes (see discussions with respect to thermal performance curves, Gilchrist, 1995; Kingsolver & Huey, 1998, 2008). Furthermore, because we found support for, e.g. DIB in species from both mesic and xeric environments, this suggests more broadly that physiological responses to drought could be adaptive in *Glossina* species, although based on the present results it seems unlikely that a single acclimation hypothesis could be common for desiccation resistance across the genus. However, we have only examined acclimation responses in four of *c.* 23 *Glossina* species and future studies should examine this question across a wider range of species.

Conclusion

In conclusion, the present study found novel support for several alternative acclimation hypotheses of desiccation resistance which were robust to a number of potential confounding factors. Little support for BAH was obtained in the present study, although other hypotheses for plastic responses were supported. Whether these alternative hypotheses might enhance fitness within the environments these species typically occupy is not obvious. As most of the acclimation hypotheses might be considered 'adaptive' under certain specific conditions by allowing individuals to achieve higher fitness in that environment (Ghalambor *et al.*, 2007; see similar discussion with respect to temperature in Cossins & Bowler, 1987), but are dependent on a number of critical steps (e.g. genetic changes) which the present study could not evaluate (discussed in Ghalambor *et al.*, 2007), we tentatively consider the results of this study to provide evidence for adaptive phenotypic plasticity of WLR in puparia of *Glossina* species from both mesic and xeric environments. Whether the acclimation responses detected have already been altered through evolution to xeric or mesic habitats is not evident but also seems likely. Clearly acclimation responses of desiccation resistance are more complex than previously suspected and further consideration should be given to the generality of these findings and their implications for fitness. Nevertheless, these results suggest an important role for phenotypic plasticity in moderating environmental variation within the life-time of these organisms for traits of water balance, with possible dramatic results for evolutionary fitness. Furthermore, this study suggests that mesic and xeric species are not necessarily constrained to different acclimation responses under varying environmental moisture conditions. The outcomes of this study have significant implications for determining physiological variation across taxa and predicting climate change responses in these and other insect species.

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Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Ceratitis capitata*)

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Abstract

Climatic means with different degrees of variability (δ) may change in the future and could significantly impact ectotherm species fitness. Thus, there is an increased interest in understanding the effects of changes in means and variances of temperature on traits of climatic stress resistance. Here, we examined short-term (within-generation) variation in mean temperature (23, 25, and 27 °C) at three levels of diel thermal fluctuations (δ = 1, 3, or 5 °C) on an invasive pest insect, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Using the adult flies, we address the hypothesis that temperature variability may affect the climatic stress resistance over and above changes in mean temperature at constant variability levels. We scored the traits of high- and low-thermal tolerance, high- and low-temperature acute hardening ability, water balance, and egg production under benign conditions after exposure to each of the nine experimental scenarios. Most importantly, results showed that temperature variance may have significant effects in addition to the changes in mean temperature for most traits scored. Although typical acclimation responses were detected for most of the traits under low variance conditions, high variance scenarios dramatically altered the outcomes, with poorer climatic stress resistance detected in some, but not all, traits. These results suggest that large temperature fluctuations might limit plastic responses which in turn could reduce the insect fitness. Increased mean temperatures in conjunction with increased temperature variability may therefore have stronger negative effects on this agricultural pest than elevated temperatures alone. The results of this study therefore have significant implications for understanding insect responses to climate change and suggest that analyses or simulations of only mean temperature variation may be inappropriate for predicting population-level responses under future climate change scenarios despite their widespread use.

Introduction

Climate change is one of the major challenges facing society, with the implications for biodiversity conservation, human and animal health, and agricultural production. Although it is generally accepted that climate is changing (IPCC [Intergovernmental Panel on Climate Change], 2007), mitigating and coping with these effects are pressing unresolved issues (Parmesan, 2006; Thuiller, 2007; Williams et al., 2008). Generally, forecasting efforts for vertebrate and invertebrate responses to climate change focuses almost exclusively on the variation in mean temperatures (e.g., Crozier & Dwyer, 2006; Deutsch et al., 2008). However, it is expected that variances and extremes of weather may also change under future scenarios (Easterling et al., 2000; Tebaldi et al., 2006; Tebaldi & Sanso, 2009) which are predicted to impact ectotherm fitness significantly (Helmuth, 2009; Hoffmann, 2010; Somero, 2010). From a functional perspective, how changes in means and variances of temperature might affect the basal (static) and plastic responses of climatic stress resistance or life-history of ectothermic animals are generally poorly understood and is critical for predicting

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physiological responses in the wild (reviewed in Gaines & Denny, 1993; Gutschick & BassiriRad, 2003; Helmuth et al., 2005; Měráková & Gvoždík, 2009; Marshall & Sinclair, 2010).

