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How plastic are the critical thermal limits of insects? Tsetse (Glossina spp.) as a case study for investigating upper thermal limits and their plasticity.

How plastic are the critical thermal limits of insects?

Tsetse (*Glossina* spp.) as a case study for investigating upper thermal limits and their plasticity.

Hester Weaving

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Thesis abstract

Animals vary in thermal tolerance, which set the limits at which survival and reproduction can occur. Thermal tolerance defines species distributions on earth and indicates vulnerability to climate change. Thermal plasticity, or the ability to respond to temperature exposure via phenotypic changes, can alter thermal limits, allowing animals to tolerate more extreme temperatures. In this thesis, I investigate the response of insects – significant as ecosystem service providers, vectors of disease, and crop pests – to warming temperatures using a multi-faceted approach, by means of comparative meta-analyses across over 100 insect species, and detailed experimental work on tsetse flies (*Glossina* spp.), vectors of human and animal African trypanosomiasis. I find that plasticity of insect thermal tolerance is generally weak, especially upper thermal limits, indicating physiological and evolutionary limits at high temperatures. Weak plasticity of upper thermal limits was mirrored in tsetse, which show limited or non-existent adult and between-generation thermal plasticity. I found considerable variation in the level of thermal plasticity among insects generally, and among tsetse species, but trends in tolerance remained obscure. I find that, thermal fertility limits, the temperature at which reproduction is prevented, occur at lower temperatures than those which kill tsetse, but, in contrast to studies on other insect species, female fertility as temperature sensitive as male fertility. These differences indicate that a diversity of species should be examined to ensure generalisations are relevant across insect species. Finally, I found that body size was important in shaping thermal tolerance limits, with high developmental temperature leading to small adult body size and, in consequence, reduced upper thermal tolerance and survival. These data support predictions of range contractions in tsetse species in response to climate change. More broadly, my findings highlight grave consequences of warming temperature for insect populations and the need for detailed experimental work on further under-studied groups of insects.

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Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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1. Chapter 1 – Introduction

1.1 Impact of climate change to life on earth

Anthropogenic induced climate change has warmed the earth by 1°C above pre-industrial levels and is likely to continue warming by 2 to 4°C by 2100 (Liu and Raftery, 2021). As warming progresses, heatwaves are becoming more frequent, prolonged and extreme (Christidis et al., 2015; Meehl and Tebaldi, 2004; Perkins et al., 2012), causing increasingly severe droughts and wildfires (Dai, 2011; Tyukavina et al., 2022). Globally, temperature records are being broken and, concerningly, the severity of heatwaves is accelerating at a faster rate than projected (Perkins-Kirkpatrick and Lewis, 2020). Heatwaves in equatorial regions, for example, have increased in frequency by 75% per 0.4°C warming (Buckley and Huey, 2016).

Effects of climate change on animals and plants are already apparent, with a wide range of effects such as biodiversity losses, shifting species' ranges, phenotypic responses, and disruptions to phenology. Global biodiversity has declined dramatically over the last two decades due to a combination of climate change, habitat loss, invasive species, pollution, and species exploitation, such as hunting and poaching (Díaz et al., 2019; Leclère et al., 2020; Maxwell et al., 2016). Heatwaves have caused mass mortality of terrestrial and marine animals such as fish, bats and corals (Robine et al., 2008; Welbergen et al., 2014, 2008). Evidence suggests that animals and plants are moving poleward and to higher elevations in response to increasing temperatures (Chen et al., 2011; Couet et al., 2022; Fossheim et al., 2015; Kelly and Goulden, 2008; Root et al., 2003), including local extinctions at range boundaries (Wiens, 2016). Animal body size has decreased in response to climate warming across several taxonomic groups (Merckx et al., 2018), such as fish (Genner et al., 2010), amphibians (Reading, 2007), and insects (Tseng et al., 2018; Wonglersak et al., 2021). As seasonal temperatures change and become less predictable, there has been a shift in phenology to earlier in the year, such as the flowering of plants and emergence of insects (Vitasse et al., 2022; Walther et al., 2002). As phenology is not affected uniformly across species, temperature change can disrupt species interactions, which cascade ecological networks (Memmott et al., 2007).

Thermal tolerance, the temperature at which growth, reproduction and survival are permitted, is an important determinant of species distributions, and can indicate vulnerability to climate warming (Kellermann et al., 2012; Overgaard et al., 2014; Pinsky et al., 2019). Upper thermal tolerance in particular may be critical for population persistence during heatwaves (Frölicher et al., 2018;

Parmesan et al., 2000; Stillman, 2019; Williams et al., 2016). Thermal tolerance varies between species, populations, individuals, and within an individual's lifetime (Bowler and Terblanche, 2008; Hoffmann et al., 2013; Kellermann et al., 2012; Lancaster and Humphreys, 2020; Nati et al., 2021; Terblanche et al., 2006). Therefore, measuring thermal tolerance and identifying trends which explain variation may indicate which species or regions are most vulnerable and the interventions required to minimise the effects of climate change (Bernardo *et al.*, 2007; Williams *et al.*, 2008; Sunday *et al.*, 2019, but see Clusella-Trullas *et al.*, 2021).

1.2 Measuring thermal tolerance and climate change vulnerability

The range of temperatures at which an animal can persist can be plotted as a thermal performance curve, where performance is measured in terms of a "rate" trait (e.g., walking speed) related to evolutionary fitness, such as survival or fecundity (Fig. 1.1; Angilletta, 2009). Terms given in bold font are defined in the Glossary in Appendix 1. **Critical thermal minimum (CT_{min})** is found at the lower thermal limit where performance declines to zero, and **critical thermal maximum (CT_{max})** at the upper limit. The **thermal tolerance breadth** is the range of temperatures at which an organism can function with at least 80% performance (Angilletta, 2009). Peak performance is considered to occur at the animal's **optimum temperature or T_{opt}**. Vulnerability indices such as **thermal safety margins** and **warming tolerances** can be calculated from these measures (see Kellermann *et al.*, 2012; Overgaard, Kearney and Hoffmann, 2014; Buckley and Huey, 2016; Clusella-Trullas *et al.*, 2021). Here, I define the thermal safety margin as the temperature difference between T_{opt} and the animal's habitat (T_{opt} - T_{hab}), and warming tolerance as temperature difference between CT_{max} and the animal's habitat (CT_{max} - T_{hab}) (Deutsch et al., 2008). Although, note that these terms are used interchangeably in the literature. Animals with small safety margins, therefore, can only cope with a small temperature increase before performance is reduced. Small warming tolerances indicate that an animal is living close to its lethal limit. Note that measures vary depending on assumptions owing to the average conditions of the habitat, i.e., depending on when the habitat is sampled spatially and temporally (Clusella-Trullas et al., 2021). Additionally, some define these limits using operative temperatures (T_e), the body temperature of an organism at equilibrium, rather than habitat temperature, which include factors such as body size and shape, and skin reflection (Clusella-Trullas et al., 2021). Operative temperatures are increasingly being used to estimate these vulnerability indices more accurately.

In the laboratory, critical thermal limits are frequently measured by a ramping assay, where temperature is increased or decreased at a constant rate, for CT_{max} and CT_{min} respectively, until a predefined behavioural endpoint, such as onset of muscle spasms, the inability of the animal to right itself, loss of the ability to cling to a surface, or actual death (e.g., Terblanche and Chown, 2006). Limits

can also be measured at a range of static temperatures to calculate lethal limits, such as LT50, the temperature at which 50% of the population dies (e.g. Enriquez and Colinet, 2017). This methodology provides a quick and relatively easy assay to measure the thermal tolerance of an organism. As a result, numerous studies form a comprehensive database over a range of animals, plants and fungi, enabling researchers to mine data for answers to broadscale questions (Bennett et al., 2018).

However, definitions of thermal tolerance limits are inconsistent among studies. Differences in methodologies may occur to accommodate for a diverse range of taxa, for example, endpoint definition, ramp rate, or assay starting temperature. Some studies may take CT_{max}, for example, at the point of muscle spasms (e.g., Belliard et al., 2019), whilst others may take the thermal limit at the point of actual death (e.g., Calosi et al., 2008). Concerningly, differences in methodology can lead to discrepancies between critical thermal limits. For example, faster ramp rates lead to more extreme CT_{max} in tsetse flies (Terblanche et al., 2007) and ants (Leong et al., 2022), but the opposite has been found for cooling rates on CT_{min} for *Drosophila melanogaster* (Overgaard et al., 2006). These differences must be considered when making comparisons across studies and species (Terblanche and Hoffmann, 2020).

Fertility is another major component of fitness and is often measured alongside mortality in thermal physiology studies (e.g. Mockett and Matsumoto, 2014; Nik Abdull Halim *et al.*, 2022). Here, and throughout my thesis, I define a sterility as the permanent inability to produce viable offspring, and infertility as the current inability to produce viable offspring, with effects that may only be temporary. Heat stress affects fertility in a wide range of animals and plants (David et al., 2005; Hansen, 2009; Sage et al., 2015; Walsh et al., 2019b). However, unlike critical thermal limit experiments, studies on the effects of temperature stress on fertility are not standardised, using a variety of traits, such as sperm count, morphology and motility, egg size and count, or offspring survival and condition (Hansen, 2009; Karaca et al., 2002; Perez-Crespo et al., 2008). Recently, a standardised measure has been suggested, the **Thermal Fertility Limit** or TFL (Walsh et al., 2019b). Thermal fertility limits are the temperature at which an animal stops producing offspring and often occur at less extreme temperatures than critical thermal limits (Fig. 1.1; Sales *et al.*, 2018; Walsh, Parratt, Atkinson, *et al.*, 2019). **Thermal Sensitivity of Fertility** or **TSF**, captures the number of viable offspring produced, which is an important measure for population viability (Baur et al., 2022). If TFLs consistently occur below critical thermal limits we might expect that population viability is more limited by losses to reproduction than mortality. Therefore, there has been a call from Walsh *et al.* (2019) to explore TFLs in more species so that comparisons can be made and TFLs used in conjunction with critical thermal limits to estimate climate change vulnerability.

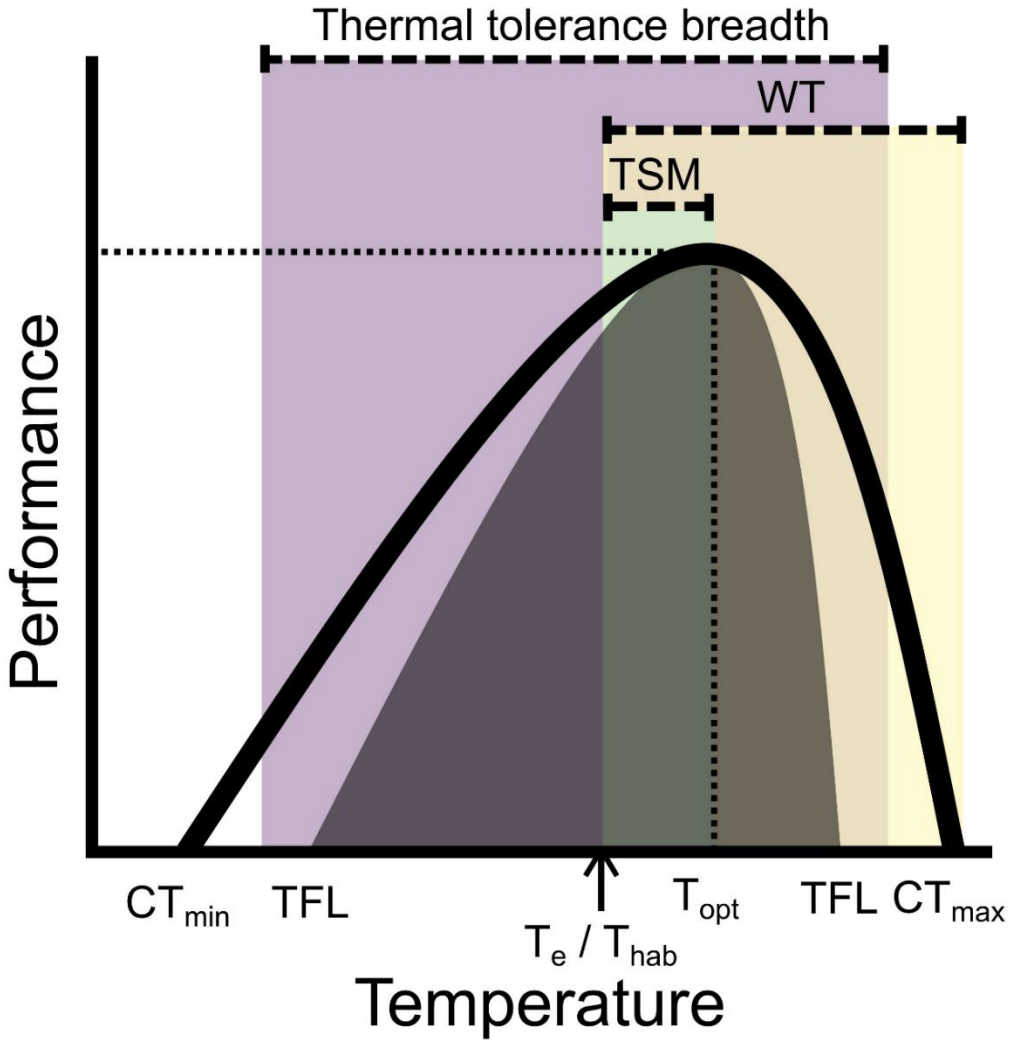

Figure 1.1 Thermal performance curve of a theoretical animal. CT_{min} is the critical thermal minimum, CT_{max} is the critical thermal maximum and T_{opt} is the optimum performance temperature. T_{hab} is the habitat temperature, and T_e is the animal's operative temperature. TFLs are the lower and upper thermal fertility limits. The TFL performance curve is shaded. WT = warming tolerance. TSM = thermal safety margin. Redrawn and adapted from Angilletta (2009).

In support, research on *Drosophila* species indicates that TFLs explain current distributions more precisely than critical thermal maxima (Parratt et al., 2021; van Heerwaarden and Sgrò, 2021). In particular, the temperature at which male *Drosophila* stop producing offspring explains distribution best, likely due to spermatogenesis being particularly heat sensitive (David et al., 2005; Hirano et al., 2022; Rohmer et al., 2004). Likewise, a range of studies indicate that male fertility is more temperature sensitive than female fertility (Hirano et al., 2022; Porcelli et al., 2017; Sales et al., 2018; van Heerwaarden and Sgrò, 2021; Vasudeva et al., 2021). However, studies that expose sexes to heat stress separately are uncommon and, in consequence, the generality of the prediction that male fertility is more heat sensitive is uncertain.

1.3 Adaptative and plastic responses to temperature change

Vulnerability indices often do not account for the possibility of adaptive potential and phenotypic plasticity so may not accurately predict warming risk (Araújo et al., 2005; Helmuth et al., 2005). Studies have investigated adaptive responses through experimental evolution in the laboratory by exposing populations to warming temperatures. However, these studies have generally found limited adaptive responses of upper thermal tolerance in *Drosophila* species to warming (Kinzner et al., 2019; Schou et al., 2014; van Heerwaarden and Sgrò, 2021), or that adaptive responses are slow to occur, requiring many generations of adaptation (Santos et al., 2023). Accordingly, a study investigating how thermal limits have evolved in over 2000 species indicates that upper thermal tolerances of ectotherms are particularly slow to adapt (Bennett et al., 2021). Therefore, the pace of warming may outrun many species ability to adapt to temperature change in evolutionary time.

Whilst evolution via natural selection is often slow, plasticity acts within an individual's lifetime and so could provide a promising avenue for fast-paced adjustment to novel environmental conditions (Laland et al., 2015; Price et al., 2003). **Phenotypic plasticity** is "the ability of an organism to react to an environmental input with a change in form, state, movement, or rate of activity" (West-Eberhard, 2002). Beneficial **thermal plasticity** is a form of phenotypic plasticity whereby an organism to responds to temperature in a way that reduces the extent to which physiological rates change (Seebacher et al., 2015). In this way, if compensation were complete, physiological rates would remain constant across temperature. Here, and throughout my thesis I use the term **acclimation** as the thermal exposure, and **thermal plasticity** or acclimation ability as the phenotypic change or response to acclimation. Acclimation can improve thermal tolerance within hours and longer exposures can result in lasting phenotypes. Resulting phenotypes can enhance performance to new conditions, such as seasonal or daily fluctuations (Bujan et al., 2020; Koštál and Tollarová-Borovanská, 2009).

Plasticity of critical thermal limits can be measured in the laboratory by acclimating populations to a range of temperatures (e.g. Terblanche and Chown, 2006). Typically, one group is maintained at basal or rearing temperature and other groups are acclimated below or above the basal temperature. Standard thermal tolerance ramping assays are then completed, as described above, and the **basal** and **acclimated critical thermal limits** compared. The temperature change in critical thermal limit over a range of acclimation temperatures can be plotted to produce a **thermal reaction norm** (Fig. 1.2). The slope of the line is equivalent to the plasticity of the thermal limit, with steeper slopes representing greater plasticity. **Acclimation response ratio (ARR)**, the change in critical thermal limit per degree change in acclimation temperature, represents the slope of the line (Cossins and Bowler, 1987). An ARR of 1, for example, would represent complete conformation of thermal tolerance to the surrounding temperature: i.e., for every 1°C change in acclimation temperature, the organism exhibits a 1°C change in critical thermal limit. However, such complete acclimation is rare (Gunderson and Stillman, 2015; Weaving et al., 2022). Additionally, often slopes over several acclimation temperatures are not linear, so several ARRs may be needed across a range of temperatures to fully capture acclimation ability (van Heerwaarden and Kellermann, 2020).

1.3.1 Types of plasticity

Plasticity, in the context of thermal responses, can be classified into three groups: **developmental plasticity**, **hardening** and **transgenerational plasticity** (Angilletta, 2009). Developmental plasticity is where thermal conditions experienced during development result in phenotypic changes in the adult stage. Such phenotypic changes often remain fixed throughout adult life. For example, the interplay of temperature and photoperiod during development results in orange or black adult morphs of the European map butterfly, *Araschnia levana* (Nijhout, 2003). When eggs of the lizard, *Bassiana duperreyi*, are incubated under high temperature, adults are faster runners than those incubated in low temperature conditions (Elphick and Shine, 1998). When harlequin bugs (*Murgantia histrionica*) develop under cooler conditions, emerging adults are more pigmented than adults raised under warmer conditions, improving their heat retention (Sibilia et al., 2018). Acclimation during development can also enhance critical thermal maximum and minimum temperatures in adult stages (Slotsbo *et al.*, 2016; Schou *et al.*, 2017a; Schou *et al.*, 2017b).

In contrast, hardening is where plastic changes are more transient, with phenotypic responses occurring on an hourly, daily or seasonal scale (Angilletta, 2009; Hoffmann et al., 2003). For example, upregulation of molecular chaperones, such as heat shock proteins, helps to prevent proteins denature at high temperature (Li and Srivastava, 2003). Heat shock proteins play an important role in maintaining protein folding and assembly, as well as their secretion and regulation (Gething, 1997).

Level of expression of heat shock proteins can be related to the degree of thermal tolerance of a species (Feder and Hofmann, 1999). At low temperatures, rapid cold hardening can result in changes to the fatty acid composition which can improve membrane fluidity at cold temperatures (Overgaard et al., 2005).

Transgenerational plasticity occurs when the thermal environment of the parent or grandparent results in phenotypic changes in offspring. Often, transgenerational plasticity is realised through changes to the mother's environment or genetics, known as maternal effects (Mousseau and Fox, 1998). This may be through epigenetic changes, such as DNA methylation leading to reduced expression of a gene, the passage of nutrients on to offspring, or hormones (Berkeley et al., 2004; Boffelli et al., 2014). Paternal effects can be realised through epigenetic changes to sperm or paternal DNA (Jiang et al., 2013). Parental temperature can affect offspring size, quality, and growth rates (Burgess and Marshall, 2011; Chang et al., 2021; Seko and Nakasuji, 2006). Transgenerational plasticity can also improve thermal tolerance. Parental acclimation of *D. melanogaster*, for example, enhances the CT_{max} of subsequent offspring (Cavieres et al., 2020; Crill et al., 1996).

Beneficial plasticity, such as the examples above, is expected to have a cost, otherwise all animals would perfectly conform to their environment. The costs of plasticity are either allocation trade-offs or genetic trade-offs (Auld et al., 2010). An allocation trade-off is the cost of producing or maintaining a molecule, such as a protein, rather than committing energy elsewhere, for example to reproduction (DeWitt et al., 1998). Genetic trade-offs are due to processes such as negative pleiotropy and epistasis, where the upregulation of one gene comes at the cost of a linked gene that has negative fitness consequences (DeWitt et al., 1998). However, costs of plasticity are rarely measured in studies due to disposing of organisms before costs are realised, such as losses to fecundity or longevity, or costs may not be observed under laboratory conditions. For example, in response to improved cold tolerance via hardening, *Drosophila melanogaster* have reduced egg to adult viability (Schou et al., 2015) and hindered ability to find food (Kristensen et al., 2008).

Plasticity can also be deleterious through negative carryover effects and environmental mismatching. **Negative carryover effects** occur when unfavourable conditions reduce the fitness of subsequent life stages or offspring. For example, there is evidence in humans that heatwaves reduce birth weight and increase the number of stillbirths (Kuehn and McCormick, 2017). Developmental heat stress during the larval stage of several species of *Drosophila* causes impaired reproduction in emerging adults (Porcelli et al., 2017; Sisodia & Singh, 2009). Environmental mismatching occurs when an expressed phenotype does not benefit the individual in the current environment. This may occur in environments which rapidly change or those that vary unpredictably (DeWitt et al., 1998). Counterintuitively, such

deleterious plasticity can be beneficial for adaptation to climate change by promoting adaptive evolution. For example, in the seed beetle, *Callosobruchus maculatus*, heat and cold tolerance evolved rapidly under progressively more stressful, variable conditions due to deleterious plasticity (Leonard and Lancaster, 2020).

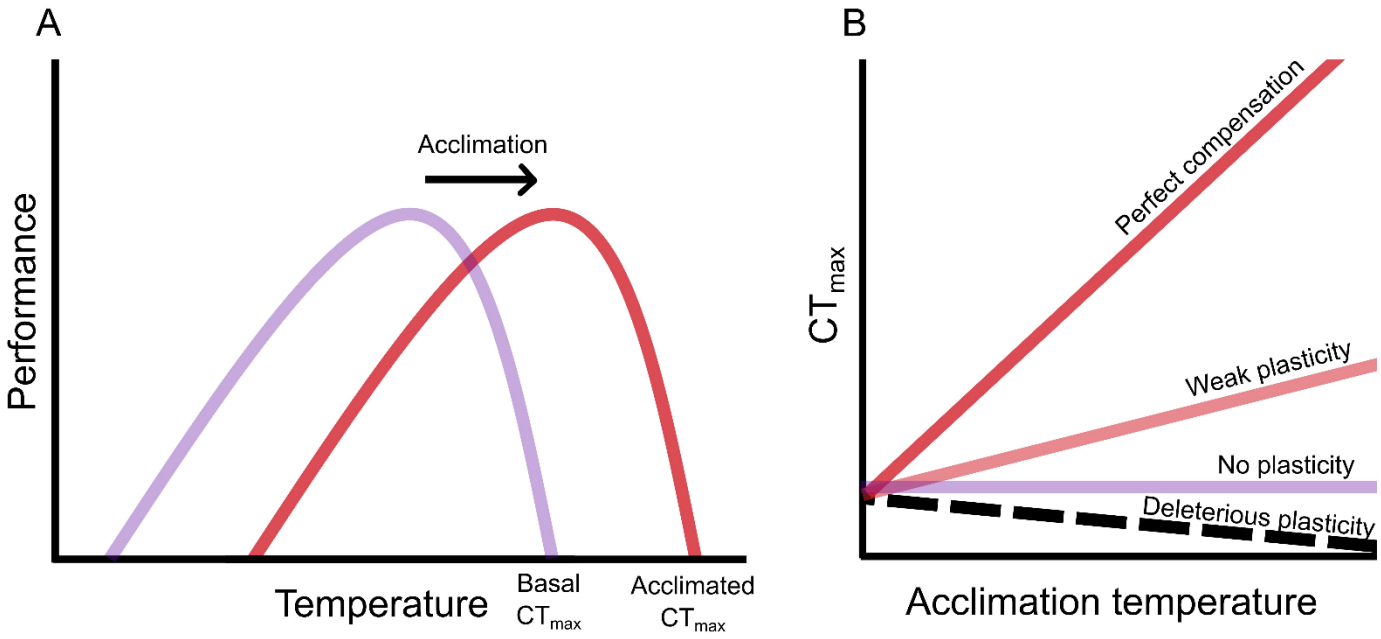

Figure 1.2 A. Thermal performance curve representing performance after acclimation at rearing/basal temperature in purple, and how the curve can shift in response to acclimation (red). B. Thermal reaction norms representing no plasticity, weak plasticity, and perfect compensation of CT_{max}, representing an Acclimation Response Ratio of 1. Deleterious plasticity is represented by the dashed black line. Note that CT_{max} often does not increase linearly with temperature but here I give a simplified example. Additionally, beneficial plasticity for some traits will be a reduction in a trait in response to temperature e.g. CT_{min}, or maintenance of a trait over a temperature range e.g. metabolic rate, heart rate. Note that CT_{min} tends show a greater plastic response than CT_{max}.

1.3.2 Clines in thermal tolerance and plasticity

Broad-scale patterns in thermal tolerance can indicate which species, populations or regions may be most at risk from climate change (Piero Calosi et al., 2008; Sunday et al., 2019). Thermal tolerance breadth of terrestrial ectotherms generally increases towards the poles and at higher altitudes as temperature variability increases. Yet, the variation in lower thermal limits is an order of magnitude greater than found in upper thermal limits (Addo-Bediako et al., 2000; Clusella-Trullas and Chown, 2014; Sunday et al., 2019). Elevational clines show a similar pattern – more extreme lower thermal limits are observed in organisms at higher elevations, but limited changes are found for upper thermal limits (García-Robledo et al., 2016; Sunday et al., 2019). This is concerning because upper thermal limits are important in defining species distribution, yet lower thermal limits are apparently more variable and evolve more quickly (Bennett et al., 2018; Sunday et al., 2011).

Clines in plastic responses of thermal tolerance, however, are more challenging to describe. In theory, beneficial plasticity evolves in a variable environment where conditions are predictable (Donelson et al., 2018; Kristensen et al., 2008). In contrast, stable environments should favour individuals with limited plasticity. Because temperature varies across spatial scales, species' thermal plasticity may increase towards the poles, at higher elevations and in terrestrial rather than marine environments, due to greater variation of temperature (Bozinovic et al., 2011; Chown et al., 2004).