Phenotypic plasticity encompasses an important suite of mechanisms that ectotherms may use to cope with short-term variation in environmental conditions. Examples of such plastic responses include seasonal or diurnal changes in physiological tolerance to climatic conditions within an organism over its lifetime (reviewed in Huey & Berrigan, 1996; Chown & Terblanche, 2007; Whitman & Agrawal, 2009). In addition, the behavioural modification (e.g., changes in daily activity patterns, or seasonal patterns of activity) (Kearney et al., 2009a) or timing of key life-history behaviours, such as phenology/diapause (e.g., Bradshaw & Holzapfel, 2006), genetic adaptation, and migration are other key ways in which ectotherms might cope with climate change. Although the relative costs and benefits of plastic responses in insect performance and fitness are debated (Thomson et al., 2001; Deere & Chown, 2006; Kristensen et al., 2008; Marais & Chown, 2008), and the importance of different acclimation or plasticity hypotheses for field fitness may not be readily apparent (Angilletta, 2009; Terblanche & Kleynhans, 2009; Clusella-Trullas et al., 2010), it is increasingly well demonstrated that phenotypic plasticity is an important component of survival in the wild. For example, phenotypic plasticity of performance is critical to foraging under adverse thermal conditions and likely improves fitness in thermally heterogeneous environments (reviewed in Kingsolver & Huey, 1998; Frazier et al., 2008; Kristensen et al., 2008; Ragland & Kingsolver, 2008a; Angilletta, 2009; Kingsolver et al., 2009). Moreover, plasticity theory suggests that the latitudinal variation in environmental variability is related to the magnitude of plastic responses, although such generalities are seldom explored from an empirical perspective (see, e.g., Ghalambor et al., 2006; reviewed in Chown & Terblanche, 2007; Angilletta, 2009; Chevin et al., 2010; Chown et al., 2010). Regardless, extreme events (i.e., the magnitude of variability) are clearly important in shaping the strength of selection and thus phenotypes in natural populations (Gaines & Denny, 1993; Gutschick & BassiriRad, 2003; Katz et al., 2005), but are poorly understood for many species, particularly insect pests of agriculture. More importantly, few studies have examined such lethal and sub-lethal physiological responses in an ecologically relevant, climate change context.

To date, most investigations of fluctuating thermal regimes (FTR) have focused primarily on the repair of organisms from chilling injury (e.g., Colinet et al., 2007; Koštál et al., 2007), life-history tradeoffs (Marshall & Sinclair, 2010), or potential energetic or life-history costs of multiple low-temperature exposures (Sinclair & Chown, 2005) rather than the impact of a range of mean/variance scenarios. Some studies have focused on variation in morphometric or life-history traits (Petavy et al., 2001; Niehaus et al., 2006; Ragland & Kingsolver, 2008b; Les et al., 2009), gene expression (Podrabsky & Somero, 2004), and physiological performance (Folguera et al., 2009; Měráková & Gvoždík, 2009) under similar mean temperatures with different levels of temperature variability. However, detailed analyses of changes in both means and variability are still lacking, and most studies make little distinction between such effects (though see, e.g., Les et al., 2009), particularly for traits of climatic stress resistance in ectotherms, and are a critical component of accurate forecasting of insect population-level responses to climate change.

Here, we test the general hypothesis that the temperature variability may affect climatic stress resistance over and above changes in mean temperature at constant variability levels for traits of climatic stress resistance within a single generation of a laboratory-reared pest insect. This is an implicit but seldom examined assumption of many insect population dynamics models and also of insect geographical range responses under climate change scenarios (Crozier & Dwyer, 2006; Deutsch et al., 2008; Kearney et al., 2009b; see discussions in Helmuth et al., 2005). In this study, we used an insect pest of global agricultural significance, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), as a model organism. A range of ecologically relevant traits indicative of climatic stress resistance and life-history are examined in adult flies in a nested experimental design, including lower- and upper- critical thermal limits to activity, phenotypic plasticity of survival at an acute temperature (rapid cold- and rapid heat-hardening), desiccation resistance, body water and lipid content, and egg production. Specifically, we investigate how traits of climate stress resistance respond to benign acclimation temperatures (i.e., well within the thermobiological range) of 10 days at varying levels of temperature fluctuation which is crucial for an accurate prediction of climate change responses.

Materials and methods

Study animals and rearing conditions

Pupae of *C. capitata* were obtained from a large culture maintained in high numbers at Citrus Research International, Nelspruit, South Africa. This large outbred culture has been maintained in the laboratory for nearly 200 generations but is regularly supplemented with wild individuals added from catches made during the summer. The culture is held indoors under variable though buffered

temperatures (annual temperature range: 15–30 °C), which is fairly representative of the natural habitat for *C. capitata*. Upon arrival in Stellenbosch, the pupae were kept at 25 °C and 76% r.h. until emergence. After emergence they were held at 25 °C and 76% r.h. for 3 days prior to the start of acclimation treatments. Acclimations lasted for 10 days which is a significant portion of the adult life span of these flies. The adult flies were transferred to 45 5-l plastic jars (15 containers per experimental block, five replicate containers per acclimation/variability group with n = 100 individuals per container) and supplied with 1:3 ratio honey:water solution and sugar. All flies used at the start of acclimation conditions were of a similar age (36–72 h-old) and had access to food and water ad libitum during acclimation. Acclimation temperatures were maintained in three computer-controlled, insulated insectary rooms programmed to one of the three mean temperatures (23, 25, or 27 °C) with each of the three variability levels (δ = 1, 3, or 5 °C) changing over a 24-h period (Figure 1). Acclimation temperatures were chosen to represent the range of seasonal temperature variation observed in South African agroecosystems from early spring to late summer. Relative humidity was maintained at 76% during all treatments using saturated sodium chloride solutions. Due to limits on the number of climate-controlled rooms available, acclimation treatments of 23 δ = 1, 25 δ = 1, and 27 δ = 1 °C were undertaken in one experimental block, followed by 23 δ = 3, 25 δ = 3, and 27 δ = 3 °C in one block, and finally followed by 23 δ = 5, 25 δ = 5, and 27 δ = 5 °C in the last experimental block.

Thermal tolerance

For all thermal tolerance assays, flies of mixed genders were used, as sex does not play a significant role with respect to thermal tolerance in this species (Nyamukondiwa & Terblanche, 2009). Fruit flies used in the thermal tolerance assays had access to food and water ad libitum and therefore feeding status was not strictly controlled, but instead we rather employed large sample sizes to better reflect population-level effects. After acclimation for 10 days, 10 *C. capitata* adults were individually placed into organ pipes (double-jacketed chambers) connected to a programmable water bath (Grant GP200-R4; Grant Instruments, Cambridge, UK) filled with 1:1 water:propylene glycol to allow for subzero temperatures. A type K thermocouple (36 SWG) connected to a digital thermometer (Fluke 54 series II; Fluke Cooperation, Beijing, China; accuracy of 0.05 °C) was inserted into the control chamber to record organ pipe temperature. For small ectothermic insects like *C. capitata*, body temperature of individuals is in a steady state with an

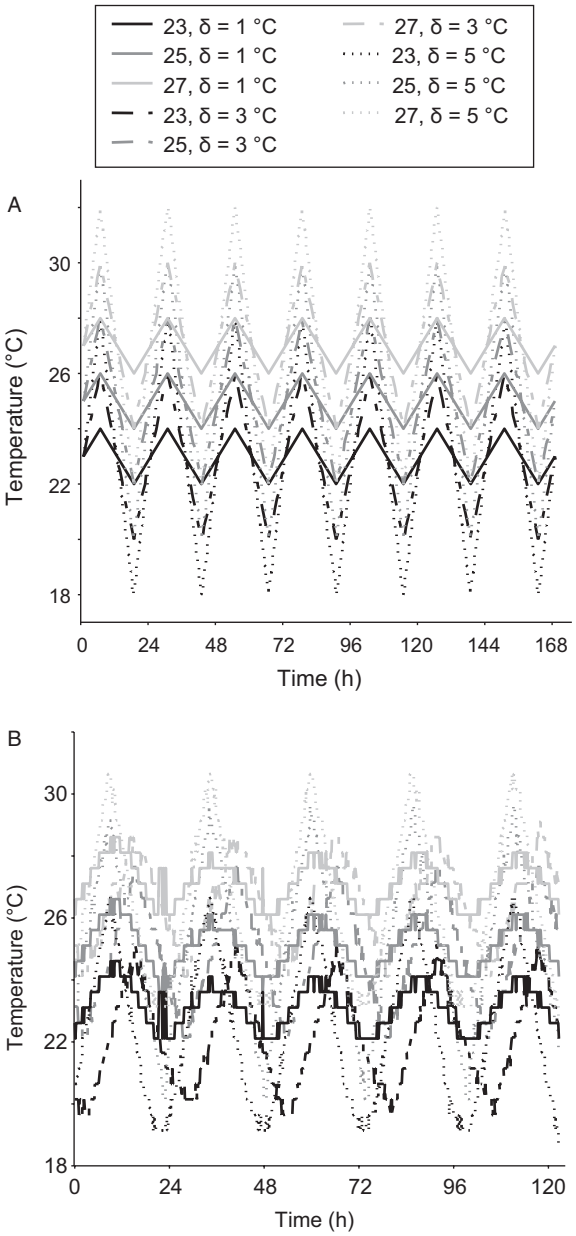

Figure 1 (A) Insectary temperatures recorded during acclimation and variability experiments and (B) a 5 day-long sample of iButton dataloggers (± 0.5 °C accuracy at 10 min sampling rate) within the fruit fly culture vials.

organ pipe chamber temperature under the experimental conditions employed, as has been shown for other, larger fly species (Terblanche et al., 2007a).

Both critical thermal limit (CTL) experiments [i.e. critical thermal maxima and critical thermal minima (CT_{max} and CT_{min}, respectively)] started at a temperature of 25 °C from which the temperature increased for CT_{max} or decreased for CT_{min} at a rate of 0.25 °C min^{-1} until all

the insects reached their CT_{max}/CT_{min}. This ramping rate was chosen as a compromise between its ecological relevance and processing all acclimation groups and replicates within a short period of time, thereby avoiding, e.g., ageing effects (Nyamukondiwa & Terblanche, 2009). This rate of temperature change is relatively slow compared with much previous CTL work undertaken to date (see discussion in Chown et al., 2009). However, the 0.25 °C min^{-1} rate used is likely to be 4–5 times faster than actual heating or cooling rates in the wild, thus perhaps limiting the interpretations of CTL results to field thermal ecology. Nevertheless, this rate allows comparison amongst treatments which is the main focus of the present study. Critical thermal limits were recorded for each individual fly, defined as the temperature at which each individual insect loses co-ordinated muscle function, consequently by losing the ability to respond to mild stimuli (e.g., prodding). Each CTL experiment was repeated twice to yield sample sizes of n = 20. The complete set of data was gathered across a period of 2 days with each treatment group run in random order on a given day to avoid diurnal effects.