Recently, meta-analysis has been used to attempt to describe these patterns. Meta-analysis is a useful tool for statistically synthesising the results of many studies which address the same research question (Koricheva et al., 2013). Results of studies are converted into effect sizes (e.g., Hedges' g indicates the difference between two groups) which assess the magnitude of an outcome on a common scale. These effect sizes are combined to produce a grand mean effect size and confidence interval to test whether the effect differs from zero. Moderators are used to describe variation among effect sizes.

Using these techniques, several studies have addressed thermal plasticity of ectotherms, describing trends with moderators such as latitude, seasonality, body size and methodologies, such as acclimation duration or ramp rate. However, these meta-analyses have not found consistent support for general predictions, and either find no evidence or conflicting results regarding clines in plasticity (Gunderson and Stillman, 2015; Rohr et al., 2018; Seebacher et al., 2015). This may be due to analyses describing a broad range of taxa, so trends specific to a group could be diluted, or conflicting selection pressures may obscure effects.

1.4 Thermal tolerance in insects

Insects are the most diverse and species-rich animal group; over a million species of insect are described, which cover a wide variety of climates and habitats (Moczek, 2010). Insects are important for ecosystem services such as pollination, waste disposal and control of pest species (Beynon et al., 2015; Calderone, 2012; Losey and Vaughan, 2006). Most insects are ectotherms, and therefore thermoregulate by seeking suitable microclimates (Woods et al., 2015). Temperature is a major determinant of insect population dynamics, controlling vital rates of growth, reproduction, and mortality, and, therefore, responses to climate change may be considerable (Halsch et al., 2021).

Insects make useful models for the study of thermal tolerance limits and their plasticity, and numerous insect species have been investigated (English and Barreaux, 2020; Weaving et al., 2022). Most insects can easily be raised in the laboratory as they are typically quick to multiply with short lifecycles. Their lifecycles have well defined stages, which is beneficial when testing between different types of plasticity. Life stages also often occupy different thermal niches so we can ask how thermal sensitivity and plasticity change over a lifetime or between different development types due to differing thermal history (Bowler and Terblanche, 2008; English and Barreaux, 2020; Fawcett and Frankenhuis, 2015). Despite their importance and usefulness as models, how insects respond to climate change via plasticity remains a topic of debate (Sgrò et al., 2016; Sørensen et al., 2016).

1.5 Vectors of disease

Insects account for approximately 700,000 deaths per year through the transmission of parasites, viruses and bacteria (WHO, 2020). Consequently, how disease vectors respond to climate change is of growing concern (Githeko et al., 2000). Several species of disease vector have moved poleward or expanded their ranges in recent decades. Asian tiger mosquitoes (*Aedes albopictus*), the vector of dengue fever, have increased their range from the western Pacific and South-east Asia into Europe, Africa, the Middle East, and the Americas (Gratz, 2004). In Europe, the biting midge, *Culicoides imicola*, has moved northward, spreading blue tongue virus in cattle (Purse et al., 2005). This year, locally transmitted cases of malaria have been reported in Florida (Florida Arbovirus Surveillance, 2023). Distributional changes cannot be attributed solely to warming temperatures - increasing globalisation allows the spread of disease between countries and continents (Knobler et al., 2006), and changing rainfall patterns alter the number of breeding sites for species like mosquitoes which lay their eggs in stagnant water (Morin et al., 2013). However, is it clear that temperature is a vital component in determining current and future distributions of vector species so an important area of investigation (Caminade et al., 2017; Moore et al., 2014; Rocklöv et al., 2019; Watts et al., 2019).

1.6 Tsetse flies

Tsetse flies are disease vectors of trypanosomiasis throughout sub-Saharan Africa. They are unusual insects – slow to reproduce, exhibiting high maternal investment, and give birth to live young (Leak, 1998) – and therefore make an interesting study organism for thermal limits and plasticity. Here, I outline their current distribution, epidemiology, lifecycle, and thermal tolerance for background to Chapters 3 to 5 of my thesis.

1.6.1 Current distribution

Tsetse flies (*Glossina* spp.) belong to the superfamily Hippoboscoidea, along with other biting Dipterans. There are 31 species and subspecies within *Glossina*, which are split into 3 subgenera: Morsitans, Palpalis and Fusca (Fig. 1.3). Tsetse occur throughout sub-Saharan Africa throughout a band named the "tsetse belt". They generally prefer a temperature of 25°C as optimum and are restricted to regions with over 500 mm of rainfall per annum (Leak, 1998; WHO, 2013). Subgenera have different distributions (Fig. 1.3), related to habitat preference, temperature and relative humidity (RH). Generally, the Morsitans group inhabit savanna and woodland, requiring approximately 50 to 60% RH. Both Palpalis and Fusca require 65 to 85% RH, with the former inhabiting environments with rivers and lakes, and the latter moist forests of West Africa (WHO, 2013). Consequently, the majority of northern Africa and parts of southern Africa, such as the Kalahari Desert, are too hot and dry for tsetse. Cooler temperature restricts distribution in certain highland areas and parts of southern Africa. High humidity is required for vegetation cover which provides shelter in cooler microclimates and protection from wind and solar radiation (Hargrove, 2004; Leak, 1998; WHO, 2013).

Comparable to other disease vectors, the distribution of tsetse species is shifting. In Zimbabwe, at the southern limit of tsetse distribution, fly numbers of *Glossina pallidipes* and *Glossina morsitans morsitans* have been declining over the last 30 years where temperatures have risen by almost 1°C since 1975 (Mangwiro et al., 1999; Thomson, 1987; Torr, Holloway, & Vale, 1992). Mechanistic modelling suggests that temperature change is responsible for declines in *G. pallidipes* (Longbottom et al., 2020; Lord et al., 2018). At the northern limit in Burkina Faso, the range of tsetse flies have declined by 70,000 km^2 due to human population growth and increasing numbers of droughts (Courtin et al., 2010). In West Africa, tsetse distribution of *Glossina palpalis* and *Glossina tachinoides* has moved 200 km southwards (Courtin et al., 2008).

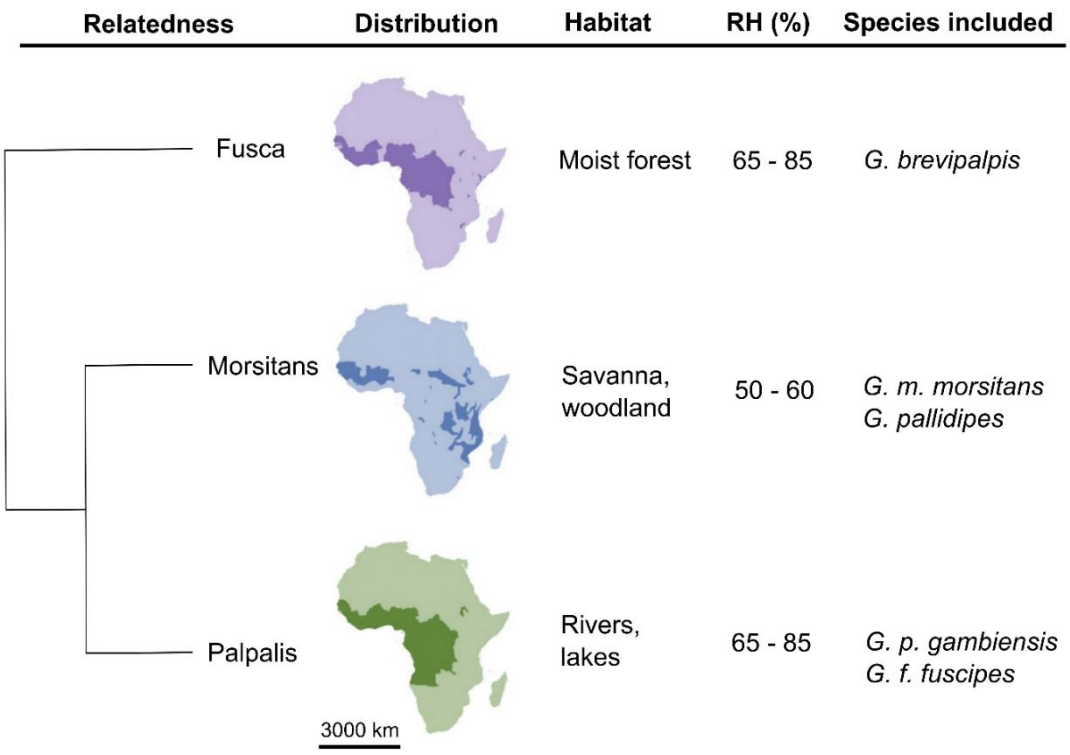

Figure 1.3 Predicted distribution of Fusca, Morsitans, and Palpalis groups using data from PAAT-Information System. From Mugenyi (2015) and Egeru *et al.* (2020). Phylogeny was constructed using Open Tree of Life and R packages 'rotl' and 'ape' in R. Species listed are those included in this thesis.

1.6.2 Epidemiology

Both male and female tsetse are obligate blood-feeders, feeding on a variety of vertebrate animals, including cattle, wild animals, and humans. They are vectors of bloodborne protozoan parasites, trypanosomes, which cause sleeping sickness or trypanosomiasis (WHO, 2013). The two human parasites are *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, which differ in their distribution and prevalence. *T. b. gambiense* is far more prevalent, explaining over 95% of cases, and is found in West and central Africa (WHO, 2013). If left untreated, the disease is usually fatal. The latest epidemic from 1970 to late 1990s was responsible for over 300,000 cases (WHO, 2013). Successful control efforts have reduced reported cases to less than 1000 in 2022 (WHO, 2023a, 2023b). Over the decade between 2007 and 2017, the disability adjusted life years (years of life lost due to mortality or healthy life lost) for African trypanosomiasis decreased by over 80% (GBD DALYs and HALE Collaborators, 2017).

Nagana is the animal disease which is caused by *Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax*. The disease prevents cattle production in 10 million square kilometres of Africa (Steelman, 1976). Economic losses are estimated at US$1–1.2 billion per year due to mortality in cattle. Total losses are estimated at US$4.75 billion per year, which include losses due to lack of cattle production in tsetse areas and inability to use cattle for ploughing (Budd, 1999). As a result, trypanosomiasis has been named as one of the most important factors in preventing socio-economic development in Africa by the Program Against African Trypanosomiasis (FAO and PAAT, 2008).

1.6.3 Life cycle

Tsetse are unusual insects as they do not lay eggs and only produce one offspring at a time. Adult females retain an egg *in utero* where it hatches into a first stage larva (Leak, 1998; Fig. 1.4). The larva is nourished by a milk-like secretion, until moulting into a second, and then third stage larva (Benoit et al., 2015). Females generally produce their first larva after 14 to 17 days post emergence and subsequently, every eight to 10 days, depending on temperature (Hargrove, 2004). Once mature, the larva is deposited by the female, buries below ground, and immediately pupates. Larva can weigh the same amount as the mother once mature, each representing a huge energetic investment (Haines et al., 2020). Adults emerge after around 30 days, having relied solely upon nourishment from the mother (Hargrove, 2004). Flies are defined as “teneral” adults until they have taken their first blood meal and the cuticle has tanned and hardened. Mating typically occurs three to five days after emergence. Tsetse males transfer sperm from the testes to the female in a tightly packed bundle which is formed into a spermatophore within the uterus of the female from male accessory gland secretions (Odhiambo, Kokwaro and Sequeira, 1983; Scolari *et al.*, 2016). Sperm are stored in the spermathecae of the female where they can be used throughout her lifetime (Fig. 1.5). This unusual form of reproduction is called adenotrophic viviparity, or “gland-fed live birth” (reviewed in Benoit *et al.*, 2015). The large energetic investment and slow production of offspring mean that tsetse only produce an average of eight to 10 offspring during their lifetime, having one of the lowest reproductive rates of all insects (Hargrove, 2004). As a result, control of the fly is a viable method to reduce prevalence of trypanosomiases, such as by mass trapping, insecticides, and, in some contexts, the sterile insect technique (Benoit et al., 2015).

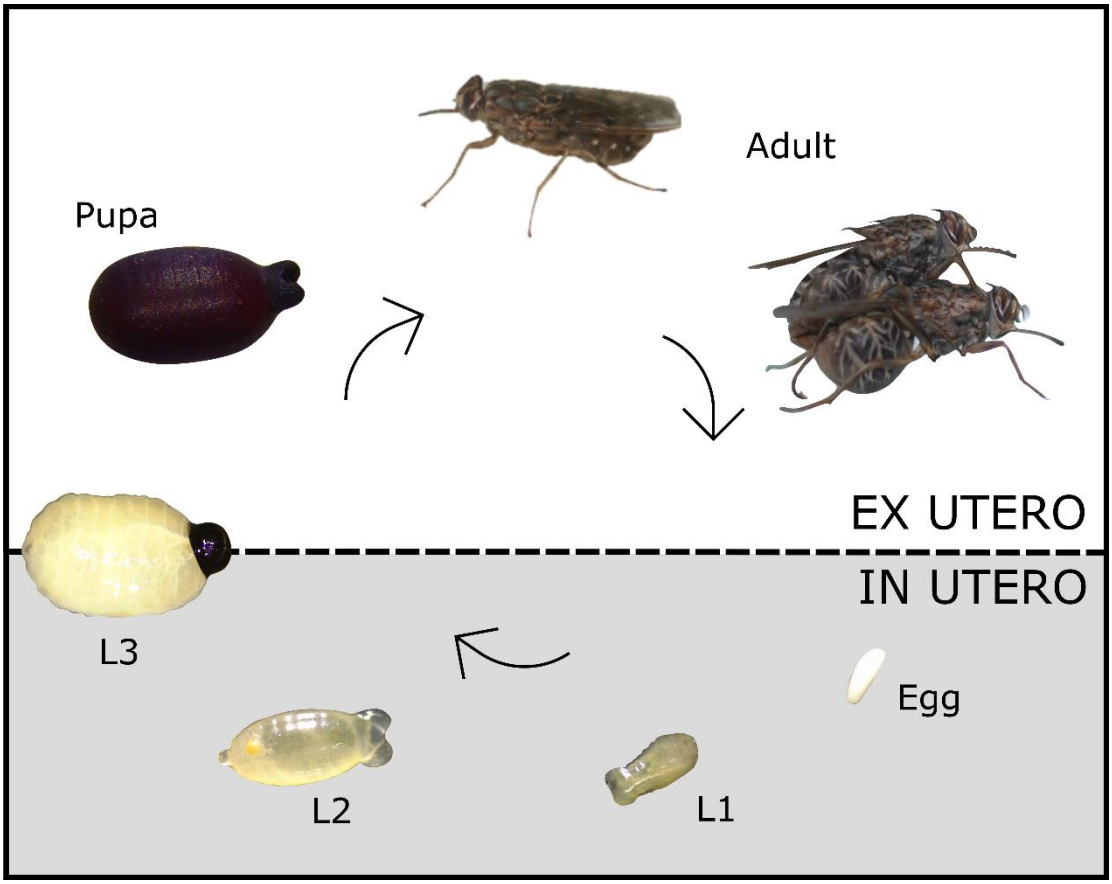

Figure 1.4 – Tsetse life cycle from egg to adult. Example photos given are of *Glossina pallidipes* and *Glossina morsitans morsitans*. The egg is retained by the female and hatches *in utero*, going through three larval moults before deposition as a third stage larva. At 25°C, egg to L3 is approximately 9 days and pupal development is ~ 30 days. Images are not to scale.

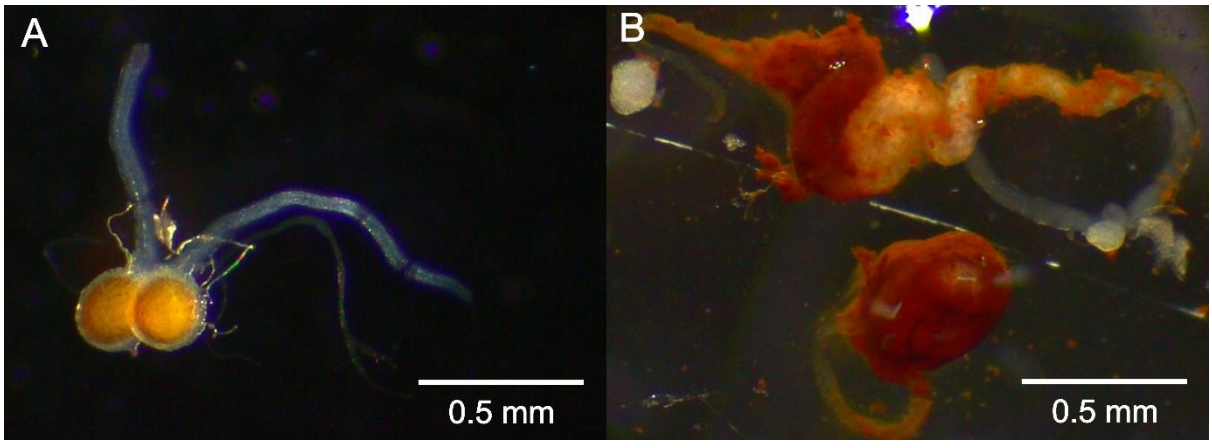

Figure 1.5 – Photos of A.) a pair of spermathecae, where females store sperm. B.) A pair of tsetse testes, with the upper testis unravelled to reveal the sperm within. Scale bar = 0.5 mm, taken on a Leica EZ4W dissecting microscope at 35 x magnification.

1.6.4 Thermal tolerance and plasticity

Tsetse are ectotherms so temperature increases their metabolic rate (Terblanche et al., 2005), and has a substantial effect on population dynamics (reviewed in Hargrove, 2004). How temperature affects reproduction, development and mortality rates is well defined in tsetse. The number of offspring a female produces increases linearly between 20°C and 30°C and declines dramatically outside these temperatures (Hargrove, 1994). Tsetse may also abort larvae before development is complete in response to high temperature. Abortion rates in the wild are low but rise exponentially with temperature (Hargrove, 2022, 1999).

Pupal development rate varies from 20 to 80 days, depending on temperature, but pupae will not complete development outside the range of 16 to 36°C. Above 36°C, pupae die from direct effects of temperature, and below 16°C pupae deplete their energy reserves before completing pupation (Hargrove and Vale, 2020; Phelps, 1973). Towards the thermal limits at which pupal development occurs, teneral tsetse may emerge with low lipid reserves, risking starvation or desiccation before they find their first blood meal (Phelps and Burrows, 1969). Therefore, starvation tolerance and desiccation resistance may be important measures of thermal tolerance in tsetse.

Around 40°C is the adult lethal limit for most species of tsetse. 39°C is lethal for *G. tachinoides* (Nash, 1936), and one hour at 40°C for *G. m. morsitans* (Potts, 1933). The LT50 for *G. pallidipes* for 1, 2 and 3 hour heat treatments are 37.9, 36.2 and 35.6°C, respectively (Terblanche et al., 2008). For *Glossina palpalis gambiensis* median survival for 31°C was four days, and for 35°C was two days (Pagabeleguem et al., 2016). In the field, mark recapture studies indicate higher adult mortality with increasing temperature (Hargrove, 1993). Population numbers are seasonal with fewer flies in the hot season (Phelps and Clarke, 1974). This relationship is unlikely due to the direct effects of temperature but may be owing to tsetse requiring more frequent, risky blood meals at high temperature, resulting in greater predation (Hargrove and Williams, 1995; Randolf et al., 1992).

Few studies have assessed the thermal plasticity of tsetse. Work has focused on *G. pallidipes*, a vector of animal African trypanosomiasis. Studies reflect patterns observed more broadly in insects – CT_{max} varies only 1°C between populations, altitudes, and seasons, but CT_{min} varies by over 9°C (Terblanche et al., 2006). Plasticity of upper thermal tolerances appears constrained; a 10-day acclimation of adult flies resulted in a 0.6°C increase in CT_{max} and 3°C decrease in CT_{min}. A study on the developmental plasticity of critical thermal limits finds similar results – there was no response in CT_{max} to developmental acclimation, while CT_{min} exhibited a plastic response (Terblanche and Chown, 2006). However, it is unknown if the limited plastic responses in CT_{max} of *G. pallidipes* are reflected across other tsetse species.

1.7 Addressing gaps in the understanding of the effect of climate change on insects and tsetse: thesis aims

In this thesis, I aim to investigate insect responses to warming temperatures using a multi-faceted approach, by means of comparative meta-analyses across insects and detailed experimental work in tsetse. Meta-analyses have examined the plasticity of species critical thermal limits (Barley et al., 2021; Gunderson et al., 2017; Gunderson and Stillman, 2015; Kellermann and van Heerwaarden, 2019; Morley et al., 2019; Rohr et al., 2018). Yet, no study has focused specifically on insects despite their ecological and economic importance. Therefore, in Chapter 2 I test the prediction that plasticity increases the resilience of insect populations to temperature extremes by enhancing their critical thermal limits. I examine broad-scale patterns in plasticity between life stages, development types and experimental methodologies, and across latitudinal and body size gradients (Fig. 1.6).

I then focus on tsetse as a case study for thermal tolerance and plasticity, to see if general patterns found in insects apply to tsetse. I first examine species variation in adult plasticity of critical thermal maximums across five tsetse species and then investigate how basal and acclimated CT_{max} relate to body size and sex across and within species (Fig. 1.6; Chapter 3). I then ask how lethal limits differ to thermal fertility limits in *G. pallidipes* and investigate differences in thermal sensitivity between males and females (Chapter 4). In this chapter, I also examine evidence for transgenerational plasticity, observing how the parent environment enhances CT_{max} in the offspring generation. Finally, I look for evidence of developmental plasticity in *G. m. morsitans*, investigating how temperature in the pupal stage affects teneral survival under blood deprivation (Chapter 5).

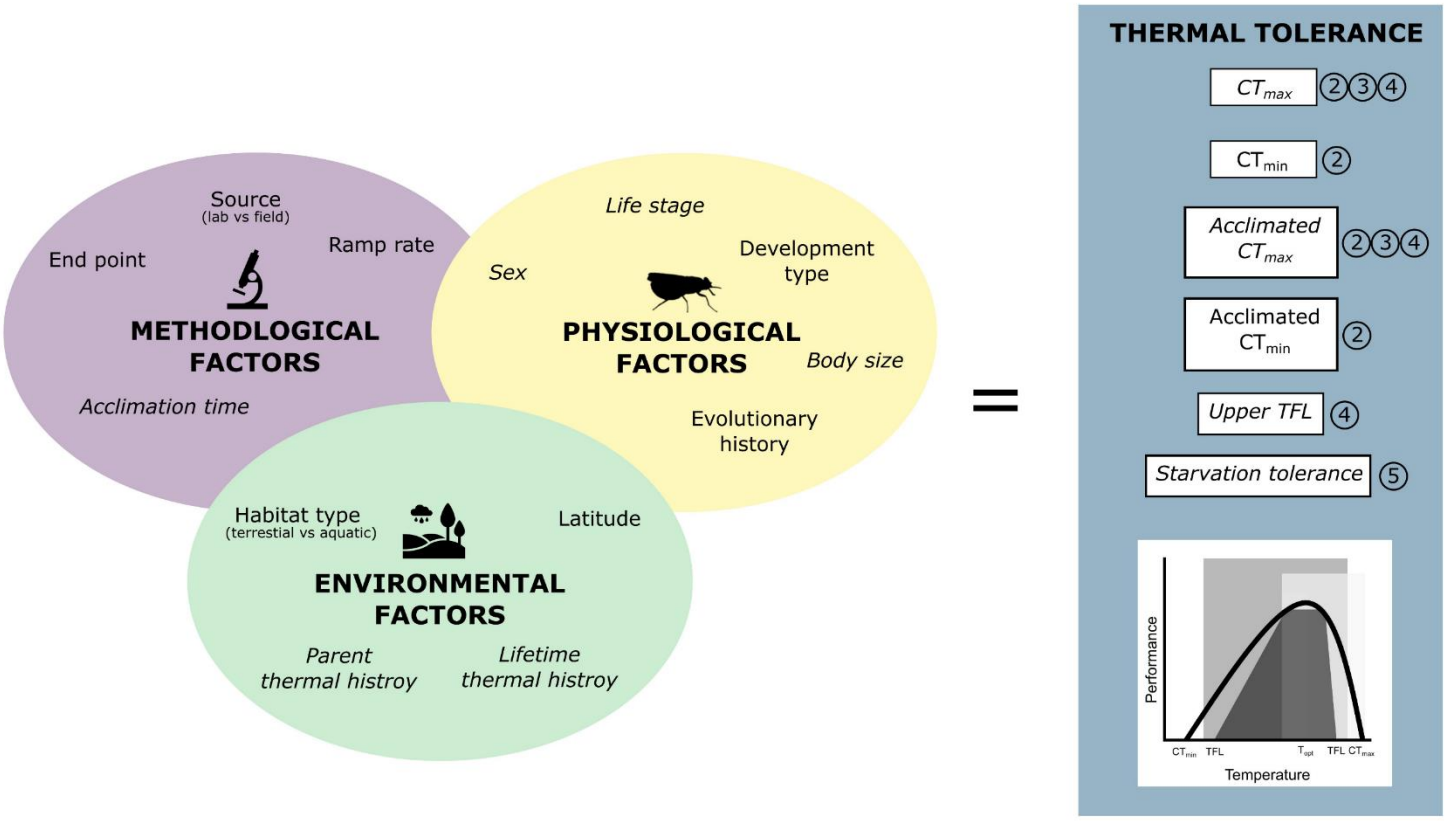

Figure 1.6 Schematic overview of thesis. I have outlined the methodological, physiological, and environmental factors I investigated, and the thermal tolerance metrics measured. Circled numbers on the right-hand side refer to the Chapter in which the thermal tolerance measure is investigated. Text in italic font refers to factors or metrics measured in tsetse, rather than insects generally.