Lower and upper discriminating temperatures of −5 and 41 °C were used to investigate rapid cold-hardening (RCH) and rapid heat-hardening (RHH), respectively, in *C. capitata*. Two hours at these temperatures causes ca. 90% mortality in adults of this species. For all rapid thermal response assays, five replicate 60-ml vials of 10 insects each were placed in a growth chamber at 25 °C for 30 min to allow equilibration, after which flies were exposed directly to 5 °C (2 h) for potential cold-hardening and 35 °C (2 h) for potential heat-hardening. Following 2 h at 5 °C, flies were then immediately transferred to the discriminating low-temperature (−5 °C for 2 h) to assess RCH. However, flies hardened at 35 °C (2 h) were given a 30-min recovery period to allow potential heat shock protein production before being subjected to the discriminating high temperature (41 °C for 2 h).

All assays were performed using plunge protocols in programmable water baths (Grant GP200-R4). After 2 h at the discriminating temperature, flies were returned to 25 °C for 24 h before scoring survival. For RCH and RHH experiments, five replicate vials of 10 flies per vial were used as the handling controls. These control flies were sorted into vials, placed at normal rearing temperatures (25 °C) in a climate chamber for 30 min, then taken out of the climate chamber, handled for similar duration and vigour as treatment flies, and placed back into climate chambers for 2 h. Next, control flies were exposed to discriminating temperatures for 2 h (at the same time as treatment flies) and then returned to climate chambers at 25 °C for 24 h before scoring survival. Both the treatment and control flies had access to food and water during the recovery period. Survival was defined as a co-ordinated response to mild stimulation (e.g., prodding), or normal behaviours such as walking, flying, feeding, or mating.

Water balance

After the 10-day acclimation period, randomly selected fruit flies were placed individually into the labelled 100-ml plastic vials. Each fly was weighed individually on an electronic microbalance (Mettler Toledo AX504; Mettler-Toledo, Greifensee, Switzerland; accuracy of 0.1 mg) and water loss rate (WLR) was scored gravimetrically at 25 °C and 76% r.h. over 12 h during their inactive period (18:00–06:00 hours) in the dark. Water loss rate was calculated for each fly as the difference in mass between initial and final mass measurements divided by time (12 h). Thereafter, sex was recorded and the flies were completely desiccated for 2 days at 60 °C. Flies were weighed again after desiccation and the difference in mass from the start of the experiment was calculated to give body water content (BWC). Subsequently, body lipid content (BLC) was estimated gravimetrically after lipid extraction was performed on each individual using a 1:1 chloroform:methanol solution (three washes, once per day) (following methods in Terblanche & Kleynhans, 2009).

Egg production

Fecundity was measured as the number of eggs deposited by the female flies over the entire duration of the experimental fluctuating temperature protocols. Bananas were placed in each of the five replicate fruit fly cages as oviposition sites and these were replenished twice during the experimental duration. Following removal of the bananas from each of the five replicate cages, the eggs and larvae on each replicate were counted under a dissecting microscope. The total number of eggs produced during the entire experimental duration for each of the five replicate cages under each of the fluctuating temperatures was thus calculated.

Statistical analysis

To examine the effects of mean acclimation temperature and δ on traits of climate stress resistance, nested analyses of variance (ANOVA) were performed. This was chosen because these responses are strongly interlinked and thus represent an environmental response as well as an assessment of the impact of different conditions on a trait. However, our primary question is whether δ exerts effects in addition to mean acclimation temperature, for which the latter are well established (reviewed in Chown & Terblanche, 2007). Acclimation temperature was assigned as a random factor and each of the three δ levels as fixed factors nested within the acclimation temperature in SAS (Proc

GENMOD). If temperature variability was also run as a random factor, qualitative results remained unchanged (results not shown). Similarly, relaxing the assumption of nestedness of the study design and re-analyses of major traits using full-factorial ANOVA showed highly significant effects in all cases (results not shown), but these violate assumptions of independence of treatments and we thus only report the nested ANOVA results. Subsequent to the nested ANOVA, variance components were also calculated for each of the major effects using Proc NESTED in SAS. In all cases, data had similar variances amongst groups and were normally distributed. However, in one case of CT_{max}, one case of WLR, one of BWC, and two cases of LC the data were not normally distributed. Log_{10} transformation, however, did not improve these skewed distributions and thus original raw data were analysed both by nested ANOVA and nested generalised linear model (GLZ) to verify results using a more conservative approach. Therefore, to maintain balanced sample sizes and allow analyses by nested ANOVA, we did not attempt to delete outliers, and instead we re-ran the nested ANOVA with a nested GLZ with a normal distribution and an identity link function for errors, with corrections for overdispersion. Here, all the qualitative results of GLZ remained similar to those of the nested ANOVA, although for brevity we only report the former results.

After independently verifying that rapid cold- and heat-hardening significantly improved survival relative to control groups, we assessed the effects of acclimation temperature and variability on survival in these experiments. Nested design GLZ were performed on proportional survival assuming a binomial distribution and a logit link function in SAS statistical software (Proc GENMOD), with corrections for overdispersion (following, e.g., Marais et al., 2009) for heat- and cold-hardening experiments separately. A treatment effect (control or hardened) was included in these analyses. For egg production results, we used a Poisson distribution and an identity link function for the GLZ analyses. Post-hoc tests of overlapping 95% confidence limits were used to identify statistically homogeneous groups.

Results

Thermal tolerance and rapid thermal responses

Acclimation temperature and variation in acclimation temperature had significant effects on CT_{min} and CT_{max} (Table 1). Under low variability scenarios ($\delta = 1$ °C), typical acclimation responses were observed for both CT_{min} and CT_{max} (Figure 2). In both traits, 47–87% of the variance could be explained by temperature variation. In both rapid cold- and heat-hardening, there was a significant improvement in survival relative to control groups, as indicated by the treatment effect (Table 2, Figure 3). Rapid cold-hardening responses were not affected by acclimation temperature or variation in temperature (Table 2,

Table 1 Summary of results for nested analyses of variance (type I sums of squares) for critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), water loss rate (WLR), body water content (BWC), body lipid content (BLC), and log_{10} fecundity scored as egg production after acclimation to three temperatures (Acc Temp) with three levels of temperature variability (Temp Variation)

	Effect	d.f.	F	P	Variance (%)
CT_{min}	Acc Temp	2	6.19	0.0025	0.00
	Temp Variation (Acc Temp)	6	19.11	<0.0001	47.52
	Error	171	–	–	52.48
CT_{max}	Acc Temp	2	21.19	<0.0001	0.00
	Temp Variation (Acc Temp)	6	135.27	<0.0001	87.04
	Error	171	–	–	12.96
WLR	Acc Temp	2	36.51	<0.0001	42.7
	Temp Variation (Acc Temp)	6	18.96	<0.0001	0.00
	Error	261	–	–	57.3
BWC	Acc Temp	2	17.17	<0.0001	44.8
	Temp Variation (Acc Temp)	6	28.06	<0.0001	0.00
	Error	–	–	–	55.2
BLC	Acc Temp	2	9.09	0.0002	11.4
	Temp Variation (Acc Temp)	6	3.45	0.0027	0.00
	Error	–	–	–	88.6
Fecundity	Acc Temp	2	2.80	0.074	0.00
	Temp Variation (Acc Temp)	6	4.81	0.0011	43.22
	Error	–	–	–	56.78

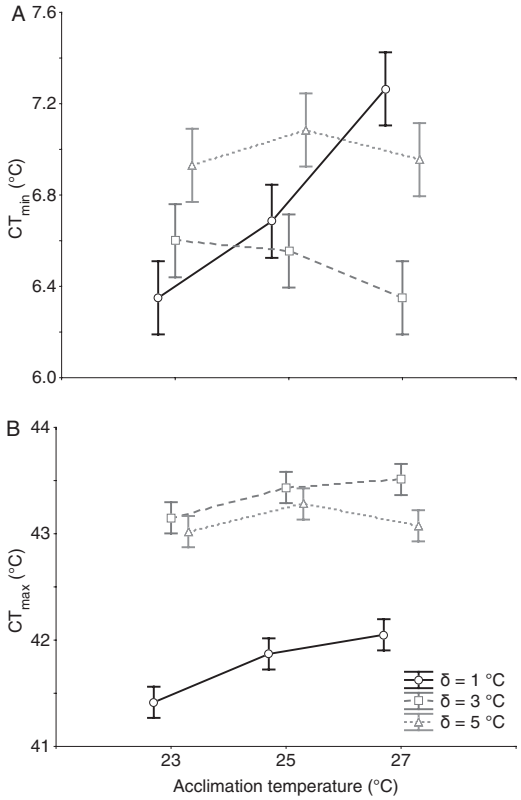

Figure 2 Mean (± SE) critical thermal (A) minimum and (B) maximum in *Ceratitis capitata* acclimated at three temperatures (°C) and three variability levels (δ).

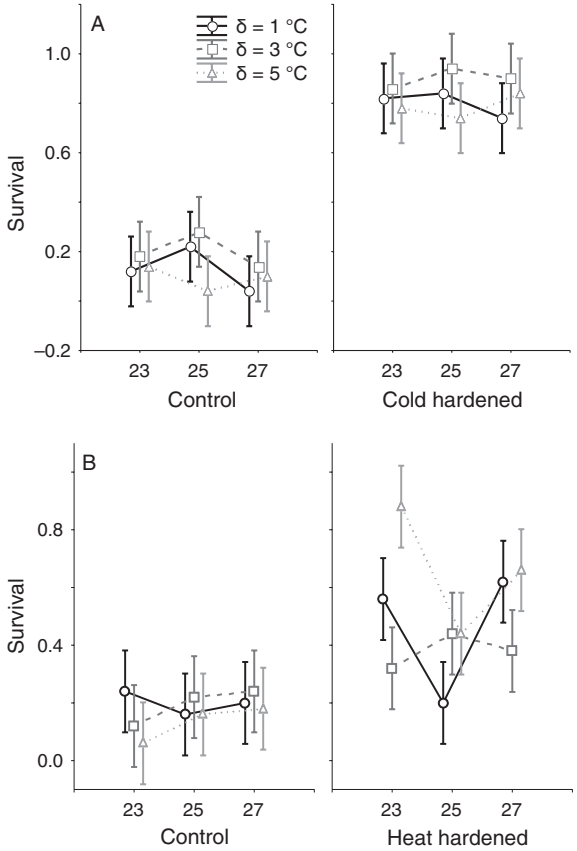

Figure 3 Mean proportion of survival (± 95% CLs) for (A) rapid cold-hardened (5 °C for 2 h) and control (direct from experimental conditions to −5 °C for 2 h), and (B) rapid heat-hardened (35 °C for 2 h) and control experimental (41 °C for 2 h) groups at three acclimation temperatures and variability levels (δ) in *Ceratitis capitata*.