2. Chapter 2 - Meta-analysis reveals weak but pervasive plasticity in insect thermal limits

2.1 Statement

This chapter is published as a paper in Nature Communications with authors: John S. Terblanche, Patrice Pottier, and Sinead English. I, Hester Weaving completed the analysis, wrote the code and led the writing of the manuscript. PP contributed code and input to the analysis. SE and JT jointly supervised the work and helped conceptualise the project. All authors contributed to the development of drafts. Find at: Weaving, H., Terblanche, J.S., Pottier, P., and English, S. (2022) Meta-analysis reveals weak but pervasive plasticity in insect thermal limits. *Nature Communications* **13**, 5292. 10.1038/s41467-022-32953-2

2.2 Abstract

Extreme temperature events are increasing in frequency and intensity due to climate change. Such events threaten insects, including pollinators, pests and disease vectors. Insect critical thermal limits can be enhanced through acclimation, yet evidence that plasticity aids survival of temperature extremes is limited. Here, using meta-analyses across 1,374 effect sizes, 74 studies and 102 species, I show that thermal limit plasticity is pervasive but generally weak: per 1°C rise in acclimation temperature, critical thermal maximum increases by 0.09°C; and per 1°C decline, critical thermal minimum decreases by 0.15°C. Moreover, small but significant publication bias suggests that the magnitude of plasticity is marginally overestimated. Juvenile insects are more plastic than adults, highlighting that insects vary in their responses to temperatures through ontogeny. Overall, I show critical thermal limit plasticity is likely of limited benefit to insects during extreme climatic events, yet more studies are needed in under-represented taxa and geographic regions.

2.3 Introduction

Extreme heatwaves are becoming more frequent and intense, whist the reverse is true for extreme cold events (IPCC, 2021). The upper and lower critical thermal limits of animals, frequently estimated as critical thermal maximum and minimum (CT_{max}, CT_{min} respectively), serve as useful proxies for inferring climate-related vulnerability (Angilletta, 2009; Sunday et al., 2014). Extreme heatwaves are expected to exceed species' critical thermal limits, so animals must adapt or move poleward to cooler climes (Hampe and Petit, 2005). As high latitudes have greater variation in surface temperature, both poleward advancement and maintenance of current ranges will expose species to a greater frequency and magnitude of extreme temperatures (Ma et al., 2021; Parmesan, 2006). Plasticity of critical thermal limits – a flexible response to changing conditions that can occur at the level of individuals, populations, or species – provides an important mechanism for populations to enhance tolerance, and cope with increasingly variable and intense temperatures (Angilletta, 2009). Such plasticity can be achieved through acclimation, whereby prior thermal exposure can cause a shift in critical thermal limits, allowing animals to perform better and/or recover from, more extreme temperatures (Hoffmann et al., 2003; Oostra et al., 2018). For example, acclimation can cause upregulation of heat shock proteins, and result in changes to phospholipid composition in the cell membrane (Overgaard et al., 2005; Štětina et al., 2015). Plasticity could therefore be important for tracking increasingly variable and intense temperatures and allow time for evolutionary responses via slower genetic change across generations (Laland et al., 2015).

Insects fulfil diverse ecological roles as pollinators, agricultural pests and disease vectors, and there is global concern over recent, rapid declines in abundance of rare, ecologically- or agriculturally-important species and, conversely, spikes in pest and disease vector outbreaks (Deutsch et al., 2018; Sánchez-Bayo and Wyckhuys, 2019). How insects will respond to climate change via plasticity remains an important topic of debate (Sgrò et al., 2016; Sørensen et al., 2016). Recent systematic reviews and formal meta-analyses across ectotherms have assessed plasticity of critical thermal limits and described broad-scale patterns of variation in plasticity (Barley et al., 2021; Gunderson et al., 2017; Gunderson and Stillman, 2015; Kellermann and van Heerwaarden, 2019; Morley et al., 2019; Rohr et al., 2018). Generally, these studies find weak plasticity of critical thermal limits, concluding that this mechanism has limited potential to aid survival of ectothermic species under climate change. Explaining broad-scale trends is, however, complicated, and contradictory findings have been presented regarding the relationship of plasticity with factors such as latitude, seasonality, and body size (Gunderson and Stillman, 2015; Rohr et al., 2018; Seebacher et al., 2015). With the general focus on ectotherms, trends specific to important assemblages – such as insects – may be obscured, and traits unique to these assemblages (such as modes of development) are typically not investigated.

Here, I undertake a systematic meta-analysis of experimental studies on the plasticity of insects' upper and lower critical thermal limits, including taxon-specific moderators to investigate variation in plasticity. I investigate plastic responses to thermal acclimation – measured as the acclimation response ratio (ARR), the change in critical thermal limit with a given change in acclimation temperature – as they are relevant to future climate change scenarios, widely reported, and have been used in previous meta-analyses on the topic (Barley et al., 2021; Gunderson et al., 2017; Gunderson and Stillman, 2015; Morley et al., 2019; Rohr et al., 2018). I examined (a) insects' ability to adjust their critical thermal limits via plasticity, (b) broad-scale trends across origin (latitude and habitat type), ecology and morphology (sex and body size), and ontogeny (life stage and development type), and (c) how diverse methodologies used in experimental studies affect plasticity estimates. A priori predictions are outlined in section 2.4.5.

Overall, I show that critical thermal limits have generally weak plasticity, in keeping with the broader literature. Evidence of publication bias, although of small effect, indicates that insects could be even less plastic than expected. Few broad-scale trends were identified, suggesting that insects express complex and heterogenous responses to their thermal environment. I also found that juvenile insects were more plastic than adults, indicating that insects can better compensate for variable temperatures during development.

2.4 Methods

2.4.1 Literature search

Each step was reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher et al., 2009). Searches were performed in Web of Science (WoS) (Core collection) and Scopus between July and November 2020. The search was limited to studies published between January 1990 and November 2020. The first search using Web of Science and Scopus used the following search terms: (ectotherm* OR insect*) AND (thermal OR heat OR cold OR chill OR temperature) AND (min* OR max* OR critical OR surviv* OR lethal) AND (plastic* OR (phenotyp* plastic*) OR acclim* OR stress OR tolerance) NOT (plant* OR tree* OR fung* OR mammal* OR marsup* OR bird* OR reptile* OR lizard* OR snake* amphib* OR frog* OR toad* OR fish* OR newt*).

As WoS only had three hits before 1990, articles were only included from the period of 1990 to 2020 to reduce bias between the two databases. The articles were limited to those published in English. Coverage of the literature was assessed using previously mentioned meta-analyses which examined acclimation in ectotherms. Of the four articles (post 1990) on insects included in an analysis by Seebacher *et al*. (2015), all were found in the present literature search (Seebacher et al., 2015). Twenty-one articles (post 1990) on insect species were included in Gunderson *et al.*'s 2015 study, nine of which were picked up by the present study (Gunderson and Stillman, 2015). Therefore, coverage of the literature was deemed to be insufficient, so an additional search was completed between October and November 2020 using more comprehensive search terms. The following were used: (insect* OR ecopteran* OR archaeognatha OR bristletail* OR ecoptera* OR ecopteran* OR *lice OR *louse OR Psocopter* OR blattodea* OR cockroach* OR ecopter* OR ecoptera* OR ecoptera* OR dermaptera* OR earwig* OR orthoptera* OR grasshopper* OR cricket* OR ecoptera* OR mantis* OR mantid* OR ephemeroptera* OR ecopt* OR ecopteran* OR phasmid* OR ecopter* OR ecopteran* OR isoptera* OR termite* OR ecopteran* OR thrip* OR hemiptera* OR *bug* OR cicada* OR aphid OR *hopper* OR ecopteran* OR webspinner* OR web-spinner* OR zoraptera* OR endopterygot* OR megaloptera* OR hymenoptera* OR wasp* OR ants OR ant OR bee OR bees OR coleoptera* OR beetle* OR lepidoptera* OR ecoptera* OR moth* OR caterpillar* OR ecopteran* OR ecoptera* OR ecopteran* OR flea* OR diptera* OR *fly OR *flies OR mosquito* OR ecopteran* OR lacewing* OR antlion* OR ecopteran* OR raphidioptera* OR strepsiptera*) AND (thermal OR heat OR cold OR chill OR temperature) AND (min* OR max* OR critical OR surviv* OR lethal) AND (plastic* OR (phenotyp* plastic*) OR acclim* OR stress OR tolerance). These search terms resulted in full coverage of the literature from the aforementioned meta-analyses.

2.4.2 **Eligibility criteria**

The exclusion procedure is summarised in Supplementary Figure 2.1. In total, the two databases found 12,139 unique results. Study abstracts were scanned manually for suitability by a single observer (HW) and selected studies were further examined by their methodology. Studies were selected for data extraction if they described dynamic tolerance assays where CT_{max} or CT_{min} was measured by ramping the temperature until a specified end point. We chose to only evaluate dynamic studies as it was a common metric used in thermal tolerance assays, removed additional sources of methodological heterogeneity, and was a metric already synthesised in other meta-analyses. Studies were required to have at least two temperature treatments (including studies where a single treatment was compared to a control), perform a temperature acclimation treatment (all durations of acclimation, including acute hardening and longer-term chronic acclimation, were included and fluctuating temperatures were allowed), and be undertaken in a laboratory. Studies were not included if any variables in addition to temperature were modified (excluding named moderators). Studies were also excluded if the endpoint was recorded for a proportion of the insects assayed only (e.g. $CT_{max}80$).

2.4.3 **Data extraction and effect size calculation**

Data were extracted (arithmetic mean, standard deviation (SD), sample size (N)) from 60 and 52 articles, comprising 92 and 74 species, for CT_{max} and CT_{min} respectively, from tables or text directly, from Supplementary Information, or directly requested from the authors when not available. Four studies where a very large number of insects were measured were removed from the CT_{max} dataset as the unusually large sample sizes (n > 700) grossly inflated the study weight and it was deemed that this number of insects could not be accurately assessed in one run. When only presented in graphical form, data were digitised from Figures using R package 'metaDigitise' (Version 1.0.1). Axes were calibrated using the longest distance possible to increase accuracy. If the error bars were obscured by the data points, the full size of the data point was taken as the error as a conservative measure. If not directly stated, sample sizes were calculated from degrees of freedom, and where the resulting numbers were non-integer, the sample size was rounded down. Where a range of sample sizes were stated, the smallest was always taken.

Acclimation Response Ratio (ARR) was calculated for CT_{max} and CT_{min} from the raw data using $\mathrm{ARR} = \frac{\mathrm{CTL}_{[\mathrm{T}_2]} - \mathrm{CTL}_{[\mathrm{T}_1]}}{\mathrm{T}_2 - \mathrm{T}_1}$, where CTL is the critical thermal limit (CT_{max} or CT_{min}) and T is the acclimation temperature (Cossins and Bowler, 1987). This results in a positive ARR if heat acclimation increases CT_{max} or if cold acclimation decreases CT_{min}. The standardized slope can be interpreted as a change in critical thermal limit for each degree change in acclimation temperature. As in Pottier *et al.* (2021), when more than two acclimation temperatures were reported, pairwise comparisons were made

(e.g., 10-12°C, 12-15°C, 15-20°C). I calculate multiple ARR measures rather than deriving a single slope per study in order to capture potential (and likely) non-linearity in the relationship between acclimation temperature and critical thermal limit. This meant that some responses were used in ARR calculations twice. To account for this, a variance covariance (VCV) correlation matrix was used to reduce the weight of dependent observations (see section '2.4.6 Statistical analysis'). The variance was calculated as: $\mathrm{Var} = \left(\frac{1}{T_2 - T_1}\right)^2 \left(\frac{SD^2_{[T1]}}{N_{[T1]}} + \frac{SD^2_{[T2]}}{N_{[T2]}}\right)$, where SD is the standard deviation and N is the adjusted sample size.

2.4.4 **Moderator variables**

Prior to the analysis, predictions were made regarding the chosen moderators and submitted to Turnitin. Moderators were extracted either from the study itself or from published studies and databases. All lengths of acclimation were included, resulting in 19% of effect sizes (k = 265) with acclimation treatments under 24 hours, 35% (k = 486) between 1 and 7 days, and 45% (k = 623) for over a week. As some studies stated the duration of acclimation treatment in life stages rather than a metric of time, 35% (k = 478) of data were missing. Unfortunately, this meant data available were biased to shorter acclimation times as longer acclimations were usually stated in life stages. The stage at which the insect was acclimated was during the juvenile stage for 23% of effect sizes (k = 310), adults for 51% (k = 694), several life stages for 24% (k = 334), and several generations for 3% (k = 36). I also recorded whether the acclimation treatment and assay were within the same life stage (k = 1004) or over different life stages (k = 370) to test for preliminary evidence of the effect of the temperature-size rule on ARR (Supplementary Table 2.3 and 2.4). For mass data, 10% (k = 132) came directly from the paper, 69% (k = 946) from the wider literature, with the remaining 21% missing (k = 296). If wet (fresh) body mass was not stated, data were first obtained from studies for the same species within the database, otherwise I searched the wider literature. References for studies from which mass estimates were extracted can be found in Weaving et al. (2022) or deposited at https://osf.io/cbhv4/. Where only dry mass was available, estimates of wet mass were made by using water balance estimates of closely related species found in Hadley (1994). For latitude, where only a place name was given, I chose a midpoint within this area and used Google Maps to drop a marker in the middle of the location specified. Some studies did not provide detail of the source of a laboratory population, meaning that 14.6% (k = 202) of data were missing for latitude.

2.4.5 **A priori predictions**

A priori predictions for meta-analysis moderators were used to examine variation in plasticity of critical thermal limits in insects. General predictions are made but it is noted that often contradictory arguments can be made and responses may be mediated by other factors (e.g. trade-offs with basal resistance, the temperature-size rule, mobility influencing the environmental experience, and organismal biochemical and physiological constraints) (Barley et al., 2021; Pincebourde and Woods, 2020; Stevenson, 1985a; van Heerwaarden and Kellermann, 2020).

i.) Origin

Theory suggests that selection drives plastic responses in animals from environments with moderate environmental variability and a degree of predictability (Donelson et al., 2018; Kristensen et al., 2008).

(a) Latitude

We expect animals from lower latitudes to show less thermal tolerance plasticity than those living at higher latitudes, with higher seasonality and thus predictable variability (Bozinovic et al., 2011; Chown et al., 2004). We acknowledge, however, that these predictions have received mixed support both in quantitative synthesises on ectotherms (Barley et al., 2021; Gunderson and Stillman, 2015; Rohr et al., 2018; Seebacher et al., 2015), and when explicitly tested in insects at either the species (Overgaard et al., 2011), or population level (Sgrò et al., 2010).

(b) Habitat type

We predict that terrestrial organisms will have greater thermal tolerance plasticity than aquatic species, where the environment is more stable. However, here too some evidence suggests the contrary (Gunderson and Stillman, 2015).

ii.) Ecology and morphology

(a) Size

Larger insects tend to be longer lived than smaller insects so are likely exposed to a greater range of temperatures throughout their lifetimes (Rohr et al., 2018). We therefore predict that ARR will increase with body size. Evidence consistent with this prediction has been found in quantitative syntheses on ectotherms (Rohr et al., 2018). First principles also suggest that plasticity of thermal tolerance could decrease with size, due to lower thermal inertia in smaller insects, and reduced ability to exploit microclimates (Brown et al., 2004; Kingsolver and Huey, 2008).

(b) Sex

Given that animals are often sexually dimorphic, critical thermal limit plasticity may differ between sexes. For example, across ectothermic animals, males are often smaller than females (Stillwell et al., 2010). However, males also tend to display more risky behaviours which could expose them to greater temperature variability, promoting selection for greater thermal tolerance plasticity (Stillwell et al., 2010; Tarka et al., 2018). Due to conflicting selection pressures, we predict no consistent difference between sexes in insects. When this hypothesis was tested across ectothermic animals by meta-analysis, either no differences were found, or females had greater plasticity of thermal tolerance (Pottier et al., 2021).

iii.) Ontogeny

Animals express different degrees of plasticity within their lifetimes (Bowler and Terblanche, 2008; English and Barreaux, 2020; Fawcett and Frankenhuis, 2015). Insect life stages often differ considerably in traits such as size and behaviour, often utilising distinct niches. As an added complication, insects under high developmental temperatures generally become smaller (the temperature-size rule (TSR) (Kingsolver and Huey, 2008)), which may act counter to predictions. A formal test of TSR could not be undertaken in the present study as most mass estimates were derived from the wider literature.

(a) Life stage at acclimation

We expect that juvenile insects will have greater critical thermal limit plasticity early in life, due to juvenile stages being less mobile than adults and so less able to regulate temperature behaviourally (English and Barreaux, 2020).

(b) Development type

We predict that hemimetabolous insects will have greater plasticity than holometabolous insects across the life stages. Developmental plasticity in holometabolous insects may be lost after metamorphosis due to morphological reorganisation (Bowler and Terblanche, 2008). This may be adaptive as in holometabolous insects cues are less comparable across life stages, where pupae are immobile and larvae often have different ecology to adults (English and Barreaux, 2020). As hemimetabolous insect do not metamorphosise, plasticity may be preserved into adulthood.

iv.) Methodology

Methodology regarding how critical thermal limits should be measured has been widely debated (Overgaard et al., 2012). Less frequently acknowledged is how these diverse measures affect

plasticity estimates (Bak et al., 2020; Rodrigues and Beldade, 2020). Typically, studies use dynamic assays, where, following a period of acclimation, temperature is ramped until a predefined endpoint (CT_{max} or CT_{min}). However, specific methodology necessarily varies widely to accommodate diverse taxa and life stages with unique behaviours, which in turn may affect comparability across studies and species (Terblanche and Hoffmann, 2020).

(a) Length of acclimation treatment

The length of time an insect is subjected to an acclimation treatment can vary from hours to weeks. As acclimation can be stressful, we expect smaller ARRs for longer acclimation times as injury accumulates exponentially with time (e.g. discussed in (Cossins and Bowler, 1987; Loeschcke and Hoffmann, 2002)). However, we acknowledge that under mildly stressful conditions more time under acclimation can allow for increased plasticity (Pintor et al., 2016).

(b) Assay ramp rate

The ramp rate can vary by a factor of 75, with some studies arguing that faster rates have greater ecological relevance (Rezende et al., 2011). We predict that a slower ramp rate will generally result in reduced tolerance plasticity due to increased time to see divergence between control and treatment groups. However, we acknowledge that the effect of ramp rate on critical thermal limit plasticity has not been well explored in the insect literature (but see (Allen *et al.*, 2016), for evidence on springtails).

(c) Endpoint definition

A temperature ramp causes a series of behavioural and physiological responses in insects, such as loss of coordination, partial paralysis, muscle spasms, and finally, total paralysis/death. We expect greater plasticity in critical thermal limits at behavioural endpoints than if the endpoint is measured as death (Lutterschmidt and Hutchison, 1997).

(d) Insect source

Multiple environmental factors vary in the field which may magnify plastic responses, in contrast to controlled conditions of the laboratory environment where plasticity may be less pronounced. Therefore, we predict that studies which use laboratory populations will capture less pronounced plasticity of critical thermal limits than those which use field-caught insects (Terblanche et al., 2006).

2.4.6 **Statistical analysis**

All analyses were completed in R version 4.0.3. Code and raw data can be found deposited at: https://osf.io/cbhv4/. The following sources of non-independence were identified and considered: phylogenetic relationships, non-phylogenetic species-related effects (e.g. shared ecology), population effects (e.g. same collection site), study effects (ARRs calculated from the same study), pairwise comparisons for ARR calculations, and within study effects (effect size ID; variability in the true effects within studies). Phylogenetic trees were constructed in the Open Tree of Life and R packages 'rotl' (Version 3.0.11) and 'ape' (Version 5.5) (for full trees, see Supplementary Fig. 2.2; Hinchliff *et al.*, 2015). A phylogenetic correlation matrix was constructed based on hypothetical relatedness of species. A VCV matrix was constructed to account for dependant observations due to pairwise comparisons during ARR calculation. Branch lengths were assigned following Grafen's method. The VCV matrix did not explain any of the variation in the data, so was excluded from subsequent models. The final random effect structure was study ID, phylogeny, species ID, and effect size ID. Although the random effect structure was not the best fit for CT_{min} data (study ID and effect size ID only), for ease of interpretation models were run with the same structure.

The R package 'metafor' (Version 3.0-2) was used to perform multi-level, random effects models (Viechtbauer, 2010). All models were run with the chosen random effect structure, with data for CT_{max} and CT_{min} run separately. Intercept models were fitted to assess the overall effect of acclimation on CT_{max} and CT_{min}. Each moderator was examined in univariate models, and then fitted in a full model with all moderators. The 'Dredge' function from the MuMIn package (Version 1.43.17) was used to assess which combination of moderators had the best fit ($\Delta AICc \leq 2$; Barton, 2020). The multivariate models excluded latitude, mass and acclimation duration as these moderators did not have a complete dataset available, which would have reduced power and may have affected the results. As differences between variables were small, conditional averages were used rather than full averages which tend to be more conservative and bias small results towards zero (Barton, 2020). Full-average model statistics can be found in the Supplementary Tables 2.8 and 2.10. Statistical significance was assumed when 95% confidence intervals (95% CIs) did not span zero or, when comparing groups, 95% CIs did not overlap. Residuals were assessed for homogeneity of variance between groups visually. Where residuals were heterogeneous the robust.rma.mv function from the metafor package was used. I^2, the proportion of variance not attributed to sampling error, was calculated for each model to assess heterogeneity. The overall amount of heterogeneity was calculated, I^2 total, as well as the heterogeneity explained by each of the random effects.

2.4.7 **Sensitivity analyses and publication bias**

Leave-one-out analyses were performed by iteratively removing one family, species, or order to determine if any influential groups affected the model outcome. Analyses were also completed without Drosophilidae, and with Drosophilidae only, and without data where the acclimation treatment was a fluctuating temperature, results for which are reported in the Supplementary Tables 2.13-2.17. Publication bias was assessed by funnel plot and Egger's regression test (Supplementary Tables 18-23). Egger's regression test was performed by fitting standard error as a unique moderator and as part of the best fit model (Nakagawa et al., 2022). As publication bias was identified, Standard Error^2 was fitted as a moderator to predict mean ARRs corrected for publication bias. A model was also fitted with study year as a moderator to exam temporal biases (Supplementary Tables 2.24-2.25). All code was adapted from Pottier *et al.* (2021) and Macartney *et al.* (2019). Figures were constructed using the orchaRd package (Version 0.0.0.9) and by adapting code from Pottier *et al.* (2021).

2.5 Results

2.5.1 Effect size dataset

A total of 803 effect sizes (from 60 studies, 92 species) were analysed for CT_{max} and 571 (from 52 studies, 74 species) for CT_{min}. Overall, the analysis for both measures comprised 102 species from 74 studies. Diptera were by far the most represented order (k = 684, with most effect sizes from Drosophilidae (k = 584)) – followed by Coleoptera (k = 261), Hemiptera (k = 150), Hymenoptera (k = 101), Lepidoptera (k = 75), Blattodea (k = 26), Trichoptera (k = 26), Ephemeroptera (k = 20), Plecoptera (k = 17), Odonata (k = 6), Grylloblatta (k = 6) and Orthoptera (k = 4).

2.5.2 Overall plasticity of critical thermal limits

Overall, there was weak, positive plasticity for both upper and lower critical thermal limits. For every 1°C rise in acclimation temperature, CT_{max} increased by 0.091°C (95% CI = 0.030, 0.153, Figure 2.1). Full statistics can be found in Supplementary Table 2.2. Lower thermal limits were 60% more plastic; CT_{min} decreased by 0.147°C (95% CI = 0.106, 0.188) for every 1°C decline in acclimation temperature (Figure 2.1; Supplementary Table 2.2).

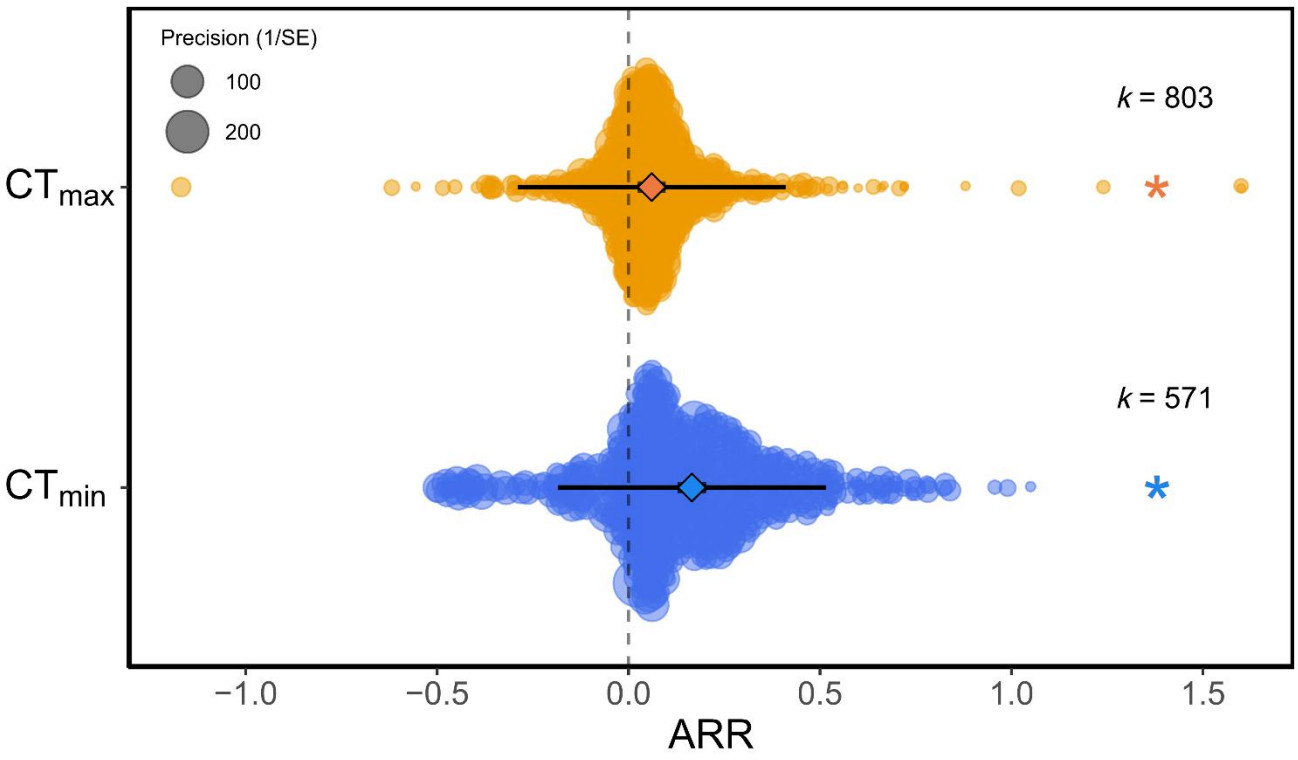

Figure 2.1 Meta-analytic mean acclimation response ratio (ARR) for upper and lower critical thermal limits, CT_{max} (k = 803) and CT_{min} (k =571). A positive ARR indicates an adaptive plastic response; heat acclimation increases CT_{max} or cold acclimation decreases CT_{min}. 95% confidence intervals (95% CIs) are depicted in heavy black lines (and partially hidden by the mean data points, depicted by a diamond symbol), prediction intervals in thin black lines. The precision of the study (1/SE (Standard Error)) is proportional to the size of each data point. k = number of effect sizes per group. Asterisk indicates that 95% CIs do not span zero.