Figure 3A). By contrast, the effects of acclimation temperature on rapid heat-hardening responses were non-significant, whereas variation in temperature was significant (Figure 3B).

Water balance

Water loss rate, BWC, and BLC of *C. capitata* were significantly affected by both acclimation temperature and the variability of the temperature within each acclimation group (Table 1, Figure 4). However, for these traits the proportion of the variance was mainly attributed to error

Table 2 Summary of results for nested generalised linear model using a binomial distribution and logit link function and corrected for overdispersion for rapid cold-hardening (RCH) and rapid heat-hardening (RHH) in *Ceratitis capitata* scored after acclimation to three temperatures (T = 23, 25, or 27 °C; Acc Temp) with three levels of temperature variability (δ = 1, 3, or 5 °C; Temp Variation) and two treatment levels [control or pre-treated (hardened); Treatment]

	Effect	d.f.	Wald χ^2	P	Variance (%)
RCH	Acc Temp	2	0.72	0.70	0.00
	Temp Variation (Acc Temp)	6	6.81	0.34	0.00
	Treatment (Temp Variation)	3	230.96	<0.0001	90.28
	Error	72	–	–	9.72
RHH	Acc Temp	2	5.14	0.077	0.57
	Temp Variation (Acc Temp)	6	13.26	0.039	0.00
	Treatment (Temp Variation)	3	71.34	<0.0001	75.29
	Error	72	–	–	24.14

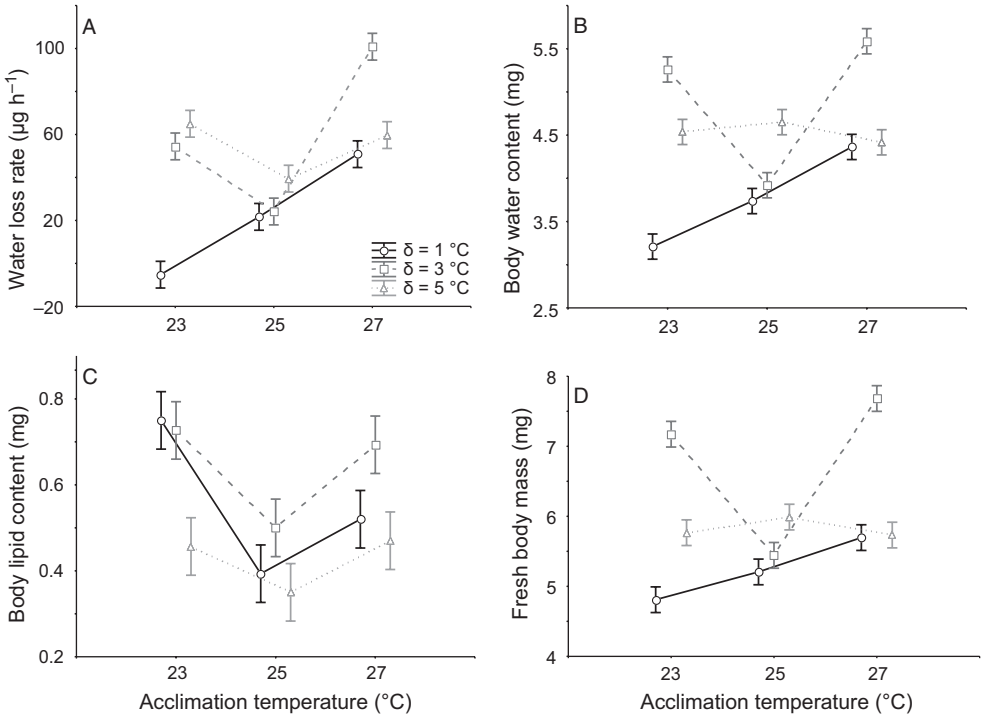

Figure 4 Mean (± SE) (A) water loss rate (μg h^{-1}) determined at 25 °C and 75% r.h. over 12 h during the night, (B) body water content (mg), (C) body lipid content (mg), and (D) fresh body mass (mg) in *Ceratitis capitata* acclimated at three temperatures and three variability levels (δ).

(55–89%) or acclimation temperature (11–45%), but almost none to variability level (0% in all three traits) (Table 1).

As fly body size may also have been affected by the acclimation treatment, the main effect of variation in WLR with acclimation and temperature variability could have simply been a result of changes in body size. Thus, we also re-ran the nested GLM model for WLR including initial body size as a co-variate. These results were qualitatively similar to the earlier uncorrected results with both acclimation temperature and temperature variability showing highly significant effects on mass-specific WLR (Mass*Acc Temp: $P<0.0001$; Mass*Temp Variation (Acc Temp): $P<0.0001$).

Fecundity

Egg production was influenced by acclimation temperature and temperature variability (Table 1). In low δ scenarios, egg production was low, whereas under higher δ scenarios the 25 °C acclimation temperature produced the most eggs per vial (Figure 5). High δ, high acclimation temperature groups produced fewer eggs, but still more than in the low variance groups at either 25 or 27 °C (Figure 5).

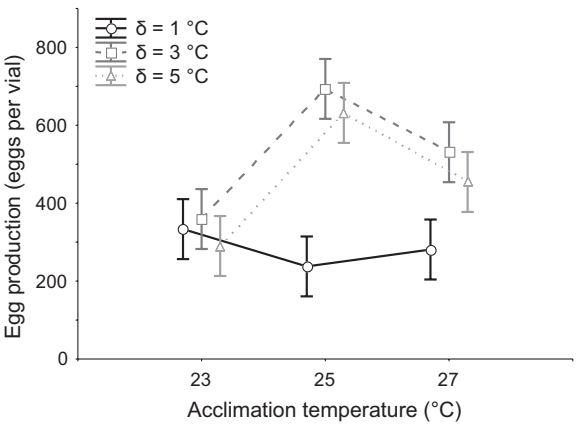

Figure 5 Mean (± SE) egg production of *Ceratitis capitata* acclimated at three temperatures (°C) and three variability levels (δ). Each datum point included for analyses represents the average number of eggs produced per 45–55 females in five replicated vials per experimental treatment.

Discussion

Based on the present results, the general hypothesis that acclimation responses of traits of climatic stress resistance

are similar at different levels of temperature fluctuations must be rejected in all traits examined. By contrast, the levels of stress resistance or tolerances differed dramatically amongst variability (δ) levels, although not necessarily in a systematic fashion and are dependent to some degree on the specific traits examined. At low δ, typical acclimation responses of critical thermal limits were detected, similar to documented responses of thermal tolerance in other insect species (e.g., Slabber & Chown, 2005; Terblanche et al., 2005; Powell & Bale, 2006; Jumbam et al., 2008; Lachenicht et al., 2010). For example, if variability was fixed at δ = 1 °C, higher CT_{min} and CT_{max} were recorded in flies acclimated to warmer temperatures, which might be interpreted as being advantageous to population fitness, as is the case in many studies of ectotherm thermal tolerance to date (e.g., Gvoždík et al., 2007; see discussion in Chown & Terblanche, 2007). However, at higher levels of temperature variability, acclimation responses varied substantially amongst the treatment groups and traits. Most significantly, CT_{min} was poorer (higher) across all acclimation groups if temperature variability was higher. On the other hand, CT_{max} was significantly higher at high variability levels (41.4–42.0 °C in δ = 1 °C scenarios vs. 43.0–43.5 °C in δ = 3 or 5 °C), and may be related to heat shock protein production (e.g., Kalosaka et al., 2009; reviewed in Chown & Terblanche, 2007). Thus, changes in mean temperature combined with increasing temperature variability can alter the direction of thermal tolerance responses which could be unfavourable to activity limits at low temperatures but advantageous at high temperatures in *C. capitata*.

Although some species do not alter traits of water balance in response to changes in temperature (e.g., Terblanche et al., 2005), and other insects can improve desiccation resistance after exposure to elevated temperatures (e.g., Hoffmann et al., 2005), our results suggest that high temperatures are detrimental for *C. capitata* as WLR was higher after acclimation to warmer conditions under δ = 1 °C variation. However, WLR were lowest after low temperature-low variability acclimation, but highest after 27 °C acclimation when δ = 5 °C. Low temperature-high variability acclimation also resulted in significantly elevated WLR. This suggests a lack of acclimation ability for WLR in *C. capitata*. However, it is clear that BWC and BLC, and possibly also body size (see discussion below), may be modified to cope with losing water more rapidly under such temperature variation. Regardless of the acclimation adjustments taking place, it is clear that water balance physiology was significantly affected by both the changes in mean temperature and temperature variability levels.

Acclimation effects on hardening responses in *C. capitata* are similar to the other work reported to date. Acclimation temperature may affect the basal survival levels (e.g., Hoffmann et al., 2005; Slabber & Chown, 2005) but it does not necessarily influence the magnitude of the hardening response, which is similar to responses observed for *Drosophila* larvae (Rajamohan & Sinclair, 2009) and other flies (Terblanche et al., 2007b; Marais et al., 2009). Thus, hardening responses may be relatively well conserved, at least over short-term acclimations within a single generation.

Life-history traits were also strongly influenced by acclimation temperature and variability levels. Egg production was highest at the optimal rearing temperature (25 °C) under δ = 3 or 5 °C scenarios but lowest at optimal temperature under δ = 1 °C variation. Generally, egg production was stable across acclimation groups under δ = 1 °C scenarios, but warmer acclimation temperatures and higher variability resulted in higher egg production. This could be some form of increased investment in egg production in response to what the flies may perceive as being the onset of summer conditions in the wild, although such a speculation naturally requires further investigation. Body size is affected by acclimation and variability levels (fresh body mass in the water balance experiment). Mass is fairly similar amongst the acclimation groups at δ = 1 °C, but much greater at δ = 3 °C in 23 and 27 °C groups. By contrast, the δ = 5 °C groups maintained relatively low and constant mass, though generally higher than the δ = 1 °C groups. These results are largely in keeping with the life-history literature on insects (e.g., Petavy et al., 2001; Marshall & Sinclair, 2010) in which the time spent within critical developmental temperatures allows optimum growth and reproduction.