2.5.3 Broad-scale patterns in critical thermal limit plasticity

We assessed whether variation in plasticity of critical thermal limits was explained by moderators using a series of univariate (Supplementary Tables 2.3-2.4) and multivariate models (Supplementary Tables 2.5-2.10). Due to 15-35% of data missing for latitude, acclimation duration and mass, these moderators were not included in the multivariate models. ARRs are stated as mean differences between groups (with the direction of comparison stated in subscript) or as meta-regressions.

We expected insects originating from environments with greater temperature variability to be more plastic, however there was no relationship between latitude and ARR (CT_{max} βARR = -0.001; 95% CI = -0.002, 0.001; CT_{min} βARR = -0.001; 95% CI = -0.002, 0.001) and no difference between aquatic and terrestrial insects (CT_{max} ARR $_{terrestrial\text{-}aquatic}$ = 0.002; 95% CI = -0.111, 0.117; CT_{min} ARR $_{terrestrial\text{-}aquatic}$ = 0.115; 95% CI = -0.067, 0.297). We predicted that insects with larger mass would have greater ARRs, however there was no relationship (CT_{max} βARR = 0.001; 95% CI = -0.001, 0.003; CT_{min} βARR = <-0.001; 95% CI = -0.001, <0.001). As expected, there was no overall difference in thermal tolerance between male and female insects (CT_{max} ARR $_{male\text{-}female}$ = 0.035; 95% CI = -0.005, 0.076; CT_{min} ARR $_{male\text{-}female}$ = -0.028; 95% CI = -0.098, 0.042; for full comparisons and individual coefficients see Supplementary Tables 2.3 and 2.4).

We predicted that plasticity of critical thermal limits would be greater in juveniles and that holometabolous insects would be less plastic than hemimetabolous insects. As anticipated, insects acclimated in adulthood were less plastic than insects acclimated during early life (Fig. 2.2; CT_{max} ARR $_{adult\text{-}early\ life}$ = -0.036; 95% CI = -0.066, -0.007; CT_{min} ARR $_{adult\text{-}early\ life}$ = -0.067; 95% CI = -0.131, -0.003), although only 1.3% and 1.6% of the variation was explained for CT_{max} and CT_{min} respectively. Additionally, upper thermal limits of holometabolous insects were less plastic than those of hemimetabolous insects, explaining 5.8% of variation (Fig. 2.3; CT_{max} ARR $_{holo\text{-}hemi}$ = -0.090; 95% CI = -0.175, -0.006). However, when Orthoptera (k = 1) was excluded from the analysis, this result was no longer significant (CT_{max} ARR $_{holo\text{-}hemi}$ = -0.064; 95% CI = -0.131, 0.004). For lower thermal limits, there was no significant difference in plasticity between the two development types, although the trend was also for lower plasticity in holometabolous insects (Fig. 2.3; CT_{min} ARR $_{holo\text{-}hemi}$ = -0.036; 95% CI = -0.132, 0.059). To investigate differences between development types before metamorphosis, juvenile insects were analysed as a subset. Again, there was no significant difference in critical thermal limit plasticity between hemi- and holometabolous insects at the juvenile stage, although, again, there was a trend for lower plasticity in holometabolous insects (CT_{max} ARR $_{holo\text{-}hemi}$ = -0.057; 95% CI = -0.137, 0.024; CT_{min} ARR $_{holo\text{-}hemi}$ = -0.063; 95% CI = -0.183, 0.057).

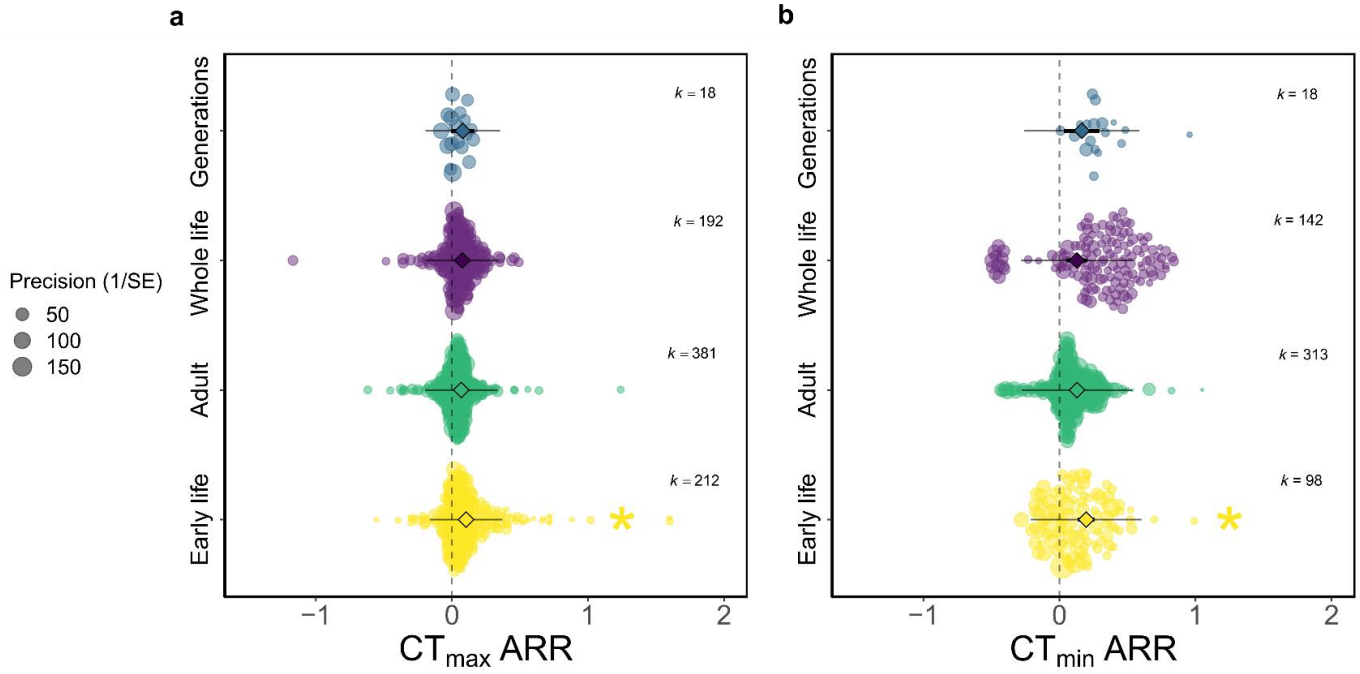

Figure 2.2 The effect of acclimation life stage on acclimation response ratio (ARR) for critical thermal limits, (a) CT_{max} (k = 803) and (b) CT_{min} (k = 571). Early life is defined as the egg or nymph stage for hemimetabolous (non-metamorphosising) insects and the egg, larval or pupal stage for holometabolous (metamorphosising) insects. 95% confidence intervals (95% CIs) are depicted in heavy black lines (often hidden by the mean data point, depicted by a diamond symbol), prediction intervals in thin black lines. The precision of the study (1/SE (Standard Error)) is proportional to the size of each data point. k = number of effect sizes per group. Asterisk indicates that 95% CIs do not overlap, comparisons are made with the adult group.

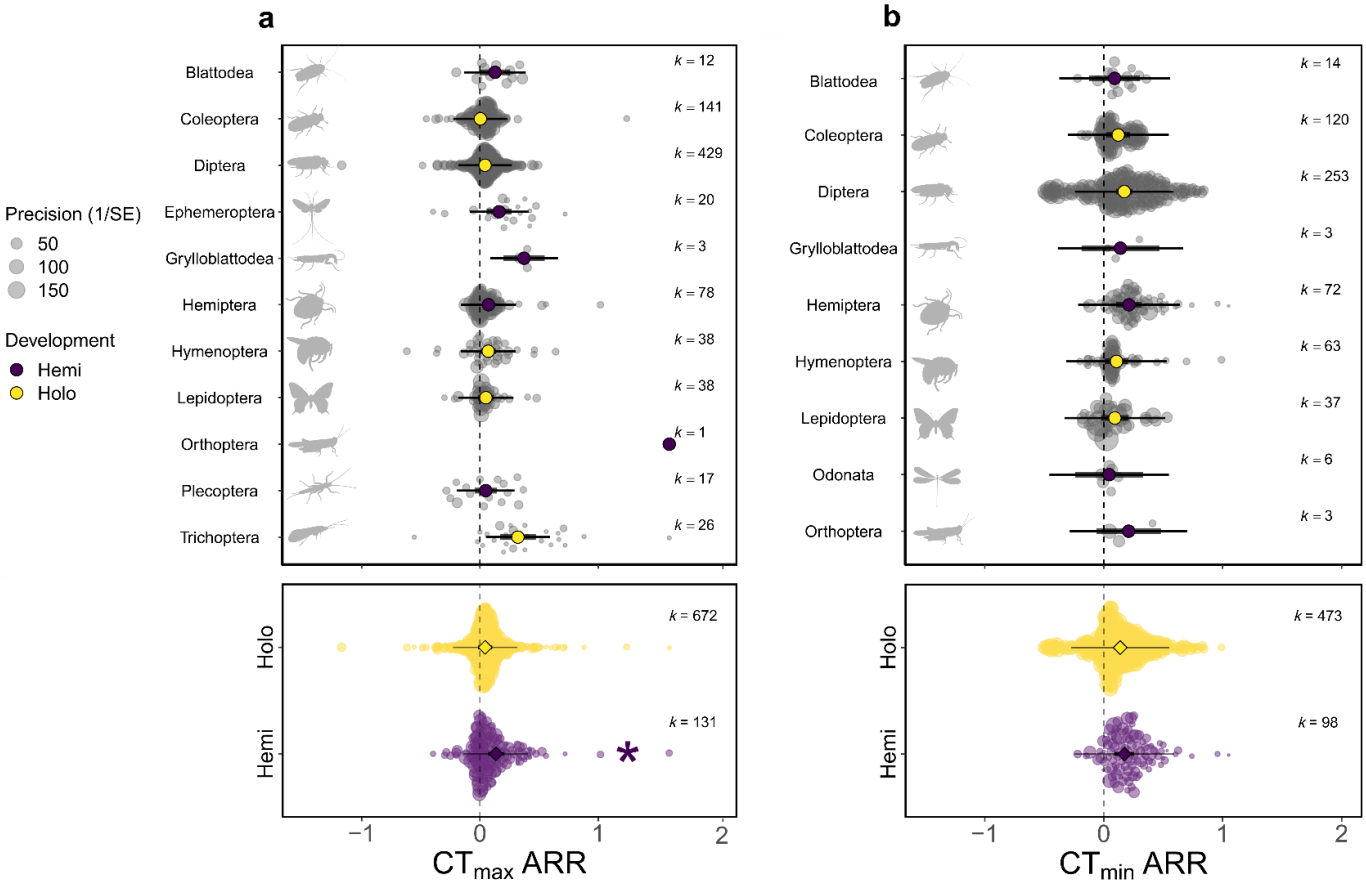

Figure 2.3 The effect of development type (holometabolous, metamorphosing insects; or hemimetabolous, non-metamorphosing insects) on acclimation response ratio (ARR) for (a) CT_{max} (k = 803) and (b) CT_{min} (k = 571). Mean ARR are arranged by Insect Order (alphabetical), coloured by development type. 95% confidence intervals (95% CIs) are depicted in heavy black lines (sometimes hidden by the mean data point, depicted by a diamond or circle symbol), prediction intervals in thin black lines. The precision of the study (1/SE (Standard Error)) is proportional to the size of each data point. k = number of effect sizes per group. Asterisk indicates that 95% CIs do not overlap, comparisons are made between the two development types. Icon credit: phylopics.

Plasticity in critical thermal limits varied depending on the assay endpoint employed. If CT_{max} was defined as when the insect lost its righting response, the plasticity was significantly greater than all other endpoints, excluding death (Fig. 2.4; for full comparisons see Supplementary Table 2.3). For CT_{min}, when the response was measured as death, critical thermal limits were less plastic than when the endpoint was measured as loss of clinging, righting, and motor control. When the endpoint was measured as loss of natural position, ARRs were lower than all other endpoints, excluding death and loss of activity (Fig. 2.4; for full comparisons see Supplementary Table 2.4). We expected longer times under stressful conditions to result in smaller ARRs, however there was no relationship with acclimation duration (CT_{max} βARR = <-0.001; 95% CI = <-0.001, <0.001; CT_{min} βARR = <0.001; 95% CI =<-0.001, <0.001), or assay ramp rate (CT_{max} βARR = 0.020; 95% CI = -0.067, 0.106; CT_{min} βARR = 0.017; 95% CI = -0.091, 0.125). We predicted that field-caught insects would be more plastic than laboratory-reared insects. However, there was no difference for upper thermal limits (CT_{max} $ARR_{lab\text{-}field}$ = 0.021; 95% CI = -0.044, 0.086) and opposing evidence for lower thermal limits, where laboratory insects were more plastic than field-caught insects and 1.2% of variation was explained (CT_{min} $ARR_{lab\text{-}field}$ = 0.052; 95% CI = 0.006, 0.098).

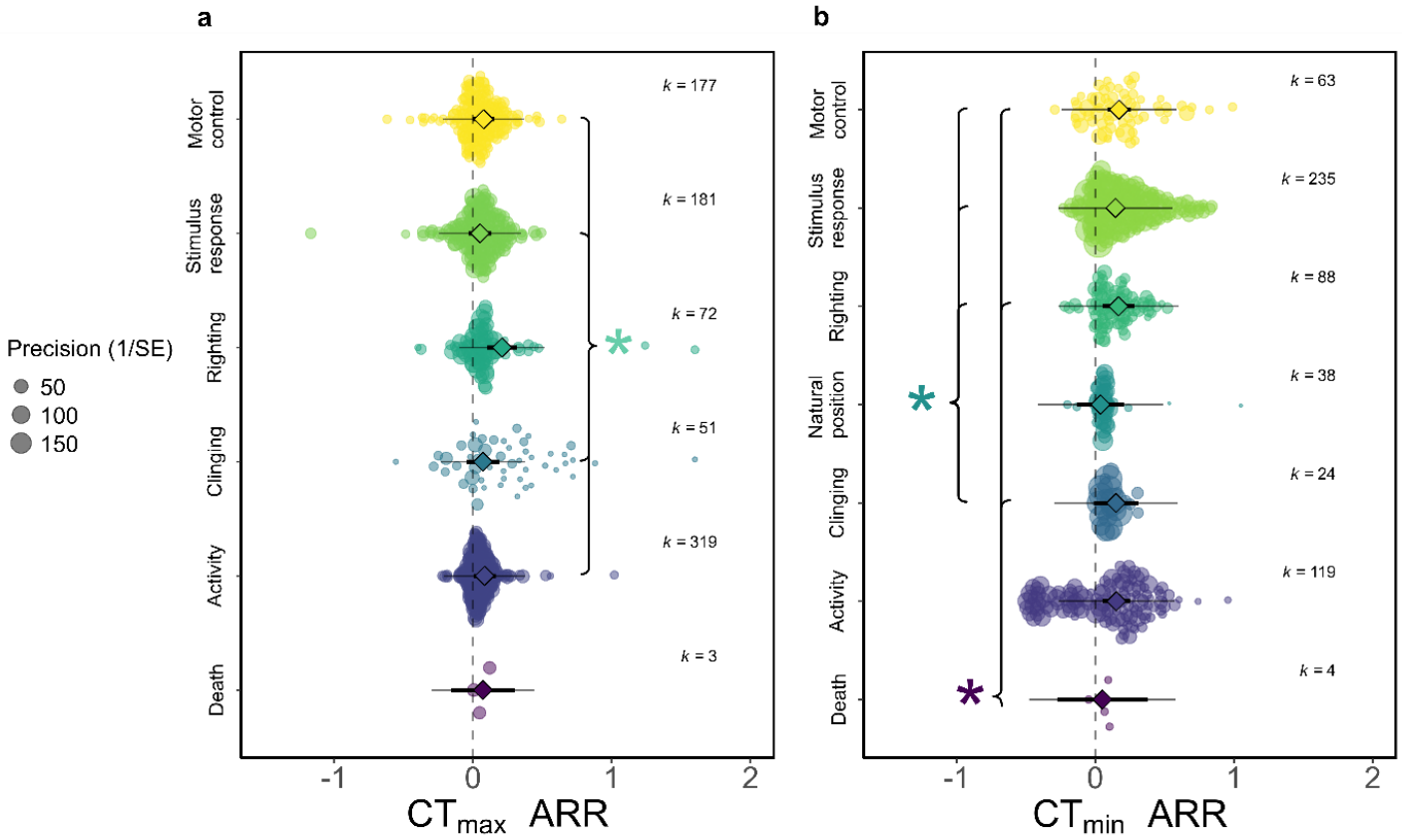

Figure 2.4 Difference in acclimation response ratio (ARR) between assay endpoint definitions of critical thermal limits, (a) CT_{max} (k = 803) and (b) CT_{min} (k = 571). The endpoint of the assay is the behaviour at which the critical thermal limit is taken. Excluding 'Death', variables can be read as 'Loss of…'. 95% confidence intervals (95% CIs) are depicted in heavy black lines (sometimes hidden by the mean data point, depicted by a diamond symbol), prediction intervals in thin black lines. The precision of the study (1/SE (Standard Error)) is proportional to the size of each data point. k = number of effect sizes per group. Asterisk indicates that 95% CIs do not overlap, brackets on the right show that the reference group is larger than marked groups, on the left, smaller.

Multivariate models were used to find the best fit models ranked by AICc. For upper thermal limits, the best model included development type as the only moderator, finding holometabolous insects were less plastic than hemimetabolous insects (Supplementary Table 2.5). The second-best model also indicated that holometabolous insects were less plastic, and found insects acclimated in early life had greater ARRs than those acclimated during adulthood, consistent with univariate models (Supplementary Table 2.5). These models explained 5.9% and 6.4% of variation respectively. The model averaging approach using conditional averages showed that the most important moderators ranked by AICc were development type, life stage at acclimation, source, and ramp rate (Supplementary Table 2.7; see Supplementary Table 2.8 for full averages). Development type and life stage at acclimation were significant moderators. Differences between CT_{max} endpoint methodologies were not robust to model averaging, indicating that differences are likely driven by other moderators. For lower thermal limits, the best model indicated that laboratory insects are more plastic than field-caught insects, consistent with the univariate model (Supplementary Table 2.6). The most important moderators using conditional averages were source, life stage at acclimation, sex, habitat, development type and ramp rate (Supplementary Table 2.9; see Supplementary Table 2.10 for full averages). Model averaging indicated that juvenile insects had greater plasticity in lower thermal limits than adult insects. This approach also indicated that insects from the unknown sex group were less plastic than female insects.

2.5.4 **Heterogeneity, publication bias and sensitivity analysis**

Heterogeneity was very high (CT_{max} I^2 = 97%; CT_{min} I^2 = 99%, for intercept models), as common in ecology and evolutionary meta-analyses (Koricheva et al., 2013). Random factors explained heterogeneity; for upper thermal limit ARR differences between studies explained 15.1%, phylogeny explained 15.3%, non-phylogenetic differences between species explained 17.7%, and effect size ID explained 49.1%. For CT_{min} ARR, phylogenetic and non-phylogenetic signals were far weaker, both explaining <0.1% of the variation. Otherwise, study ID and effect size ID explained 36.1% and 63.0% of heterogeneity respectively.

The leave-one-out sensitivity analysis showed that no species, family or study had a disproportionate impact on results (Supplementary Tables 2.11 and 2.12). Sensitivity analysis excluding Drosophilidae and studies with fluctuating temperatures during acclimation also showed no disproportionate impact of these studies (Supplementary Tables 2.13 and 2.14).

Funnel plots for plasticity in CT_{max} and CT_{min} are shown in Figure 2.5. Egger's regression test revealed significant publication bias for CT_{max} ARR intercept model (Supplementary Fig. 2.3; CT_{max} βARR = 0.288; 95% CI = 0.028, 0.548) and best model (CT_{max} βARR = 0.288; 95% CI = 0.028, 0.548). The mean ARR

corrected for publication bias was 0.0907°C (rather than 0.0913 without the correction) (Supplementary Table 2.19; 95% CI = 0.030, 0.152). There was significant publication bias for CT_{min} ARR, with the model predicting 0.144°C per degree change, rather than 0.147°C (Supplementary Table 2.22; 95% CI = 0.102, 0.185). This result was found for both the intercept model (Supplementary Fig. 2.3; CT_{min} βARR = 0.621; 95% CI = 0.068, 1.174), and the best model (CT_{min} βARR = 0.695; 95% CI = 0.140, 1.249). For full model outputs see Supplementary Tables 2.18-2.23. There was no evidence of a relationship between year and ARR for either CT_{max} (Supplementary Table 2.24; CT_{max} βARR = -0.001; 95% CI = -0.005, 0.003) or CT_{min} (Supplementary Table 2.25; CT_{min} βARR = -0.002; 95% CI = -0.009, 0.005).

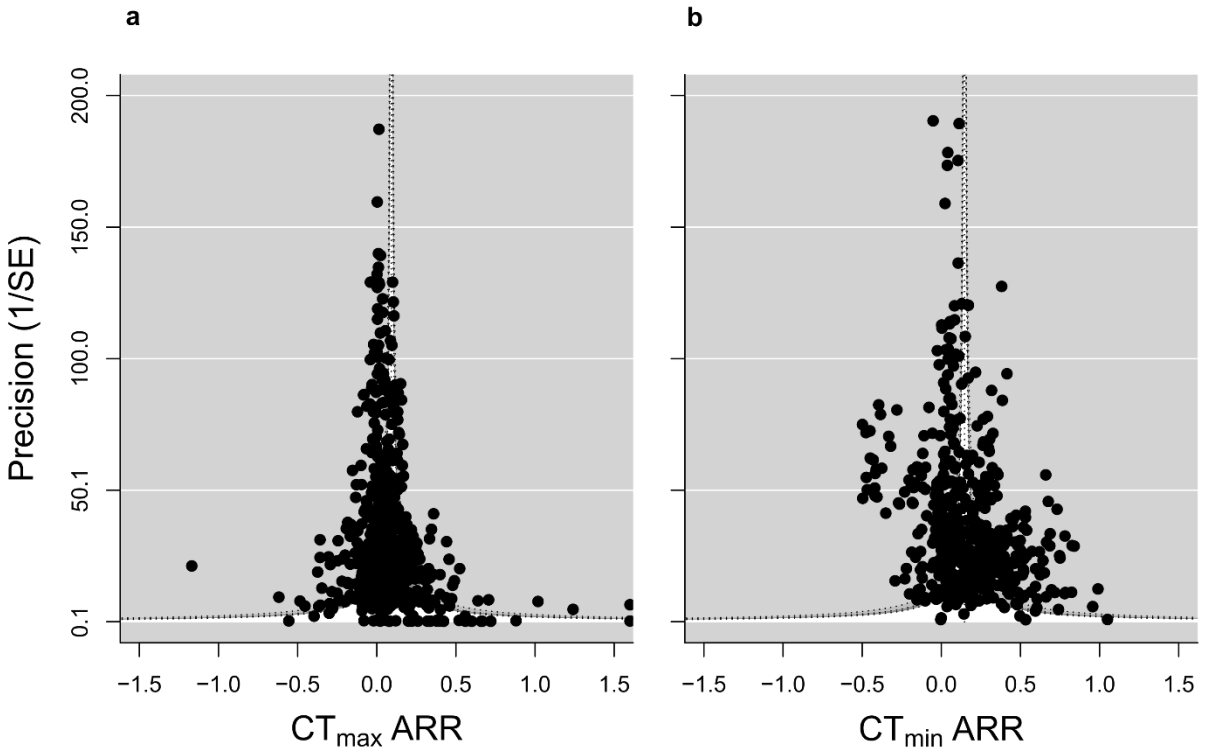

Figure 2.5 Publication bias in acclimation response ratios (ARR) of critical thermal limits, (a) CT_{max} (k = 803) and (b) CT_{min} (k = 571). More precise studies (those with higher 1/SE (Standard Error) are located at the top of the plot, and less precise studies are located at the bottom. Egger's regression test (two-sided) showed slight significant positive publication bias for CT_{max} (CT_{max} βARR = 0.288; 95% CI = 0.028, 0.548; p = 0.030) and CT_{min} (CT_{min} βARR = 0.621; 95% CI = 0.068, 1.174; p = 0.028) intercept models, indicating that data points are missing from the left-hand side of both plots. Shadings indicate ($p < 0.05$, $p < 0.01$, $p < 0.001$).

2.6 Discussion

Upper critical thermal limits of insects had weak but pervasive plasticity, with a mean shift of 0.092°C in response to a 1°C adjustment in acclimation temperature. Lower critical thermal limits were around 60% more plastic, responding with a 0.147°C change per 1°C acclimation temperature. Evidence for small but significant publication bias suggests that responses are likely to be a fraction more modest than reported here and in the wider literature (where such bias has not been previously investigated). These findings are in agreement with broader comparisons across ectotherms, showing upper thermal limit plasticity is generally weak (Gunderson et al., 2017; Gunderson and Stillman, 2015; Morley et al., 2019; Rohr et al., 2018). Indeed, in Gunderson and Stillman's 2015 analysis, insects had the weakest responses of all ectothermic groups and Morley *et al.*'s 2019 analysis illustrated a similar pattern (when excluding high latitude species). Under our current climate, some evidence suggests that the majority of ectothermic species are close to or without a thermal safety margin, as operative body temperatures in exposed environments often match or exceed physiological limits (albeit requiring a number of simplifying assumptions) (Sunday et al., 2014). With once-in-a-decade heatwave events expected to be at least four times more likely, most ectotherms – and given the current evidence, especially insects – will need to rely on other compensatory mechanisms (IPCC, 2021; Sunday et al., 2014). For example, insects can behaviourally thermoregulate using microclimates e.g. leaf shade in a forest canopy can reduce maximum air temperature by 5°C (Pincebourde and Woods, 2020; Suggitt et al., 2011). Poleward migration has also been documented in numerous insect species, and is favoured by their short generation times, fast reproduction and high mobility (Parmesan, 2006). Generally weak plasticity of insect critical thermal limits may be of some added benefit when working in combination with these mechanisms, particularly in species with range shifts into more variable poleward regions (Ma et al., 2021; Parmesan, 2006).

While most ecological and morphological moderators did not significantly explain variation in critical thermal limit plasticity, this study indicates a potentially important role of ontogeny. Insects acclimated in early life had greater plasticity than those acclimated in adulthood, providing support for the hypothesis that juvenile stages are more plastic than adults. This indicates the presence of a sensitive window, where acclimation elicits a greater response in early life stages. Variation in plasticity over an insect's lifetime reflects changes in the costs and benefits of plasticity. For example, plasticity early in ontogeny may have evolved due to juvenile insects being less able to behaviourally thermoregulate since they have generally lower motility than adults (Bowler and Terblanche, 2008). This may mean that juvenile insects are exposed to greater variation in temperature, thereby promoting selection for greater plasticity (Donelson et al., 2018; Kristensen et al., 2008). There also may be differences in the frequency and reliability of cues earlier in life, particularly apparent in insect

life cycles where juvenile and adult stages utilise entirely different niches (English and Barreaux, 2020). More generally, phenotypic adjustments earlier in life are likely to have a greater impact on fitness, because fewer individuals survive or reproduce later on in life, thus selection decreases with age (Fawcett and Frankenhuis, 2015). Overall, these findings suggest that plasticity in juvenile insects may be critical to later thermal tolerances and suggest that developmental effects should be further investigated for their relevance to insect climate change responses.

We also found some evidence that hemimetabolous insects have greater plasticity of upper thermal limits than holometabolous insects. Any developmental plasticity in holometabolous insects may be lost through metamorphosis, due to dramatic cell, tissue and whole-animal reorganisation, contributing to lower plasticity (Bowler and Terblanche, 2008). This may serve an adaptive function, as cues are less comparable across life stages in holometabolous insects, where juveniles are immobile in the pupal stage and larvae often have different ecology to adults (English and Barreaux, 2020). However, evidence for differences between developmental types was not robust to the exclusion of Orthoptera (k = 1), despite all four models (including subset data, see Supplementary Tables 2.3 and 2.4) returning results in the same direction. There is a clear need for more studies on Orthopterans, and hemimetabolous insects in general, to determine whether differences between development types are robust or an artifact of low sample sizes.

There was variation in plasticity of critical thermal limits between some types of methodology. The definition of the endpoint used in any given study led to significant differences in plasticity for both upper and lower critical thermal limits. However, differences were not found in model averages so are likely driven by other sources of variation. Additionally, contrary to expectations, there was evidence for greater plasticity of lower thermal limits in laboratory-reared insects than in field-caught insects. This could be due to more factors influencing and interacting with tolerance in field-collected individuals, while in the laboratory, more factors are controlled (e.g. diet, age, thermal history all affect estimates) and thus the signal is clearer (more distinct from the 'noise' or variation). Notably, no relationship was found between ARR and acclimation duration or ramp rate, perhaps owing to complex interactions which were not investigated in this analysis, such as those between ramp rate and acclimation, nutrition and body condition, and interval time between the acclimation treatment and endpoint (Allen *et al.*, 2016; Oyen and Dillon, 2018). The preliminary analyses investigating the temperature-size rule (Supplementary Table 2.3 and 2.4) found no difference between groups where acclimation treatment and critical thermal limit assay were within a life stage, or over different life stages. However, it would be interesting to investigate this further where study-specific mass/size data are available. These findings indicate the need to consider diverse aspects of methodology and population history in future comparative analyses.

This study adds to evidence that upper thermal limits are less plastic and more evolutionarily constrained than lower thermal limits (Bennett et al., 2021). CT_{max} was ~60% less plastic than CT_{min} which may reflect the distinct physiological and biochemical responses at the two extremes of temperature. CT_{max} is often lethal, occurring just before or at the same temperature as heat death. There are relatively few studies on the mechanisms of heat death, but the most likely causes are protein denaturation and the breakdown of membrane integrity leading to neuronal silencing. Spreading depolarisation in the central nervous system is caused by a failure to maintain ion gradients which leads to depolarisation of neurones. A surge of potassium ions prevents neural activity and causes the central nervous system to shut down (Bowler, 2018; Jørgensen et al., 2020). In contrast, CT_{min} usually results in a non-lethal chill coma, whereafter an insect may recover fully. The mechanisms of chill coma are also poorly understood. As temperature decreases, an insect will slow down in activity, become immobile and, finally, enter chill coma. Chill coma is a state of neuromuscular paralysis which may be caused by disruption to electrical or chemical signalling which connects the central nervous system to the muscles (Overgaard and Macmillan, 2017). This is likely driven by the breakdown of ionic homeostasis and depolarised membrane potential due to the effect of low temperature on ATPases, ion channels and the lipid membrane (MacMillan and Sinclair, 2011; Overgaard et al., 2021). However, whether chill coma is caused by failure of neurones, synapses or muscles is unclear (Overgaard and Macmillan, 2017).

A phylogenetic signal in CT_{max} was detected in the models, which was not observed for CT_{min}. This may reflect evolutionary constraints for CT_{max}, such as high fitness costs or substantial genetic changes required to modify upper thermal limits, causing related species to share similar thermal responses (Hoffmann et al., 2013). If upper thermal limits cannot evolve easily due to these constraints, an organism's current thermal limits will dictate the kind of environments in which it can survive. These differences create a 'concrete ceiling' for CT_{max}, where physiological barriers prevent extensive evolution and perhaps also restrict plasticity (Sandblom et al., 2016). As extreme heatwaves are becoming more frequent and intense, and extreme cold events less so, concrete ceilings will likely create strong barriers to adaptation for insect species. Understanding which species have hidden or multivariate adaptive capacity would then be essential to forecasting species responses to climate change.

Here, I focused on critical thermal limits because they are considered an important predictor for climate change and are well-studied. However, there is some evidence that critical thermal limits do not correlate well with species abundance or distribution, and therefore might not be the best predictors for assessing climate change impacts (but see (Sunday et al., 2014)) (Addo-Bediako et al., 2000; Maclean et al., 2019). In contrast, thermal fertility limits, the temperature at which an animal

becomes infertile, can be far more sensitive to temperature, with evidence in ectotherms suggesting that these occur at less extreme temperatures (see Chapter 4; Sales *et al.*, 2018; Walsh, Parratt, Atkinson, *et al.*, 2019). This study also highlights the need for greater taxonomic diversity in critical thermal limit measures, representative of broad biogeographic regions and development types, as nearly one third of the effect sizes in this study were from Drosophilidae species (k = 584 out of 1,374 total; note that a comprehensive exploration of effect sizes within Drosophilidae species is presented in Supplementary Tables 2.15-2.17), and hemimetabolous insects were not well-represented (k = 229).

Here, insect thermal tolerance plasticity was positive but weak, supporting previous findings for ectotherms more broadly. Detection of a phylogenetic signal for upper, but not lower, thermal limits indicates that evolutionary adaptation may also be constrained for CT_{max}. Ontogenetic variation in critical thermal limit plasticity suggests that a developmental window may be important in shaping insects' responses to changes in temperature and these effects should be incorporated in climate vulnerability assessments. Overall, most insect species will need to rely extensively on distributional changes and behavioural regulation if they are to buffer the effects of climate change.

3. Chapter 3 - How plastic are upper thermal limits? A comparative study in tsetse (*Glossina* spp.) and wider Diptera

3.1 Statement

This chapter was published in Journal of Thermal Biology on 30/10/23. Authors are Hester Weaving, John S. Terblanche, and Sinead English. I, Hester Weaving completed the analysis, wrote the code, and led writing of the manuscript and revision of drafts. SE and JT jointly supervised the work and contributed to the development of drafts. All authors conceptualised the project. Find at: Weaving, H., Terblanche, J.S., and English, S. (2023) How plastic are upper thermal limits? A comparative study in tsetse (family: Glossinidae) and wider Diptera. *Journal of Thermal Biology*, **118**, 103745. 10.1016/j.jtherbio.2023.103745

3.2 Abstract

Critical thermal maximum (CT_{max}) describes the upper thermal tolerance of an animal where biological functions start to fail. A period of acclimation can enhance CT_{max} through plasticity, potentially buffering animals from extreme temperatures caused by climate change. Basal and acclimated CT_{max} vary within and between species and may be explained by traits related to thermal physiology, such as body size and sex. Differences in CT_{max} have not been established among tsetse flies (*Glossina* spp.), vectors of animal and human African trypanosomiasis. Here, basal CT_{max} and its plasticity were investigated in five tsetse species (*Glossina* spp.) following adult acclimation at constant 25 or 30°C for five days. Findings are then set in context using a subset of 33 species of Diptera from the meta-analysis conducted in Chapter 2. Of the five tsetse species considered, *Glossina palpalis gambiensis* and *Glossina brevipalpis* showed plasticity of CT_{max}, with an increase of 0.12°C and 0.10°C per 1°C acclimation respectively. Within some species, higher basal CT_{max} values were associated with larger body size and being female, while variation in plasticity (i.e. response to the acclimation temperature) could not be explained by sex or size. The broader meta-analysis across Diptera revealed overall plasticity of CT_{max} of 0.06°C per 1°C acclimation, versus a similar 0.05°C mean increase in tsetse. In contrast, there was greater CT_{max} plasticity in males compared to females in Diptera. This study highlights that CT_{max} and its plasticity varies even among closely related species. Broader patterns across groups are not always reflected at a finer resolution, I thus emphasise the need for detailed experimental studies across a wide range of insect species to capture their capacity to cope with rapidly warming temperatures.

3.3 Introduction

Thermal tolerance can be defined by upper and lower critical thermal limits, CT_{max} and CT_{min} (Angilletta, 2009). CT_{max} is one of the most important predictors of species' distributions (Kellermann et al., 2012; Overgaard et al., 2014), so can be used as an indicator of vulnerability to climate change. A period of acclimation can enhance CT_{max} through plastic responses (Allen et al., 2012; Belliard et al., 2019), which could act as a mechanism to buffer animals during periods of extreme heat and increased temperature variability, two phenomenon becoming more prevalent due to climate change (Christidis et al., 2015; Meehl and Tebaldi, 2004; Perkins et al., 2012). CT_{max} can be assessed by a dynamic assay where temperature is ramped until a performance endpoint – for example, no response to prodding, the onset of muscle spasms or the inability to cling to a surface (Terblanche and Chown, 2006). Thermal plasticity can be estimated as the difference between the CT_{max} of a population held under benign (optimal) conditions, compared to a population which was acclimated in an elevated, but nonlethal, temperature before the assay. Thermal reaction norms are the relationship between these two measures, with plasticity equivalent to the slope (Fig. 1.2B). Acclimation response ratio (ARR) is the change in critical thermal limit per degree change in acclimation temperature, which describes this slope (Angilletta, 2009).

Comparative analyses across ectothermic species have shown that thermal tolerance varies predictably across seasonal (Clusella-Trullas and Chown, 2014; Oliveira et al., 2021), latitudinal (Addo-Bediako et al., 2000; Clusella-Trullas and Chown, 2014), and elevational clines (García-Robledo et al., 2016). There is less consistent evidence about the relationship between body size and thermal tolerance. Studies suggest that a wide variety of animals are decreasing in size in response to rising temperatures (Gardner et al., 2011; Sheridan and Bickford, 2011), and past extinction events due to warming have selected for smaller bodied marine and terrestrial animals (Sheridan and Bickford, 2011; Smith et al., 2009). Paradoxically, larger animals may have higher basal CT_{max} due to their slower metabolic rate relative to body size and the ability to store more resources (Brown et al., 2004; Kingsolver and Huey, 2008). Additionally, surface area to volume ratio scales negatively with size, so large animals may suffer less from water loss (Addo-Bediako et al., 2000; Bergmann, 1847; Steven L Chown et al., 2011). The association between large body size and high CT_{max} has been found both within and between species in ants (Baudier and O'Donnell, 2018), frogs (von May et al., 2019), and fish (Zhang and Kieffer, 2014). However, lower values for CT_{max} have been found with increasing body size for fish (Recsetar et al., 2012), crustaceans (Verberk et al., 2018), and other marine animals (Peck et al., 2009). This relationship may be exclusive to aquatic animals because a small surface area-to-volume ratio limits large bodied animals to extract oxygen from water at temperature extremes (Chapelle and Peck, 1999; Pörtner, 2010). An analysis of over 328 species (including marine and

terrestrial ectotherms) found a more complex relationship where large-bodied animals are less tolerant than small animals to acute heat, but were more heat tolerant during long exposure times (Peralta-Maraver and Rezende, 2021).

Broad-scale comparative analyses of upper and low thermal tolerance plasticity have found mixed support for the hypothesis that selection promotes plasticity in variable thermal environments, such as with increasing latitude (Donelson et al., 2018). These studies often show opposing trends, or fail to explain much variation in ARR, as found in Chapter 2 (Gunderson and Stillman, 2015; Seebacher et al., 2015; Weaving et al., 2022). Once more, few studies have investigated the relationship between thermal tolerance plasticity and body size (but see Rohr et al., 2018), despite its potential relationship to thermal experience (Pincebourde et al., 2021). Larger animals have greater thermal inertia so change body temperature more slowly and therefore may be slower to acclimate (Rohr et al., 2018). Additionally, lifespan tends to increase with body size, and longer lifespans may be subject to a greater thermal range e.g., over annual rather than seasonal scales (Rohr et al., 2018). A meta-analysis examining over 500 species of ectotherms by Rohr et al. (2018) found that ectothermic animals with larger body sizes had greater plastic responses at longer acclimation times, and at slower assay ramping rates. However, I found no relationship between plasticity of insects and body size in Chapter 2.

Upper thermal tolerance and its plasticity can also vary according to an individual's sex, due to sexual dimorphism and behavioural differences. Males tend to express more risk-taking behaviours and inhabit larger ranges, which could expose them to greater temperature variability, promoting greater plasticity (Stillwell et al., 2010; Tarka et al., 2018). However, female ectotherms tend to be larger, perhaps acting in opposition to this trend due to, for example, greater resources and more efficient resource use, as outlined above (Bulté and Blouin-Demers, 2010). In a meta-analysis of 44 ectothermic species, Pottier *et al.* (2021) found that females were more plastic than males, but only in field-caught individuals. However, in Chapter 2, my meta-analysis specific to insects (102 species), found no sex differences in thermal tolerance plasticity.

Forecasting responses to climate change is particularly important for vectors of disease, as changes to distribution may result in altered disease transmission (Hay et al., 2004; Rogers and Randolph, 1993; Simarro et al., 2012). Many disease vectors, such as mosquitoes, have short generation times and high population growth rates which promote evolutionary adaptation (Burger and Lynch, 1995; Couper et al., 2021). In contrast, tsetse flies (*Glossina* spp.), vectors of trypanosome parasites, are slow to reproduce and population persistence is highly sensitive to temperature (Buxton, 1955; Hargrove, 2004). Therefore, in a warming world, tsetse may need to rely on within-lifetime plastic thermal

tolerance, rather than across-generation changes. However, thermal tolerance and its plasticity have only been quantified in *Glossina pallidipes* by Terblanche and Chown (2006), who found limited or non-existent adult and developmental plasticity in CT_{max}. It is unknown if patterns observed in *G. pallidipes* reflect the entire genus indicating constraints to CT_{max} plasticity or if there is systematic variation across the genera. There are 31 species and subspecies of *Glossina*, which are split into 3 subgenera: Morsitans, Palpalis and Fusca, with differing habitat preferences (Fig. 1.3; Leak, 1998). The various tsetse species also cover a range of body sizes, for example the body mass of *G. brevipalpis*, one of the largest species, is five times greater than the smallest species, such as *Glossina austeni* (Leak, 1998), making them an interesting group in which to explore thermal tolerance variation (Fig. 3.1).

Here, I measure the critical thermal maximum (CT_{max}) and its plasticity across five tsetse species (*G. brevipalpis, G. pallidipes*, *Glossina fuscipes fuscipes*, *Glossina morsitans morsitans, Glossina palpalis gambiensis*). The largest species is *G. brevipalpis*, which is three times greater in body mass than the smallest species measured, *G. p. gambiensis* (Fig. 3.1). These species cover all three subgenera, originating from a range of locations (Supplementary Table 3.1). I investigate within and between species differences in basal and acclimated CT_{max} and ask how these relate to body size and sex. I expect larger body sizes to give rise to higher basal CT_{max}, and greater plasticity to be associated with large body size. I expect no differences in plasticity between sexes due to competing selection pressures. I then set my results in the context of the meta-analysis conducted in Chapter 2, using a subset of 33 species of Diptera to confirm if patterns across the five species in this unique family reflect more broad findings across the order.

3.4 Methods

3.4.1 Pupal development and adult emergence

Approximately 300 early-stage pupae (within around one week of deposition) of five tsetse species (*G. brevipalpis, G. m. morsitans, G. pallidipes*, *G. f. fuscipes*, *G. p. gambiensis*; Fig. 3.1) were ordered from the International Atomic Energy Agency (IAEA), Vienna, between October 2022 and February 2023. IAEA colony conditions are 24-25°C and 75-80% Relative Humidity (RH) for adult tsetse and 23-24°C and 75 RH for pupae (Opiyo et al., 2006). Laboratory-reared individuals were used to eliminate the possibility that differences in plasticity come from varying thermal history e.g. developmental plasticity from different rearing environments (van Heerwaarden and Kellermann, 2020). Once delivered, pupae were kept at 25°C and 80% RH in a climate-controlled room, monitored by an iButton at a sampling frequency of 30 minutes. Adults and pupae were kept in the same climate-controlled room, so the same conditions were used for both stages. The light:dark cycle was 12:12, 9am – 9pm using dimmed lighting. Pupae were housed in emergence cages (approx. 150 pupae per cage) and covered with sterilised sand.

Upon emergence, adults were separated from pupae and transferred to a chest fridge to be sorted into single sex cages (maximum 25 flies per cage). The fridge was maintained between 2 and 6°C using a RS Pro Dual Datalogger with T type thermocouples, and flies were held at this temperature for no longer than five minutes. Cages were made from modified plastic piping (16 cm diameter x 8 cm depth) with mesh fabric (2.5 mm holes) on the top and bottom, with a circular opening bunged with a cork. Females emerge before males so approximately eight cages of females were collected on days one and two, and eight cages of males were collected on days three and four, although actual numbers varied per species. Adult flies were kept in the climate-controlled room on racks at the above-mentioned conditions.

3.4.2 Feeding

Defibrinated horse blood (TCS Biosciences, Buckingham, UK) was ordered in 500 ml quantities and decanted mechanically (Rota-filler 3000) using a 50 ml serological pipette (Sarstedt) into 25 ml universals (Sterilin) under a laminar flow hood. Blood was stored in the fridge at ~4°C for no more than 3 weeks. Flies were fed the day after being sorted and then three times weekly on Monday, Wednesday, and Friday at approximately 9:00 am. 200 µl of ATP was added to each 25 ml vial of blood using a 100 – 1000 µl pipette (Eppendorf) as a feeding stimulant. ATP was made by diluting 5.51g of adenosine 5’-triphosphate disodium per 100 ml of Reverse Osmosis water and mixed using a Corning Stirrer PC-353 with magnetic flea.

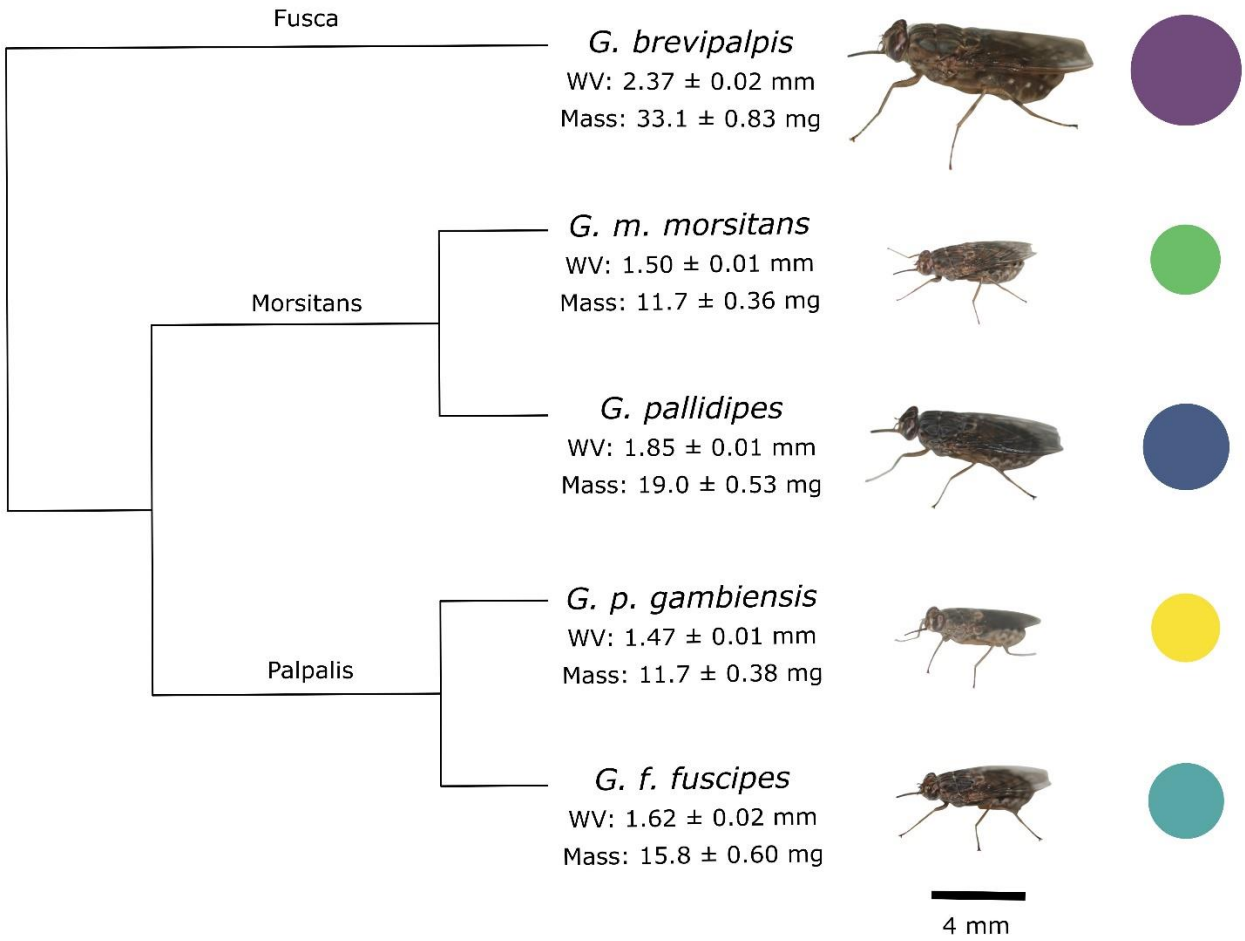

Figure 3.1 Photographs of each tsetse species (*Glossina* spp.) used in the experiment, to scale by wing vein (WV; mm). Dry mass is given in mg. Pictured are adult females of each species. These species represent the full range of subgenera: Fusca (*G. brevipalpis*), Morsitans (*G. m. morsitans, G. pallidipes*), and Palpalis (*G. f. fuscipes, G. p. gambiensis*). Phylogeny was constructed using Open Tree of Life and R packages 'rotl' and 'ape' in R and shows the five species measured in this experiment of the total 31 species and subspecies. Circles represent relative body size by wing vein and colours used in Figures throughout Chapter 3.

Blood was poured on to metal trays (25 ml per 47cm x 40cm tray), covered with a silicon membrane, and heated to 36°C using heated mats (Flexible heated hoses, Birmingham, UK). A thermocouple was used to monitor temperature. After feeding, trays and membranes were rinsed with cold water, scrubbed, and sterilized at 110°C overnight in an oven (Gallenhamp, Hotbox Oven).

3.4.3 **Acclimation**

Flies emerged on day zero, were fed on day one and then were transferred to their acclimation treatments at 11:00 am. Treatments were 25°C as the control (i.e., basal) temperature and 30°C for the acclimation temperature for a period of five days. An acclimation treatment of 30°C was used because it is near to the constant upper temperature at which tsetse can survive, being around 32°C for *G. p. gambiensis* and *G. m. morsitans* (Are and Hargrove, 2020; Pagabeleguem et al., 2016), but depends on species). Temperatures within and above this range are regularly experienced in the field, for example, at Rekomitjie Research Station, Zimbabwe, maximum air temperatures can reach 44°C (see temperature data in Supplementary data for Lord et al. (2018)). Half of the flies were transferred to 30°C, 80% RH in an incubator (Snijder Micro Clima-Series) with 12:12 light:dark conditions, and half remained at 25°C in the climate-controlled room. Flies housed in the incubator were kept in a large box drilled with holes for ventilation. The box was covered with blue roll to create similar dim lighting conditions as the climate-controlled room. Flies were only removed from the acclimation treatment to feed, three times weekly, as described above. Feeding occurred within the climate-controlled room at 25°C. For all species, the actual mean temperature (°C) and relative humidity (RH %) experienced in the 25°C treatment was 24.9 ± 0.2 and 78.2 ± 0.8, and in the 30°C acclimation treatment was 30.6 ± 0.7 and 79.9 ± 4.3, respectively. Mean temperature and humidity data for individual species are given in Supplementary Table 3.1.

3.4.4 **CT_{max} assay**

CT_{max} assays were undertaken using two programmable Grant LTC4 refrigerated circulating liquid baths with TX150 heating circulators, attached to a set of Perspex organ pipes with rubber tubing (Figure 3.2), and filled with water. The temperature program was set using Grant Labwise software (Version 2.1.2, Grant Instruments, Cambridge, UK) and consisted of 10 minutes acclimation at 25°C followed by a ramping treatment at a rate of +0.1°C/min. Ramping rates within this range have been used for tsetse in previous studies and this rate can be considered ecologically relevant from microsite temperature profiles in the field (Terblanche et al., 2007). Two thermocouples (Type T) monitored temperature in one empty tube per water bath during the experiment. Fly temperature was considered the same as tube temperature due to the small body size of tsetse, as previously determined (Terblanche et al., 2007). Four runs were completed for each species (n = ~80 flies per

species across four runs), half of individuals were male and half female, with an equal number from each acclimation treatment.