Two potential confounding factors limit the interpretation of these experiments to the field situation. First, we used a laboratory-reared culture which may not reflect field populations as laboratory adaptation may take place rapidly, thereby changing trait means and the magnitude of plasticity over time (reviewed in Chown & Terblanche, 2007). However, although it is clear that traits can change in laboratory cultures (e.g., Kingsolver et al., 2009) such responses tend to be highly trait-specific and not necessarily accompanied by a loss of plasticity (e.g., St. Juliana & Janzen, 2007; discussed in Terblanche & Kleynhans, 2009). Moreover, the fly culture used in the present study is maintained under variable, though buffered, temperatures and the specific laboratory holding effects on the traits examined have yet to be determined. Thus, while it is unclear how such changes in means and plasticity for the traits scored may have affected our results, similar work on field-collected species or a recently collected population, and across multiple life-stages and generations, would be a valuable future direction.

Second, the range of mean temperature variation and associated variability levels used in our experiments may be unrealistic of the field temperature regimes experienced by *C. capitata*, and are perhaps damaging in some way to the flies. We therefore considered the range of thermal regimes *C. capitata* may experience in the field. Mean temperatures (±SD) of 21.9 (±5.3), 20.7 (±5.6), and 25.1 (±7.9) °C were recorded at three different localities during January 2010, and coincided with the peak activity time for *C. capitata* in these sites. Furthermore, a comparison of central Kenya, California (USA), and Ceres in the Western Cape (South Africa) found mean monthly temperature ranges of ca. 16, 11, and 13 °C, respectively, in comparison with our experimental ranges of ca. 2, 6, and 10 °C. Similarly, our laboratory means (±SD) were 23 (±0.5), 25 (±1.7), and 27 (±2.9) °C. Seasonally, the SD of field temperatures within an apple tree in a single South African orchard inhabited by *C. capitata* varied from 5.3 to 7.9 °C between winter and summer of a single year (2009). However, it is largely unclear for this species how much they use behaviour to avoid or mitigate such temperature variation (see discussions in Huey et al., 2003; Kearney et al., 2009a). Thus, in many respects the range of temperatures we exposed flies to experimentally, both in terms of means and associated variability levels, was probably very modest and likely to occur reasonably often in natural or agricultural habitats occupied by *C. capitata*. However, closer examination of the actual body temperatures adult flies are exposed to naturally would be valuable.

Therefore, assuming that these results are a reasonable reflection of natural population responses to changing climates, the results of these novel experiments allow two important conclusions. First, different temperature variability levels do not necessarily have the same outcome, which might be expected as sub-lethal, stressful conditions and possibly also extreme, lethal temperatures are more frequently encountered. However, even the highest (27 °C) temperature group with the highest thermal variance in our experiments probably did not reach lethal levels: only a few hours a day are in the region of 32 °C and short periods at these temperatures are still well within the limits of viability for most vinegar and fruit flies (Hoffmann, 2010). However, these modest temperatures may well influence the mating behaviour, fertility, egg and sperm sterility of *C. capitata* adults (Myburgh, 1969; Prokopy & Hendrichs, 1979; Hendrichs & Hendrichs, 1990), larval development rates (Grout & Stoltz, 2007), and thus overall population growth rates. Indeed, Marshall & Sinclair (2010) recently demonstrated that, at least for low-temperature fluctuating thermal regimes, *Drosophila melanogaster* trade-off immediate survival against future reproductive effort. Thus, fluctuating temperatures may result in important trade-offs in life-history in *C. capitata*, and, as documented by our results, could also affect physiological tolerance and resistance to climate extremes in largely unpredictable ways.

Second, these results generally suggest that larger fluctuations in temperature which are expected under future climates (Easterling et al., 2000; Tebaldi et al., 2006; Zhou et al., 2009) might obscure, limit, or alter plastic responses such as beneficial acclimation (e.g., Marais & Chown, 2008), and could even produce plastic changes in the opposite direction to those which might be considered beneficial, as predicted by plasticity theory (see discussions in Deere & Chown, 2006; Chown & Terblanche, 2007; Angilletta, 2009; Chevin et al., 2010; Chown et al., 2010). Such effects could therefore amplify the reduction in fitness of insects faced with climate change under high-temperature variability scenarios. Instead, the insects faced with more extreme temperatures in future may be forced to rely more extensively on behavioural responses to cope with climate variability (Huey et al., 2003; Kearney et al., 2009a). This in turn could alter the form of phenotypic plastic responses to temperature over longer timescales (Marais & Chown, 2008), thereby affecting population dynamics and fitness (van Buskirk & Steiner, 2009; Chevin & Lande, 2010), and may involve complex cost-benefit tradeoffs (Huey, 1974; Angilletta, 2009). The results of the present study therefore have broad implications for understanding phenotypic plasticity of insects and the responses of insects to future climate change. Most significantly, this study provides novel evidence from short time-scales to suggest that simple analyses of mean temperature variation may be inappropriate for predicting population-level responses under climate change scenarios despite their popular usage. Further work over longer time-scales (multiple generations) and additional species would strengthen the validity of these conclusions.

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