Figure 3.2 Organ pipe set up for CT_{max} experiments. Organ pipes are connected by tubing to Grant LTC4 circulating water baths. Temperature of the inside of the pipes was taken by T type thermocouple. Seen here, *G. pallidipes.*

Flies were fed on the last day of the acclimation treatment (day five) so that all individuals had taken a bloodmeal on the previous day. Assays began at approximately 11 am on day six, although this varied depending on run (9:30 – 13:30). This meant that flies were approximately one week old on the day of the thermal assay. Flies were knocked down using 100 % Sevoflurane inhalation anaesthetic (SevoFlo, Zoetis, Belgium). Sevoflurane was chosen as an anaesthetic as it has minimal effects on survival and reproduction in comparison to cold anaesthetic in *Drosophila* (MacMillan et al., 2017). In a separate experiment, to ensure that sevoflurane did not negatively affect tsetse, cages of sevoflurane-treated and non-treated flies were assessed for mortality after one week. We used a glm with quasibinomial family and logit link. We found no significant difference between the two groups (mean difference ± SE = - 0.15 ± 0.43, z value = -0.34, p = 0.74). Sevoflurane (350 µl) was applied to cotton wool for one cage of approx. 25 flies in an enclosed plastic container (20 x 25 x 10 cm) for 10 minutes. Ten flies were randomly selected per treatment and rapidly transferred into the organ pipes using stork bill forceps and bunged with cotton wool and a cork. Flies allocated to each treatment

were placed in the pipes alternately. Tsetse were allowed to recover from anaesthetic knock down, so that all flies were standing upright before the temperature program began. In all cases, standing occurred less than 10 minutes post anaesthetic. Occasionally (n = 2/397) dead flies were selected from cages by mistake, and these individuals were excluded from the assay.

HW took all CT_{max} measurements to avoid observer bias. Programs on the two water baths began in 20–30-minute tandem so that only 10 flies were assessed at once. CT_{max} was defined as the temperature at which the fly was knocked down or lost locomotor ability and stopped responding to a stimulus (disturbance by rocking the organ pipes). After the temperature rose to 40°C (known to be near to tsetse CT_{max} from pilot trials and previous research on *G. pallidipes* under these experimental conditions), flies were checked every 30 seconds for movement. Once all flies were knocked down, they were removed from the pipes and placed into 50 ml conical falcon tubes to assay subsequent mortality, each drilled with a hole in the lid for ventilation.

Mortality was determined by shaking the tube 24 hours after the assay. Flies were considered dead if they could not right themselves. Mortality was expected to be close to 100% as CT_{max} is usually near or the same as lethal temperature in insects (Vorhees and Bradley, 2012). Flies were frozen at -18°C for 24 hours and then dried overnight in an oven at 70°C. Dry mass was taken on a Ohaus Explorer EX124 balance (accurate to 1/1000 mg). The left wing was removed and photographed using a Leica EZ4W dissecting camera microscope at 35x magnification and LAS EZ (Version 3.4.0). ImageJ (Version 1.53) was used to take the size of the hatchet cell wing vein length, known to indicate fly size (Jackson, 1946), and this was calibrated using a graticule with 0.1 mm divisions.

3.4.5 **Statistics**

All analyses were completed in R (version 4.2.3; R Core Team, 2023). Full raw data and code can be found deposited at: https://osf.io/b3m28/. Mixed-effect linear models using the lme4 package (version 1.1-31; Bates et al., 2015) were used with experimental run as a random effect. Starting with a maximal model each term was excluded and removed if it did not significantly improve model fit (Bradburn et al., 2003). The difference between models was tested using analysis of variance (ANOVA). Where interactions were significant, data were split into separate models so that interactions could be investigated fully. These models did not contain experimental run as a random effect as there were only four runs per treatment. Post-hoc pairwise comparisons were calculated using the `lsmeans` package (Version 2.30; Lenth, 2016) and p-values were adjusted using the false discovery rate method (FDR).

Linear mixed-effects models were validated by plotting standardised residuals against fitted values to check for heteroscedasticity. Normality of residuals was validated using a Q-Q plot and Shapiro-Wilk

test. Influential data points were identified using Cook's distance. One *G. m. morsitans* outlier was removed from the data set due to being highly influential. Once this outlier was removed all model residuals were normal. Wing vein length and dry body mass were highly positively correlated ($t_{1, 393}$ = 39.8, $p < 0.001$, $R^2 = 0.80$). Wing vein was used preferentially in models as mass varies due to the quantity of the blood meal last taken. There was also a relationship between size and sex, with male flies smaller than female flies (mean difference ± SE = -0.22 ± 0.03, $t_{1, 393}$ = 40.3, $p < 0.001$, $R^2 = 0.093$), so all regressions were rerun, replacing sex with size to eliminate nonindependence issues.

3.4.6 **Basal heat tolerance**

First, variation in basal CT_{max} according to species, sex, and body size was assessed. Data from individuals kept at 25°C were considered. CT_{max} as a function of species and size (using wing vein), and the interaction between these variables was modelled. Species differences were considered using species as a fixed effect term rather than conducting a phylogenetic analysis, due to relatively low statistical power (due to measuring five of the total 31 tsetse species and subspecies for logistical reasons). The analyses were then repeated using sex rather than body size, as explained previously.

3.4.7 **Acclimation responses**

To determine within and between species differences in adult plasticity of CT_{max}, data was used from individuals across both acclimation treatments. Treatment (acclimation at 25°C or 30°C), body size, species, and interactions between these variables (up to three-way) were considered as fixed factors. A significant interaction between treatment and size, or treatment and species, would indicate size- or species-dependent plasticity in CT_{max}. Regressions represent the reaction norm for each species, with the slope of the line equivalent to the degree of plasticity. Here it is assumed that the reaction norm between 25°C and 30°C groups is linear, although I acknowledge this is commonly not the case (van Heerwaarden and Kellermann, 2020). Future studies could explore a wider range of acclimation temperatures to test this assumption.

3.4.8 **Acclimation responses within Diptera**

Acclimation response ratio (ARR) was calculated an in Chapter 2 using $\mathrm{ARR} = \frac{\mathrm{CTL}_{[\mathrm{T}_2]} - \mathrm{CTL}_{[\mathrm{T}_1]}}{\mathrm{T}_2 - \mathrm{T}_1}$ for each tsetse species (Cossins and Bowler, 1987). $CTL_{[T1]}$ and $CTL_{[T2]}$ are the CT_{max} at 25 (T_1) and 30°C (T_2). ARR represents the change in CT_{max} per 1°C temperature increase. A comparison was made to other Dipterans and within the *Glossina* genus, using data from Weaving *et al.* (2022). An additional literature search was completed to find any new or missing literature for tsetse and mosquitoes, as similar blood feeding vectors of disease. I searched Web of Science using the following terms: *(mosquito*) AND (thermal OR heat OR temperature) AND (CTmax* OR critical thermal max*) AND (plastic* OR (phenotyp* plastic*) OR acclim* OR stress OR tolerance) NOT (mosquitofish*)*. This

resulted in seven additional articles, one of which had useable data. For Google Scholar and Scopus, the following search terms were used*: "mosquito" ctmax plasticity.* These differed due to the first set of Boolean terms not being accepted in these search engines. Scopus had nine results and one had appropriate data for extraction. Abstracts on the first three pages of Google Scholar were examined, two of which had useable data. Hits after the first three pages became irrelevant so were not examined for further articles. The same search was performed for tsetse, replacing "mosquito" with "tsetse" in the search terms, and removing "*NOT "mosquitofish""*. No new articles were found for tsetse on the three search engines. Digitizer was used to extract data from graphs (Rohatgi, 2010). For further detail on methodology, see Chapter 2.

The R package 'metafor' (Version 3.0-2; Viechtbauer, 2010) was used to perform a multi-level, random effects model comparing ARR within Diptera. The analysis was run as in Chapter 2. Family group, sex and body mass were used as moderators to explain variation in ARR. Dipteran families included: Glossinidae (tsetse flies), Culicidae (mosquitoes), Drosophilidae and Tephritidae (fruit flies), and Ceratopogonidae (biting midges).

3.5 Results

3.5.1 Basal heat tolerance

Overall, basal CT_{max} differed among species by a maximum of 1.8°C (Fig. 3.3; $\chi^2 = 11.19$, df = 4, p = 0.02), for which mean values are presented in Supplementary Table 3.2. Basal CT_{max} was ordered from highest to lowest by species as follows: *G. f. fuscipes, G. pallidipes,* equally *G. m. morsitans and G. p. gambiensis*, and finally *G. brevipalpis.*

I expected higher basal CT_{max} in larger flies due to greater resources and reduced metabolic rate per unit mass. Indeed, larger tsetse had greater basal CT_{max} ($\chi^2 = 4.31$, df = 1, p = 0.04), but a significant interaction between species and body size improved model fit, indicating within species relationships ($\chi^2 = 9.03$, df = 4, p = 0.06). Single species models illustrated that basal CT_{max} increased with size for *G. m. morsitans* (F = 2.77, df = 1, p < 0.001), *G. pallidipes* (F = 8.40, df = 1, p = 0.006), and there was a non-significant trend for *G. brevipalpis* (F = 3.35, df = 1, p = 0.08). There was no significant relationship for *G. f. fuscipes* (F = 0.76, df = 1, p = 0.39) or *G. p. gambiensis* (F = 1.74, df = 1, p = 0.20; Supplementary Figure 3.1).

Overall, males had lower basal CT_{max} than females ($\chi^2 = 7.50$, df = 1, p = 0.006). However, again, this relationship was dependent on species ($\chi^2 = 1.61$, df = 4, p = 0.003). Single-species models showed that within species, females had greater basal CT_{max} than males for *G. m. morsitans* (F = 26.0, df = 1, p < 0.001), *G. pallidipes* (F = 6.69, df = 1, p = 0.01), *G. brevipalpis* (F = 4.55, df = 1, p = 0.04). *Glossina fuscipes fuscipes* females also had greater CT_{max}, but the trend was non-significant (F = 3.06, df = 1, p = 0.09). These results are consistent with those found for CT_{max} and body size, as female tsetse tend to be larger. There was no relationship between sex and CT_{max} for *G. p. gambiensis* (F = 0.42, df = 1, p = 0.52).

3.5.2 Acclimation responses

I investigated how a five-day acclimation at 30°C affected $CT_{max.}$ Overall, CT_{max} increased by 0.06°C per 1°C rise in acclimation temperature ($\chi^2 = 26.7$, df = 1, p < 0.001), but there was variation in plasticity among species (Supplementary Table 3.2, 3.3; $\chi^2 = 24.5$, df = 4, p = p < 0.001). Single species models showed that acclimation increased CT_{max} for *G. p. gambiensis* (F = 36.8, df = 1, p < 0.001) and *G. brevipalpis* (F = 13.6, df = 1, p < 0.001) by 0.12°C and 0.10°C per 1°C acclimation respectively (Fig. 3.3). There was no change in CT_{max} for *G. m. morsitans* (F = 2.68, df = 1, p = 0.11)*, G. pallidipes* (F = 0.37, df = 1, p = 0.55), and *G. f. fuscipes* (Fig. 3.3; F = 1.27, df = 1, p = 0.26) of 0.04, 0.01, and 0.02 respectively. Post-hoc analysis examining species-scale differences between reaction norm slopes found that *G. p. gambiensis* was the most plastic species, with a significantly steeper reaction norm than *G. f. fuscipes*,

G. m. morsitans and *G. pallidipes* (Table 3.1). *Glossina brevipalpis* was the second most plastic, with a significantly steeper reaction norm than *G. f. fuscipes* and *G. pallidipes* (Table 3.1).

I predicted that larger flies may be more plastic, however there was no relationship between plasticity and body size (χ^2 = 0.006, df = 1, p = 0.94), therefore I present the relationship between size and both basal and acclimated CT_{max} data in Figure 3.4. Acclimated and basal CT_{max} increased with body size (χ^2 = 4.50, df = 1, p = 0.03), but this relationship depended on the species tested (χ^2 = 12.3, df = 4, p = 0.01). Single species models revealed that larger flies had greater CT_{max} for *G. pallidipes* (F = 19.0, df = 1, p < 0.001)*, G. m. morsitans* (F = 32.0, df = 1, p < 0.001), and *G. brevipalpis* (F = 3.91, df = 1, p = 0.05). However, there was no relationship for *G. f. fuscipes* (F = 0.06, df = 1, p = 0.80) or *G. p. gambiensis* (F = 0.24, df = 1, p = 0.26), in accordance with models based on only basal CT_{max}. For comparison, I also give these relationships with dry body mass (rather than wing vein) in Supplementary Figure 3.2.

I found no difference in plasticity between sexes (χ^2 = 0.54, df = 4, p = 0.46). However, there was a significant interaction between species and sex (χ^2 = 24.1, df = 4, p < 0.001), and species and treatment (χ^2 = 24.7, df = 4, p < 0.001). When these data were split into single species models, I found similar patterns as between body size and CT_{max} – females had greater CT_{max} for *G. pallidipes, G. m. morsitans* and *G. brevipalpis* (Fig. 3.4; Supplementary Table 3.3).

3.5.3 **Acclimation responses within Diptera**

A total of 488 effect sizes (from 25 studies, 33 species) were calculated to examine the effect of acclimation on CT_{max} in Diptera. References for which are uploaded to OSF: https://osf.io/b3m28/. Drosophilidae were by far the most represented family (k = 384), followed by Culicidae (mosquitoes; k = 50), Tephritidae (fruit flies; k = 32), Glossinidae (tsetse flies; k = 18), and Ceratopogonidae (biting midges; k = 4). Overall, for every 1 °C rise in acclimation temperature, CT_{max} increased by 0.048°C (Table 3.2; 95% CI = 0.024, 0.072). Therefore, plasticity of *Glossina* species (0.06°C) is similar to Diptera (0.05°C). I assessed whether variation in plasticity of CT_{max} in Diptera was explained by moderators (sex, body mass, family) using a series of univariate models. ARRs are stated as mean differences between groups (with the direction of comparison stated in subscript) or as a meta-regression for body mass. Males were slightly more plastic than females ($ARR_{male\text{-}female}$ = 0.026; 95% CI = 0.003, 0.048), but found no differences in plasticity between families or a relationship with dry body mass (Table 3.2; Fig. 3.5). There was no significant publication bias (βARR = 0.019; 95% CI = -0.45, 0.49; Supplementary Figure 3.3).

Table 3.1 Reaction norm slope pairwise comparisons for five species of tsetse (*Glossina* spp.). Slopes indicate the plasticity of each species at the population level as means of each acclimation group per species. Mean differences between slopes are given ± Standard Error (SE). The Tukey method was used for p-value adjustment, comparing a family of five estimates. Species are presented in order of most to least plastic.

Species	Contrast	Mean difference ± SE	DF	t-statistic	p-value
G. p. gambiensis	*G. brevipalpis*	0.05 ± 0.15	378	0.34	>0.99
	G. pallidipes	**0.56 ± 0.15**	**378**	**3.77**	**0.002**
	G. f. fuscipes	**0.48 ± 0.15**	**378**	**3.24**	**0.01**
	G. m. morsitans	**0.42 ± 0.15**	**378**	**2.80**	**0.04**
G. brevipalpis	*G. pallidipes*	**0.50 ± 0.15**	**378**	**3.41**	**0.006**
	G. f. fuscipes	**0.43 ± 0.15**	**378**	**2.89**	**0.03**
	G. m. morsitans	0.37 ± 0.15	378	2.45	0.10
G. m. morsitans	*G. pallidipes*	0.14 ± 0.15	378	0.92	0.89
	G. f. fuscipes	0.063 ± 0.15	378	0.42	0.99
G. f. fuscipes	*G. pallidipes*	0.074 ± 0.15	378	0.50	0.99

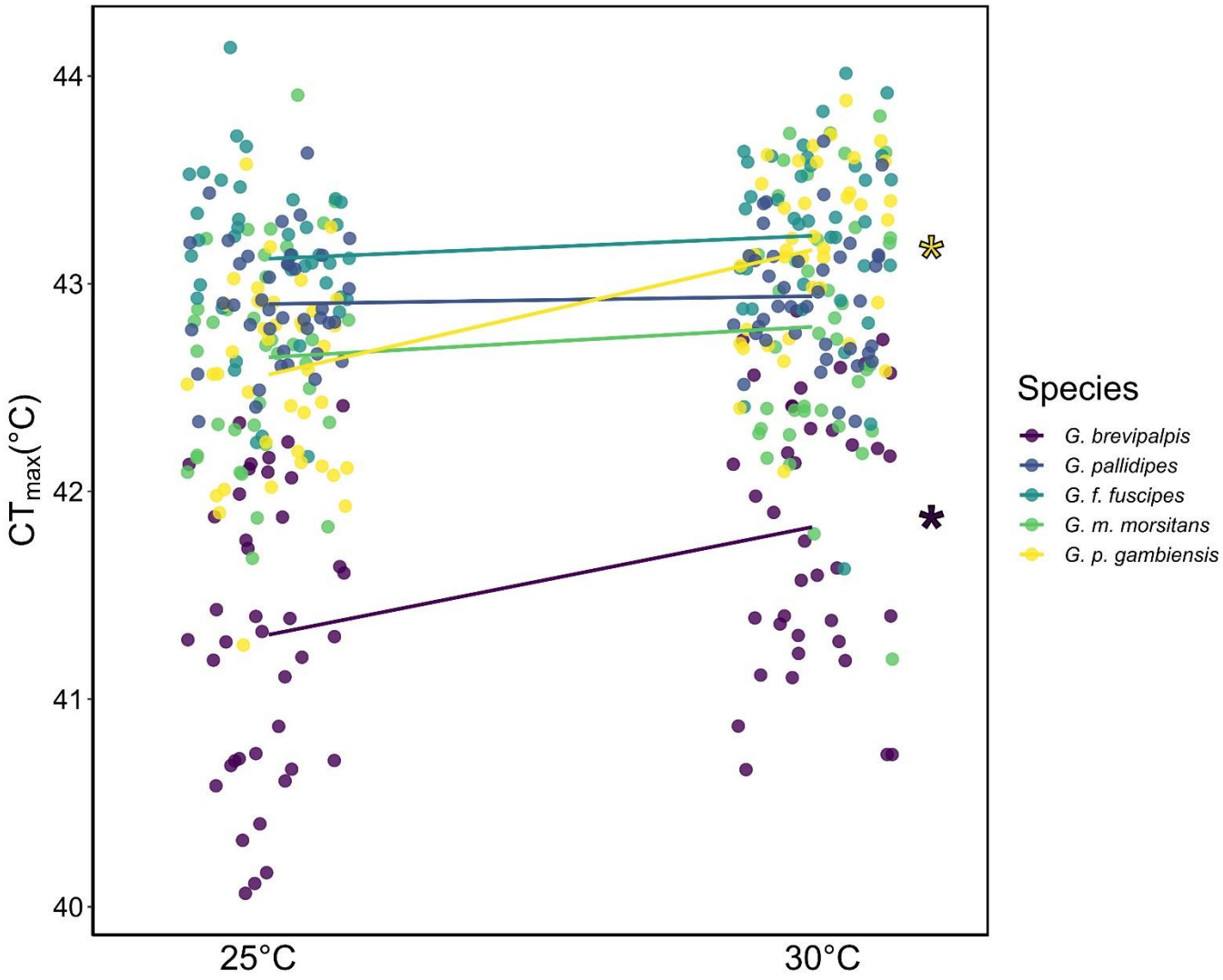

Figure 3.3 Critical Thermal Maximum (CT_{max}) after acclimation at basal (25°C) and acclimated (30°C) temperature for five days. *Glossina* spp. are distinguished by different colours and are presented in the legend in size order by wing vein. Lines represent the reaction norm of each species with the slope equivalent to plasticity. Significant differences between the CT_{max} of the two acclimation temperatures is indicated by an asterisk, which is seen in *G. brevipalpis* and *G. p. gambiensis*. N ~ 40 per treatment/species.

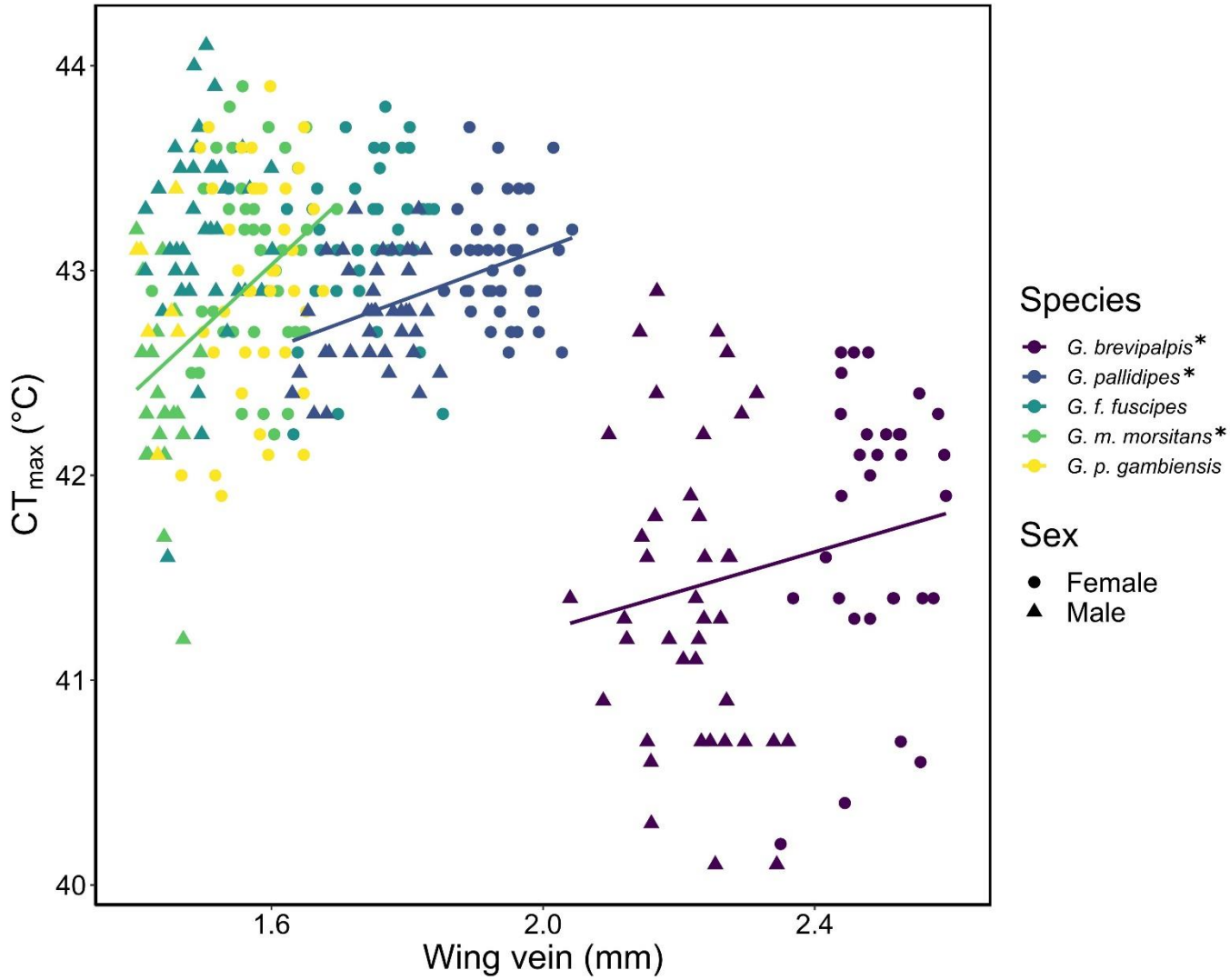

Figure 3.4 Relationship between CT_{max} and wing vein length (mm). *Glossina* spp. are distinguished by different colours and given in size order in the legend from largest to smallest by wing vein. Lines represent linear regressions for these species groups where significant, i.e., *G. brevipalpis*, *G. pallidipes* and *G. m. morsitans*. The same three species had females with significantly greater CT_{max} than males, which is denoted by an asterisk in the legend. Circles resemble female flies and triangles resemble males. CT_{max} is represented for individuals acclimated at both 25°C and 30°C. N ~ 40 per treatment/sex/species.

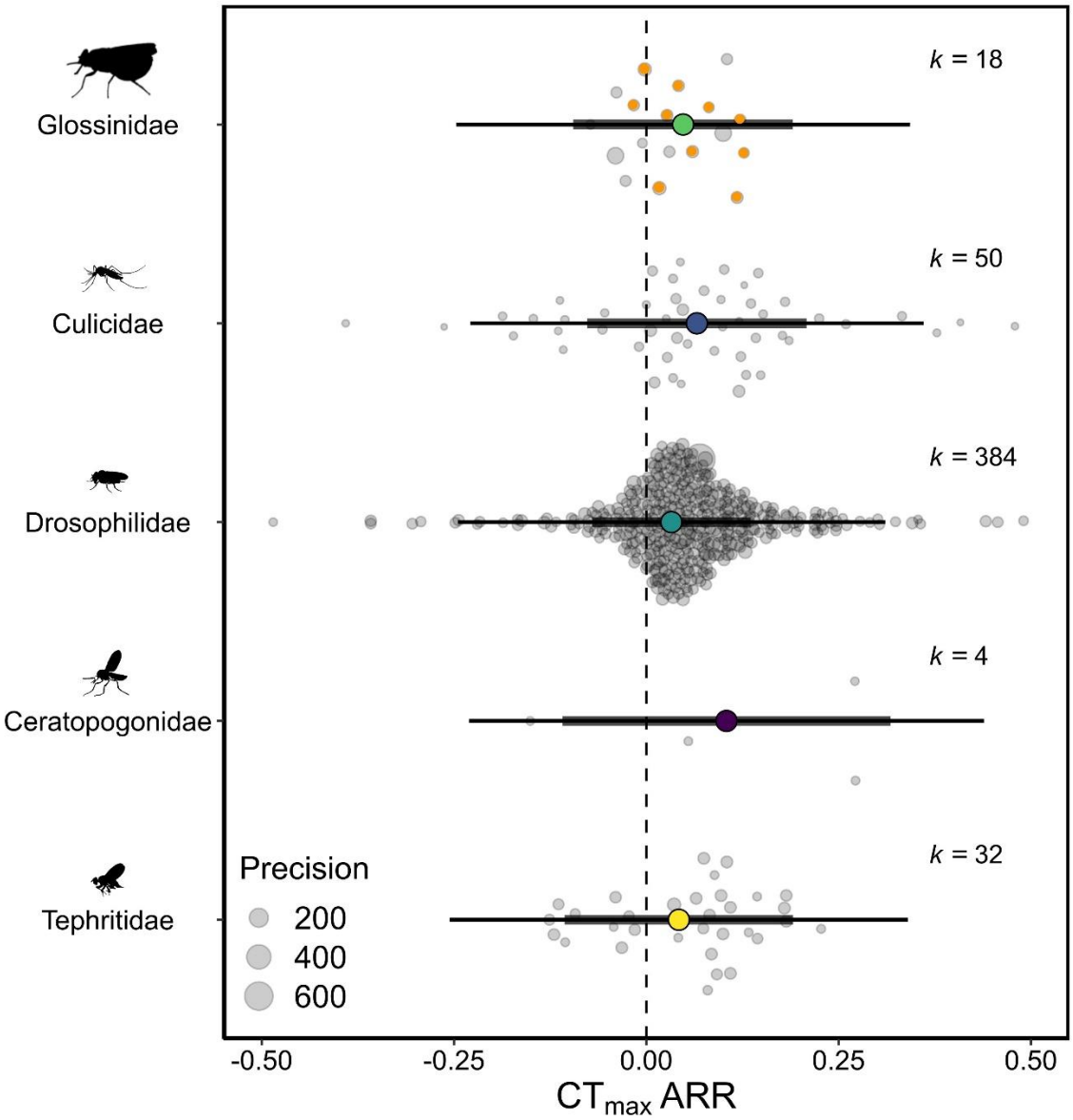

Figure 3.5 Acclimation Response Ratio (ARR) of Critical Thermal Maximum (CT_{max} in °C) across five Dipteran families, 33 species. Effect sizes from the current study are highlighted in orange, males and females are displayed separately giving k = 10. A positive ARR indicates an adaptive plastic response, whereby heat acclimation increases CT_{max}. 95% confidence intervals (95% CIs) are depicted in heavy black lines, prediction intervals in thin black lines. The size of each data point is proportional to 1/SE (Standard Error), indicating the precision of the study. k = number of effect sizes per group. One effect size from Drosophilidae was excluded from the Figure so a smaller axis could be presented. Icons are roughly scaled by the size of the family group, icon credit: phylopics.

Table 3.2 Main intercept and univariate multi-level meta-analytic, random effects models for (CT_{max}) critical thermal maximum. The main model tests whether ARR (Acclimation Response Ratio) is significantly different from zero. The univariate models are regressions or compare differences between moderator groups. Results for intercept models are displayed. Results are highlighted in bold where 95% CIs do not overlap between groups or where regressions are significant for continuous variables. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. I^2 is the proportion of heterogeneity explained by each of the random effects. R^2 marg.: R^2 marginal, the variance explained only by moderators. R^2 cond.: R^2 conditional, the variance explained by moderators and random effects.

Model	Group	Est.	t	CI.lb	CI.ub	AICc	k	I^2				R^2	
								study	phylogeny	species	row	marg.	cond.
Main	**Intercept**	**0.048**	**4.07**	**0.024**	**0.072**	-691.9	488	1.47	<0.001	16.2	79.6	-	0.18
~mass	**Intercept**	**0.046**	**3.17**	**0.016**	**0.077**	-615.2	453	1.41	<0.001	18.4	77.7	<0.001	0.20
		0.0002	0.16	-0.002	0.002								
~sex	**Intercept**												
	(female)	**0.038**	**2.96**	**0.011**	**0.064**	-688.3	488	<0.001	<0.001	18.0	79.2	0.013	0.20
	Male	**0.026**	**2.20**	**0.003**	**0.048**								
	Both	-0.002	-0.08	-0.061	0.056								
~family	Intercept												
	(Glossin.)	0.048	0.71	-0.093	0.188	-679.4	488	1.78	23.0	10.4	62.6	0.009	0.37
	Culic.	0.018	0.19	-0.180	0.216								
	Drosophil.	-0.014	-0.20	-0.166	0.137								
	Ceratopog.	0.057	0.47	-0.197	0.310								
	Tephrit.	0.005	0.09	-0.195	0.185								

3.6 Discussion

Acclimation to an elevated temperature (30°C versus 25°C) across five days in early adulthood enhanced CT_{max} in two of the five tsetse species measured, but differences in plasticity across or within species were not associated with body size or sex. Within some tsetse species, higher basal CT_{max} values were associated with larger body size and being female, but these differences were not found among species i.e., the largest species *G. brevipalpis* actually had the lowest basal tolerance. The broader meta-analysis revealed similar mean acclimation responses between tsetse and Diptera, of 0.05 and 0.06 per 1°C acclimation respectively. In contrast to tsetse, there was greater CT_{max} plasticity of male Dipterans compared to females.

The two of the five species that responded to acclimation were *G. p. gambiensis* and *G. brevipalpis*, with a 0.12°C and 0.10°C increase in CT_{max} per 1°C increase in acclimation temperature, respectively. Previously, Terblanche and Chown (2006) found a limited, or non-existent, response of CT_{max} to temperature acclimation at 21, 25, and 29°C in *G. pallidipes*. Therefore, my findings indicate that thermal tolerance plasticity is not fully constrained among tsetse species. In general, CT_{max} of ectotherms responds relatively weakly to thermal acclimation (Gunderson and Stillman, 2015). Insects on average show a 0.09°C rise in CT_{max} per 1°C acclimation temperature (Chapter 2; Weaving *et al.*, 2022). Studies have found that CT_{max} is constrained within a narrower range than CT_{min}, which may reflect hard physiological limits at high temperature (Sandblom et al., 2016). For example, both CT_{max} and CT_{min} decline with increasing latitude, but CT_{max} is an order of magnitude less responsive (Sunday et al., 2011). Evolutionary and plastic constraints to CT_{max} are worrying for insects and other ectotherms given ever increasing mean and maximum temperatures.

Differences in CT_{max} plasticity could not be explained by body size or sex in tsetse. However, the meta-analysis of Diptera indicated that males were more plastic than females by 0.03°C per 1°C increase in acclimation temperature. This is in opposition to a recent meta-analysis on acclimation in ectotherms which found that females are more plastic than males in wild-caught populations (Pottier et al., 2021) and my findings in Chapter 2 that neither sex is more plastic. Male-associated behaviours, such as large home ranges and increased risk taking (Tarka et al., 2018; Todd and Nowakowski, 2021), may be more sexually divergent in Diptera than in other ectothermic species. Broad-scale analyses on many phylogenetic groups may obscure trends if there are opposing selection pressures between groups.

Basal CT_{max} values are comparable to wider literature. For example, I found basal CT_{max} was 42.9°C in *G. pallidipes* at a ramp rate of 0.1°C/min. Terblanche *et al* (2008) found *G. pallidipes* had a CT_{max} of approximately 43.5°C at a slightly faster ramp rate of 0.12°C/min (Terblanche et al., 2008). Given that

faster ramp rates tend to give rise to higher values of CT_{max} (Chown et al., 2009; Terblanche et al., 2007), these values are comparable.

Differences between CT_{max} values were related to intra-species relationships with body size and sex. Larger flies generally had greater CT_{max} within *G. pallidipes, G. m. morsitans* and *G. brevipalpis*. Correspondingly, differences between male and female tsetse mirrored trends for body size, indicating that differences are likely related to female tsetse being larger than male tsetse, a rule common across insects (Honěk, 1993). These patterns may have been due to larger individuals having more energy reserves and a slower metabolic rate to size ratio, or alternatively, greater thermal inertia of larger individuals may have slowed the rate at which their body temperature increased (Brown et al., 2004; Stevenson, 1985b). In addition, insects raised under high temperature tend to be smaller adults (Kingsolver and Huey, 2008), which is also true for tsetse (Chapter 5). Therefore, high temperatures may have two effects – first, high developmental temperature will likely result in the emergence of small-bodied flies, and secondly, these flies may subsequently have lower basal CT_{max}. Indeed, field studies show that in hotter months, small bodied tsetse are selectively eliminated (Bursell & Glasgow, 1960; Jackson, 1948).

I note, however, that body size trends did not apply among species: *G. brevipalpis* is the largest species but had the lowest basal CT_{max}. Species differences may be due to different distributions and, therefore, thermal history. Source locations of the five tsetse species considered here are presented in Supplementary Table 3.1 and range from approximately zero to 20° latitude. There was no clear evidence linking latitude to CT_{max} and, given that only five of the total 31 tsetse species and subspecies were measured, I caveat that any broader generalisations from my findings are speculative. *Glossina palpalis gambiensis* and *G. brevipalpis* had the lowest basal CT_{max} but showed the largest acclimation response (Fig. 3.3). Individuals with lower basal tolerance may exhibit greater plasticity, known as the tolerance-plasticity trade-off hypothesis (van Heerwaarden and Kellermann, 2020). The implications of this hypothesis are that species with the highest basal tolerances may be more vulnerable to temperature rises due to their lack of plasticity. However, findings in support of this hypothesis (e.g. Comte and Olden, 2017; Faulkner et al., 2014; Vinagre et al., 2018) have recently come under scrutiny due to statistical issues of collinearity and regression to the mean, and if true, these findings may be artifacts of experimental design and statistical analysis (Gunderson, 2023; Gunderson and Revell, 2022; van Heerwaarden and Kellermann, 2020). Undoubtedly, I would recommend testing more tsetse species before coming to any conclusions.

Overall, there were intra- and inter-specific differences in CT_{max} and its plasticity across tsetse species. In general, plasticity of CT_{max} was weak, in agreement with studies which show a reduction in the range

tsetse are likely to inhabit due to climate change (Are and Hargrove, 2020; Longbottom et al., 2020). Moreover, I argue that warming temperatures will result in smaller body sizes, which is associated with reduced CT_{max}, and thus will further constrain capacity to cope with climate change across multiple tsetse species. This study highlights that broad patterns are not always reflected within closely related species or even within species, therefore detailed experimental studies are needed to capture the capacity of insects to cope with rapidly warming temperatures.

4. Chapter 4 - Transient effect of heatwave on tsetse female fertility

4.1 Statement

This chapter was published in Proceedings of the Royal Society B on 13/03/2024. The work went through significant changes after peer review, so **readers should refer to the published version**. I, Hester Weaving, am the lead author. Other authors are John S. Terblanche, and Sinead English. I completed the analysis, wrote the code, and led writing of the manuscript and revision of drafts. SE and JT jointly supervised the work and contributed to the development of drafts. All authors conceptualised the project. Find here: Weaving, H., Terblanche, J.S., and English, S. (2024) Transient effect of heatwave on tsetse female fertility. *Proceedings of the Royal Society B*, **291**, 20232710. 10.1098/rspb.2023.2710

4.2 Abstract

Heatwaves are increasing in their frequency and intensity due to climate change, pushing animals beyond critical physiological limits. While most studies focus on lethal limits, sublethal effects on fertility tend to occur below lethal thresholds, and subsequently can be equally as important for population viability. Typically, male fertility is more heat-sensitive than female fertility, yet direct comparisons are limited. Here, I measured the effect of an experimentally simulated heatwave on tsetse flies, *Glossina pallidipes*, disease vectors and unusual live-bearing insects. I exposed male or female flies to a three-day heatwave peaking at 36, 38, or 40°C, and a control of 25°C, monitoring mortality and reproductive output for six weeks. A heatwave peaking at 40°C killed 100% of individuals, while at 38°C, acute mortality was only 8%. I found 38°C heatwave-exposed females were infertile for one week, but a doubling in death risk over the six-week experiment had a greater effect on population viability. In contrast, males which experienced a 38°C heatwave had no fertility loss, but equivalent death risk. This study highlights that female fertility limits should not be overlooked but indicates that effects can be transient. Ultimately, both lethal and fertility limits should be considered when assessing population vulnerability to climate change.

4.3 Introduction

Heatwaves, defined as at least three consecutive days with maximum temperatures above the daily 90th percentile (Russo et al., 2014), are becoming more frequent, prolonged and intense due to climate change (Christidis et al., 2015; Meehl and Tebaldi, 2004; Perkins et al., 2012). Extreme temperatures are likely to have a greater impact than rising mean temperatures alone, as they force animals towards or beyond physiological limits (Sheldon and Dillon, 2016; Stillman, 2019). For many species, these limits are already being regularly surpassed (Sunday et al., 2014; Vinagre et al., 2019), which can result in mass mortality events. For example, a single day of Australian heatwave caused the death of 45,000 flying foxes (Welbergen et al., 2014, 2008), and, in Europe, a 2003 heatwave caused an excess of 70,000 human mortalities (Robine et al., 2008). Forecasting which species are most vulnerable to extreme temperature events can help predict further biodiversity losses and distributional shifts (Arneth et al., 2020; Pacifici et al., 2015).

Species' thermal vulnerability can be estimated using a variety of indices, one example is warming tolerance, the temperature difference between upper thermal tolerance and the animal's habitat (Buckley and Huey, 2016; Clusella-Trullas et al., 2021; Kellermann et al., 2012; Overgaard et al., 2014). Widely used to estimate upper thermal limits, is Critical Thermal Maximum, or CT_{max}, which is the maximum temperature at which biological function is lost (loss of coordinated movement, response to stimulus (e.g., Terblanche and Chown, 2006), and lethal temperature, where LT80 indicates the temperature at which there is 80% mortality in a population (Stillman and Somero, 2000). However, recently the usefulness and accuracy of these indices has come into question due to the lack of incorporation of factors such as evolutionary adaptation, acclimation capacity, and thermoregulatory behaviours (Clusella-Trullas et al., 2021). Additionally, these measures do not consider sublethal effects on major fitness components such as reproduction and growth. Damage to these traits often occurs at less extreme temperatures than those which kill animals (Sales et al., 2018; Walsh et al., 2019a), so such sublethal effects can potentially have more prevalent and sustained impact on populations than lethal temperatures (Clusella-Trullas et al., 2021). For this reason, a multiple trait-based approach has been suggested to estimate species vulnerability that allows for factors such as adaptation and includes sublethal limits for more robust estimates (Blackburn et al., 2014; Clusella-Trullas et al., 2021; Walsh et al., 2019b).

The temperature at which fertility of an animal declines tends to occur well below upper thermal limits (Sales et al., 2018; Walsh et al., 2019a). Here, I define sterility as the permanent inability to produce viable offspring, and infertility as the current inability to produce viable offspring, with effects that may only be temporary. Studies show that high temperature affects sperm count, viability and motility, oocyte development and maturation, and causes damage to DNA, RNA and protein synthesis

(Hansen, 2009; Karaca et al., 2002; Perez-Crespo et al., 2008). Damage to male fertility typically occurs at lower temperatures than for females (Porcelli et al., 2017; Sales et al., 2018; van Heerwaarden and Sgrò, 2021), likely due to a requirement of low temperature for spermatogenesis (Hirano et al., 2022). As a result, a population may become inviable due to sterility or infertility even if CT_{max} is not reached, meaning that vulnerability indices such as warming tolerance could underestimate the effects of warming on population dynamics (Walsh et al., 2019b). A recent study on 43 *Drosophila* species found that for around half of species infertility occurs below lethal limits, and, moreover, that temperatures which define male infertility better match species distributions than LT80 or CT_{max} (Parratt et al., 2021). The study also found that CT_{max} and fertility measures are not well correlated, indicating that it will be necessary to estimate fertility limits experimentally even for those species where CT_{max} is known.

Fertility studies use a variety of methodologies and trait measurements (Walsh et al., 2019b), making it difficult to compare species and incorporate fertility estimates into vulnerability indices. In contrast, upper thermal limits are better defined (although see e.g. (Ørsted et al., 2022; Terblanche et al., 2007)), so can be used in large-scale global analyses to identify traits which make species most vulnerable to climate change (Gunderson and Stillman, 2015; Sunday et al., 2011; Weaving et al., 2022). Therefore, two standard metrics of fertility have been proposed: first, the Thermal Fertility Limit or TFL (Walsh et al., 2019b), which is a binary measure of the temperature and duration of thermal exposure at which viable offspring production is prevented, considering individuals as fertile or infertile; and, second, the Thermal Sensitivity of Fertility or TSF, which captures the number of viable offspring produced (Baur et al., 2022). Within this framework, there are further methodological considerations. Thermal exposure can be static or variable, with the former easily repeatable and useful for calculating thermal tolerance curves, but the latter potentially more ecologically relevant. Life stages also vary in sensitivity, for example in the flour beetle, *Tribolium castaneum*, heat stress in the larval stage results in adult male sterility, but there is no fertility change if pupae are exposed to heat stress (Sales et al., 2018). Unlike CT_{max}, which typically results in death in insects (but not necessarily in vertebrates), fertility can recover (Nguyen et al., 2013). Therefore, monitoring animals over a suitable time frame offers insights into population viability. Finally, TFL data are limited largely to model organisms, and there are few studies which compare male and female fertility directly (but see (David et al., 2005; Kellermann et al., 2012; Parratt et al., 2021). Therefore, researchers are calling for measurements on a wider variety of species where heat-treated sexes are measured independently under similar conditions (Walsh et al., 2019a).

Here, I investigate the effect of a three-day heatwave on lethal limits and fertility limits of *Glossina pallidipes*, a disease vector of animal African trypanosomiasis in sub-Saharan Africa (Buxton, 1955). I expose either males or females to simulated experimental heatwave designed to mimic a daily

fluctuation in maximum, but not minimum, ambient temperature. Tsetse only produce around ten offspring in their lifetimes meaning that population persistence is vulnerable to any increases in mortality or changes to reproductive output (Phelps and Burrows, 1969), making them an interesting study for thermal limits. I had three main aims: first, to determine if TFLs occur at temperatures below lethal temperatures in tsetse; second, to disentangle differences between male and female TFLs and sensitivity, and monitor recovery time, and third, to investigate how parental thermal stress affects offspring fitness by measuring traits such as sex ratio and CT_{max} in the F1 generation.

4.4 Methods

4.4.1 Colony conditions

Batches of approximately 900 *G. pallidipes* pupae were ordered from the International Atomic Energy Agency (IAEA) in August 2022 and December 2022, for replicate one and two respectively. IAEA colony conditions are 24-25°C and 75-80 % Relative Humidity (RH) for adult tsetse and 25°C and 75 % RH for pupae (Opiyo et al., 2006). Pupae were kept in a climate-controlled room at 25°C and 80 % RH. Upon emergence, adults were briefly chilled using a chest fridge (maintained between 6 and 2°C using a RS Pro Dual Datalogger with T type thermocouples) and sorted daily into single sex cages (20 x 25 x 10 cm) consisting of no more than 10 individuals. Daily collection ensured flies were virgins and any deformed or undersized individuals were discarded. Flies were fed 24 hours post cold exposure and then three times weekly on sterile, defibrinated horse blood (TCS Biosciences, Buckingham, UK), containing 200 µl of ATP (adenosine 5'-triphosphate disodium) per 25 ml as a feeding stimulant. Blood was covered with a silicon membrane and heated to 36°C (Flexible heated hoses, Birmingham, UK).

4.4.2 Heatwave treatment

For each trial (1 and 2), approximately 200 flies underwent a thermal treatment of 25°C (control), 36°C, 38°C, or 40°C for two hours for three consecutive days to mimic heatwave conditions (Fig. 4.1). The remaining flies underwent no thermal treatment but were walked around the laboratory as a handling control. Emergence dates were split between treatment groups as evenly as possible. These thermal treatments were chosen because a heatwave is defined as a minimum of three consecutive days with maximum temperatures above the daily 90th percentile temperature (Russo et al., 2014), and *G. pallidipes* CT_{max} is around 43.5°C (Terblanche et al., 2007). Air temperatures within and above this range are regularly experienced in the field, see e.g. (Lord et al., 2018) at Rekomitjie Research Station, Zimbabwe.

Treatments occurred between 11:00 and 15:00 in Grant LTC4 refrigerated circulating liquid baths with TX150 heating circulators (Grant Instruments, Cambridge). Only two water baths were available for the four temperature treatments, so treatments were alternated daily between morning and afternoon to minimise any potential diurnal effects. Cages were placed in zip lock bags so they could be submerged in the water baths. Flies were returned to standard colony conditions at 25°C once the treatment had finished. iButtons were used to record temperatures of the water baths at a sampling rate of every 30 seconds, for which data can be found in the Supplementary Table 4.1. All heatwave temperatures were within 0.5°C of the target set temperature, except the 40°C temperature during trial 1, where on day three mean temperature + SD was 37.14 + 2.54°C, therefore data for these flies were excluded from the analysis.

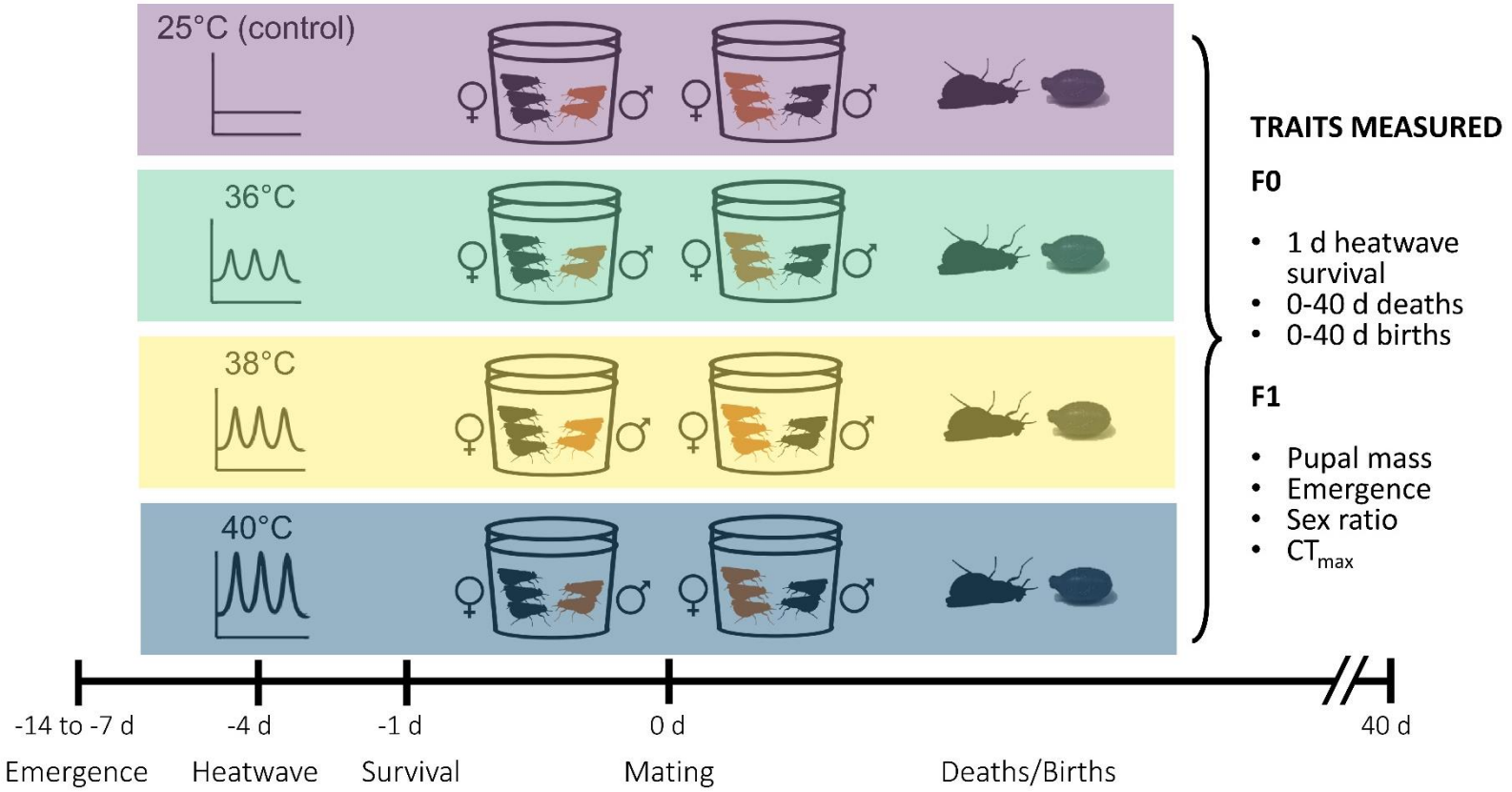

Figure 4.1 Experimental overview. Tsetse flies (*Glossina pallidipes*) were exposed to heatwave for three days and then mixed with non-treated members of the opposite sex in a ratio of three females to two males. Thereafter, the number of deaths and births were recorded for 40 days three times weekly. Further measurements were taken in the offspring (F1) generation. Temperatures indicate heatwave maximums for two hours each day during the three-day water bath exposure, after which flies were maintained at 25°C. Coloured tsetse silhouettes show that either male (♂) or female (♀) tsetse have been exposed to heatwave. Timeline is given in d days.

4.4.3 Sex mixing

As the aim was to determine the difference in thermal fertility limits and sensitivity between males and females, I mixed treated flies with untreated individuals of the opposite sex. Males were a minimum of one week old at the time they were mixed with females, to ensure they were sexually mature (Foster, 1976). Files were mixed into a ratio of 3:2 female:male the morning after the last day of heatwave using the chest fridge described above. This ratio was selected as female longevity is reduced in equal sex ratio cages due to male harassment (Clutton-Brock and Langley, 1997). Cages were made from modified 150 ml cylindrical containers (7.0 cm x 7.3 cm) with mesh fabric (2.5 mm) on the top and bottom (Supplementary Figure 4.1). Each container was placed on top of a pot to catch deposited pupae which are small enough to fall through the mesh in their larval form.

4.4.4 Thermal lethal limits

Dead flies were counted 24 hours after the heatwave treatment had finished. Thereafter, once flies were put into mixed sex cages, I scored mortality in each cage three times a week (Monday, Wednesday, Friday) for six weeks after the sexes were mixed on day zero (Fig. 4.1). Dead individuals were removed from cages and frozen at -20°C.

4.4.5 Thermal Fertility Limits

Pupal production was counted three times weekly to establish the first deposition date and pupal production per cage. Pupae were removed from the pots and housed in 50 ml conical tubes, each with a hole drilled into the middle of the lid for gas exchange.

4.4.6 Transgenerational effects

Under-sized larvae, at the first or second larval stage were recorded as abortions and stored in 70% ethanol (see stages L1 and L2 in Fig. 1.4). Once a week, F1 pupae were weighed using an Ohaus Explorer EX124 balance (Ohaus Europe, Switzerland; accurate to 1/1000 mg). A final tally of F1 emergence rates and sex ratio was taken six weeks after the last pupa was deposited, as at 25°C flies take approximately one month to pupate (Phelps and Burrows, 1969).

A random selection of F1 flies were chosen (n ~ 20 per treatment group and sex) for CT_{max} measurement. CT_{max} experiments were run using the two water baths described previously, connected to Perspex organ pipes which were pumped with water. Grant Labwise software (Version 2.1.2, Grant Instruments, Cambridge, UK) was used to set a program of 10 minutes acclimation at 25°C followed by ramping at a rate of +0.1°C/min, which is considered ecologically relevant from temperature data (Terblanche et al., 2007). Two thermocouples (Type T) monitored temperature in empty pipes which is equivalent to body temperature due to the small body size of tsetse (Terblanche et al., 2007).

Flies were transferred into pipes using 8 µl of sevoflurane pipetted into the 50 ml conical tube through the drilled hole. Previous experiments have shown that sevoflurane does not affect tsetse mortality up to one week after admission (Chapter 3; Weaving et al., 2023), and is known to have limited off-target effects in *Drosophila* (MacMillan et al., 2017). The hole was covered with cotton wool until flies were knocked down (around 2 minutes). Once all flies were standing the program commenced. CT_{max} trials were run by one researcher (HW). Programs on the two baths began in 20–30-minute tandem so a maximum of 10 flies were assessed at once. CT_{max} was defined as the temperature at which the fly stopped responding to disturbance of the organ pipes.

4.4.7 **Statistics**

All data analysis was completed in R Studio (Version 3.5.1; R Core Team, 2023). Data sets and code can be found deposited at: https://osf.io/3yxc9/. Models were verified by examining residuals for normality (where appropriate), heteroscedasticity and outliers, as well as model deviance and collinearity. For model simplification, the 'drop one' method was used whereby, starting from a maximal model, each term was excluded and removed if it did not significantly improve model fit (Bradburn et al., 2003). The difference between models was tested using analysis of variance (ANOVA) or a likelihood ratio test (LRT). Where interactions were significant, the data were split into separate models so that interactions could be investigated fully.

4.4.8 **Lethal limits**

The difference in proportion survival per cage for each treatment group 24 hours post heatwave, was calculated using a generalised linear model with binomial distribution. The proportion of dead individuals was compared between heatwave temperature and sexes, and the interaction between these variables was considered. 100% of individuals died in the 40°C treatment so the brglm2 (version 0.9) package was used to reduce bias due to complete separation in the model (Kosmidis et al., 2020).

A Cox proportional hazards model was used to test how heatwave affected longevity over the six weeks of the experiment. Packages 'survival' (Version 3.2.13; (Therneau and Grambsch, 2000)) and 'coxme' (Version 2.2-18.1 (Therneau, 2023)) were used to fit and visualise the data. No flies survived a 40°C heatwave so they were not included in the analysis. Only flies that experienced the heatwave were included in the model i.e. those that were the untreated sex were not included. Individuals that were still alive by the end of the experiment were censored. The effect of sex and heatwave temperature (25, 36, 38°C), and the interaction between these variables, on mortality were tested. Cage code was used as a random factor to account for variation between cages.

4.4.9 **Thermal Fertility Limits**

Time until the production of the first pupa per cage was modelled as a mixed effect Cox proportional hazard survival analysis. The number of days until the first pupa was deposited per cage was the response variable; while temperature treatment and whether females or males were exposed to heatwave, hereafter reported as "exposed sex", and their interaction, were independent variables.

Total pupal production per female over the six-week experiment was calculated by dividing the total number of pupae per cage by the total number of females per cage at the start of the experiment. To account for female mortality over the course of the experiment, a time-step analysis was completed where, for each recorded day, the number of females successfully producing pupae was the response variable and the log of the number of living females at that time point was included as an offset (as in (Brooks et al., 2017)). As 68% of the observations were zero values (1505/2220), a hurdle model was used with Poisson distribution. The non-zero values were investigated as count data, representing the Thermal Sensitivity of Fertility (TSF), and the success or failure of fertility of each female was represented binomially to investigate the Thermal Fertility Limit (TFL). Exposed sex, heatwave temperature and time were included as fixed factors for both parts of the model. I used cage code as a random factor, allowing for different slopes between cages over time (time | cage code) to account the auto-correlation due to repeated measures of the same cage.

To investigate population viability, the effect of treatment on total fly population was measured over time in a general linear model with Gaussian distribution and "identity" link function. I calculated the total fly population as births + total living individuals per day per cage. Births were only included where offspring successfully emerged. The three-way interaction between heatwave temperature, exposed sex, and time on total population was investigated, with cage code as a random factor.

4.4.10 **Transgenerational effects**

A series of generalised linear models were run to assess the difference between treatment groups in offspring mass, emergence rate, sex ratio and CT_{max}. The effect of parent heatwave treatment, treated parent sex, and their interaction, on these traits were considered. The difference in pupal mass between groups was assessed by general linear model with Gaussian distribution and "identity" function. Cage code was used as a random factor. The proportion of successfully emerged offspring and their sex ratio were calculated per cage. Generalised linear models with binomial distribution were used. Due to model overfitting, the random factor of cage code was not used in the sex ratio model. Finally, the effect of parent heatwave treatment on offspring CT_{max} was assessed by general linear model with Gaussian distribution and "identity" link function. Pupal mass was included as a factor because size can affect CT_{max} estimates (Chapter 3; Rohr *et al.*, 2018).

4.5 Results

4.5.1 Lethal limits

A three-day heatwave peaking at 38°C caused 8% direct mortality in flies 24 hours after exposure, and steeply increased to 100% when the heatwave peaked at 40°C (Fig. 4.2; Supplementary Table 4.2; glm: $\chi^2 = 277.4$, df = 3, $p < 0.001$). There was no difference between male and female mortality (glm: $\chi^2 = 0.16$, df = 1, $p = 0.69$), and no interaction between sex and temperature on mortality (glm: $\chi^2 = 2.70$, df = 3, $p = 0.44$).

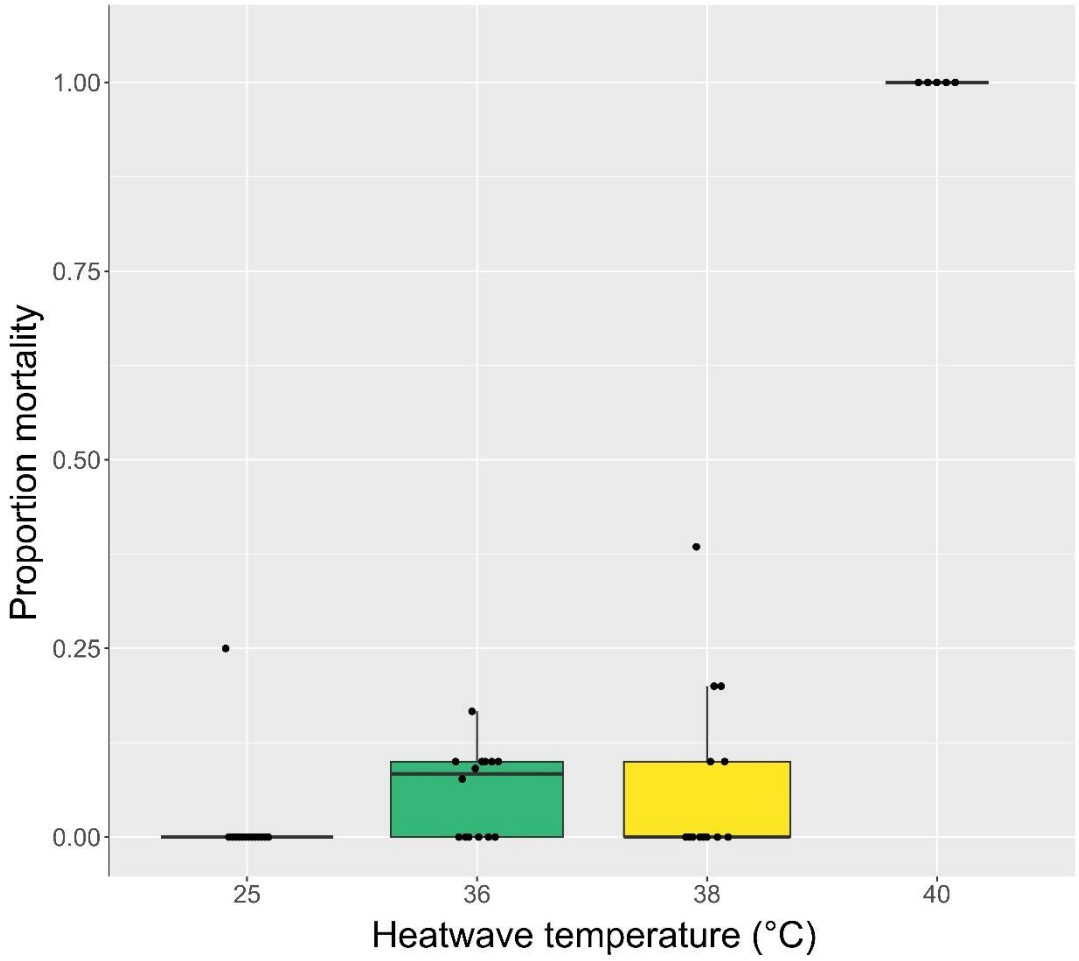

Figure 4.2 Proportion mortality of adult tsetse (*G. pallidipes*) 24 hours following heatwave exposure. Proportion survival was taken per cage, left to right: n = 14, 14, 13, 5. Each cage contained approximately 10 flies. N is lower in the 40°C group due to failure of the water bath to reach a suitable temperature in trial one. Significant values are 0 '***' 0.001 '**' 0.01 '*'.

Survival was assessed over the six-week period following the heatwave by Cox proportional hazards model. Flies exposed to a heatwave peaking at 38°C were more than twice as likely to die over this period than control flies (Fig. 4.3A; Hazard Ratio (Confidence Intervals) = 2.32 (1.27 – 4.23); $\chi^2 = 7.55$, df = 2, p = 0.02). Overall, risk of death was halved in males compared to females (Fig. 4.3A; Supplementary Table 4.3; $\chi^2 = 6.97$, df = 1, p = 0.008). There was no interaction between exposed sex and heatwave temperature ($\chi^2 = 0.80$, df = 2, p = 0.67), indicating that the effect of heatwave temperature over the six-week experiment did not depend on whether males or females were exposed.

4.5.2 **Thermal Fertility Limits**

The effect of heatwave on delay to first larviposition event was investigated. The interaction between exposed sex and heatwave temperature marginally improved model fit ($\chi^2 = 4.79$, df = 2, p = 0.09), demonstrating sex differences in response to heatwave. A heatwave of 38°C delayed the larviposition of the first pupa, when females were exposed to heatwave, from a median (IQR) of 12 (11 - 15) to 18 (17 - 19) days (Fig. 4.3B; Supplementary Table 4.4; $\chi^2 = 19.16$, df = 2, p < 0.001), but there was no delay when males were exposed (Fig. 4.3B; Supplementary Table 4.4; $\chi^2 = 2.65$, df = 2, p = 0.27).

To assess the Thermal Sensitivity of Fertility (TSF), total production of pupae per female over the six-week experiment was calculated (Fig. 4.4). Mortality was not accounted for in this model, meaning that some of the decline in pupal production is due to greater mortality in the high temperature treatments. An interaction between exposed sex and heatwave temperature was found ($\chi^2 = 6.64$, df = 2, p = 0.04), implying sex differences in thermal sensitivity. Temperature had a significant effect on pupal production when females underwent a heatwave (Fig. 4.4; $\chi^2 = 8.48$, df = 2, p = 0.01), but not when males were exposed to heatwave (Fig. 4.4; $\chi^2 = 1.77$, df = 2, p = 0.41). Females that underwent a 38°C heatwave had fewer offspring than the control group (mean difference ± SE = -0.78 ± 0.27, t = -2.87, p = 0.006), but there was no significant difference between the number of offspring produced in the 36°C heatwave treatment compared to the control (mean difference ± SE = -0.26 ± 0.27, t = -0.97, p = 0.34).

Fertility across time was assessed in a hurdle model to account for mortality of females during the experiment. The zero-part of the model assessed Thermal Fertility Limits (fertile = 1, infertile = 0), whereas the count part of the model (pupal production) assessed Thermal Sensitivity of Fertility. A three-way interaction between heatwave temperature, exposed sex, and time marginally improved model fit, indicating that the effect of the female or male heatwave on fertility likely depended on time since exposure (LRT = 8.31, df = 4, p = 0.08). The zero-part model indicated a non-significant trend that the likelihood of infertility decreased over time by 3% per day in females exposed to a heatwave

peaking at 38°C (odds ratio (CIs) = 0.97 (0.94 – 1.00); z = -1.79; p = 0.07; Supplementary Table 4.5). In contrast, if males were exposed to a 38°C heatwave there was no interaction between fertility and time (log odds ratio (CIs) = 1.01 (0.98 – 1.04); z = 0.85; p = 0.40; Supplementary Table 4.6). The count part of the model indicated no difference in pupal production if females were exposed to a heatwave peaking at either 36°C (odds ratio (CIs) = 1.02 (0.30 – 3.50); z = 0.04; p = 0.97) or 38°C (odds ratio (CIs) = 2.61 (0.66 – 10.24); z = 1.37; p = 0.17; Supplementary Table 4.5), showing that once females had regained fertility they suffered no loss in reproductive output. Heatwave treated males also showed no differences in pupal production for heatwave peaking at either 36°C (odds ratio (CIs) = 0.95 (0.46 – 1.95); z = -0.14; p = 0.88) or 38°C (log odds ratio (CIs) = 1.10 (0.57 – 2.13); z = 0.29; p = 0.77; Supplementary Table 4.6).

To assess population viability, mortality and birth rates over time were modelled to calculate changes in the mean population per cage. A three-way interaction between time, exposed sex and heatwave temperature did not significantly improve model fit (χ^2 = 0.81, df = 2, p = 0.67). However, time interacted significantly with heatwave temperature (χ^2 = 10.31, df = 2, p = 0.006) and exposed sex (χ^2 = 7.50, df = 1, p = 0.006), indicating that the effect of heatwave changed over time (Fig. 4.5). However, there was no interaction between exposed sex and heatwave temperature (χ^2 = 3.02, df = 2, p = 0.22). When females were exposed, the interaction between time and heatwave temperature improved model fit (Fig. 4.5; Supplementary Table 4.7; χ^2 = 6.87, df = 2, p = 0.03). If females were exposed to a 36°C heatwave there was a mean reduction ± SE of -0.007 ± 0.004 (t = -1.89) in the number of flies per cage per day, whereas if females were exposed to 38°C there was a larger reduction in population size of -0.010 ± 0.004 (t = -2.53) compared to the control. When males were exposed to heatwave, the interaction between time and heatwave temperature was of smaller effect, but still improved model fit (Fig. 4.5; Supplementary Table 4.8; χ^2 = 4.72, df = 2, p = 0.09). Male exposure to a 36°C heatwave reduced the cage population by -0.010 ± 0.005 (t = -2.10) flies per cage per day whereas male exposure to a 38°C heatwave caused a reduction of -0.007 ± 0.004 (t = -1.57) in the number of flies per cage per day.

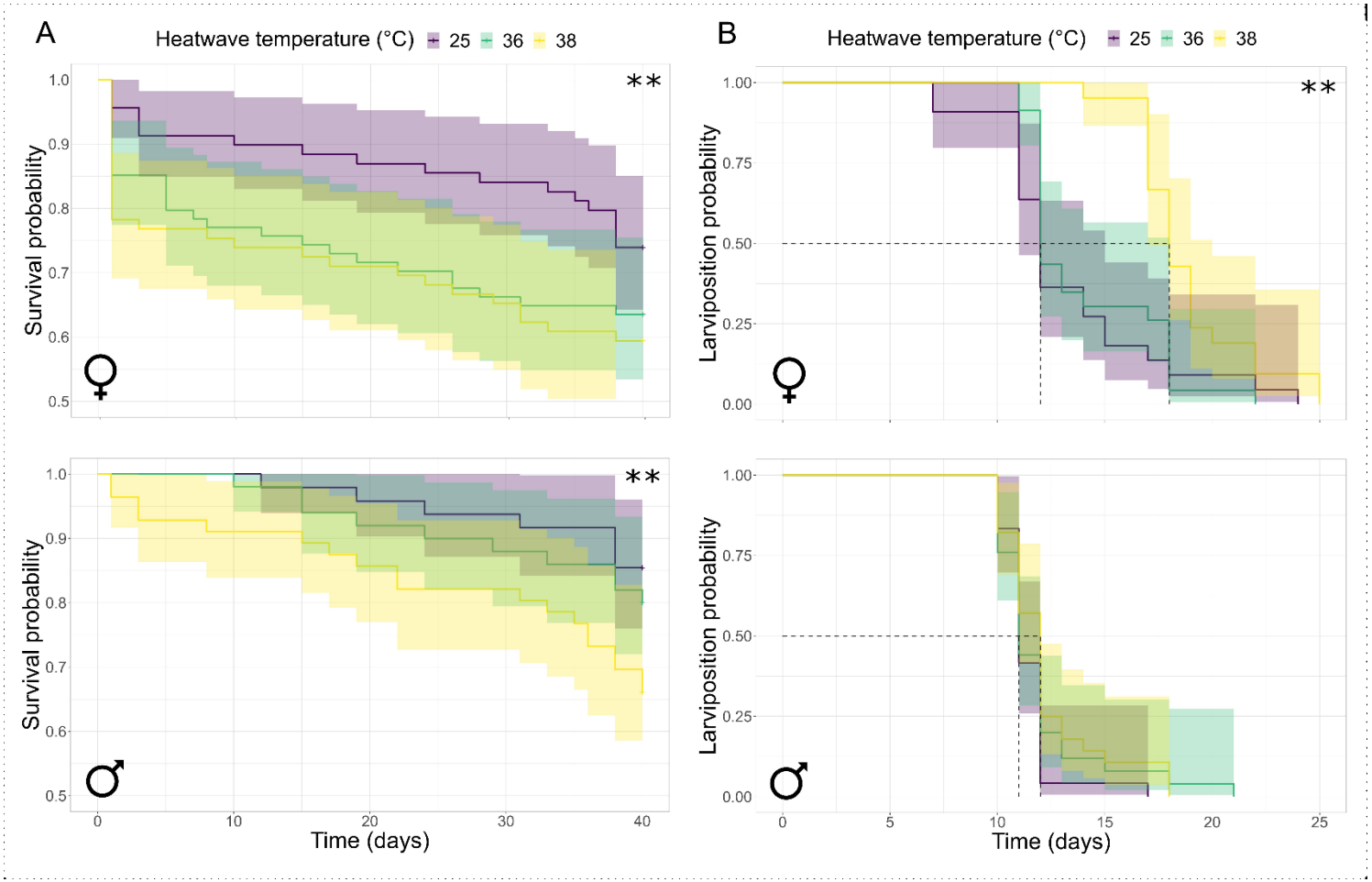

Figure 4.3 Cox survival analyses for A. Probability of survival for adult tsetse (*G. pallidipes*) over six weeks after either female (♀) or male (♂) adult flies were exposed to heatwave (n = 212 females, 154 males). Crosses indicate censored individuals which survived until the end of the experiment. B. Probability of first larviposition event for adult tsetse after heatwave (n = 143 cages). Significant values are 0 '***' 0.001 '**' 0.01 '*' and indicate differences between groups where the heatwave peaked at 38°C and the control. 95% confidence intervals are shaded.

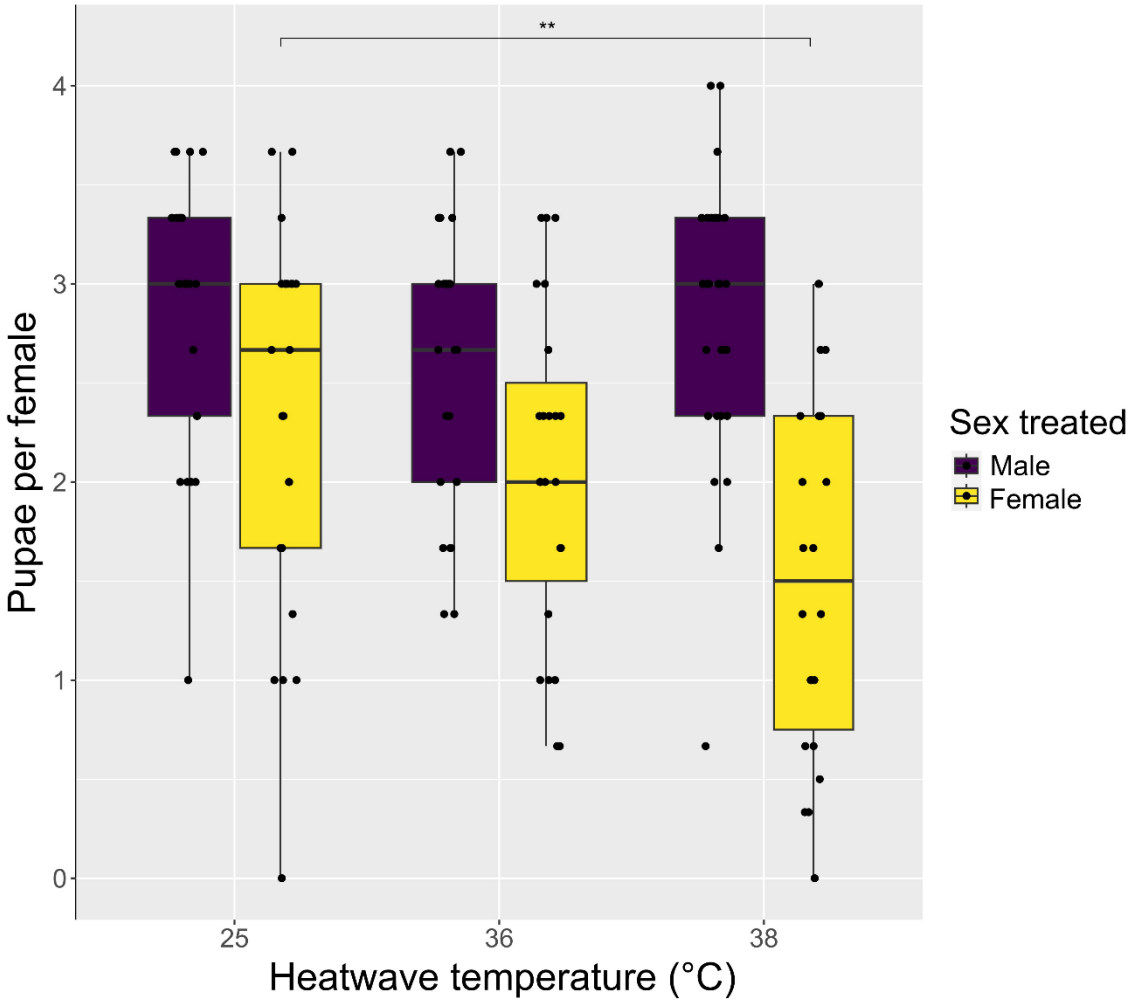

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Figure 4.4 Average pupae per female tsetse (*G. pallidipes*) over six weeks after heatwave. The number of pupae per female was calculated by dividing total pupae per cage by the total number of females at the beginning of the experiment. Significant values are 0 '***' 0.001 '**' 0.01 '*'.

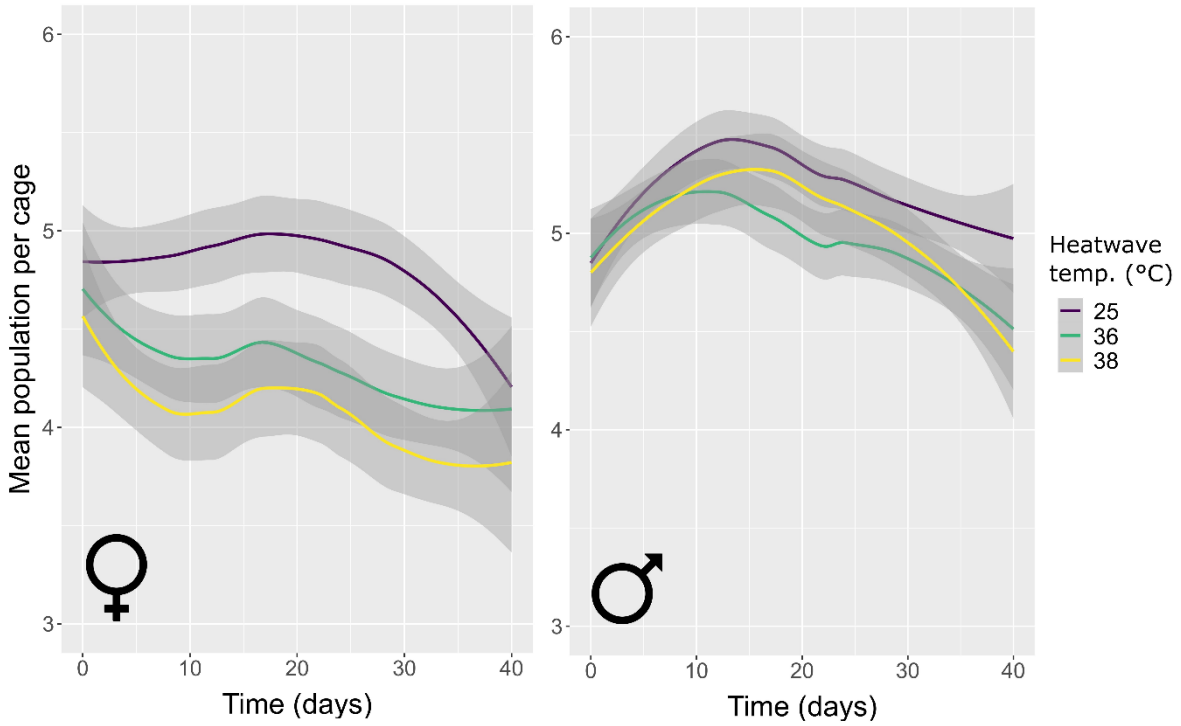


Figure 4.5 Mean tsetse (*G. pallidipes*) population per cage after female (♀) or male (♂) heatwave. The number of individuals per cage was calculated by adding the number of living flies to the number of births at each time point. Lines are drawn with geom_smooth using formula = y ~ x and method = 'loess' and are displayed with 95% confidence intervals.

4.5.3 **Transgenerational effects**

There was no effect of parent heatwave temperature on the weight of pupae (Fig. 4.6A; $\chi^2 = 1.03$, df = 2, p = 0.60) or an interaction between temperature and which parent was heatwave-exposed ($\chi^2 = 3.05$, df = 2, p = 0.22). Overall, offspring from exposed fathers were significantly heavier than those from exposed mothers (Fig. 4.6A; mean difference ± SE = 0.15 ± 0.05, t = 2.93, p = 0.003; $\chi^2 = 8.57$). There was no effect of parent heatwave temperature on emergence rate of offspring (Fig. 4.6B; $\chi^2 = 0.90$, df = 2, p = 0.63), whether mothers or fathers were exposed (Fig. 4.6B; $\chi^2 = 0.86$, df = 1, p = 0.35), or interaction between these variables ($\chi^2 = 0.95$, df = 2, p = 0.62).

At higher heatwave temperatures, there was a significant skew towards the production of females (Fig. 4.6C; $\chi^2 = 8.75$, df = 2, p = 0.01). More females were produced by parents which experienced a 38°C heatwave compared to the 25°C groups (-0.27 ± 0.09; z = -2.89; p = 0.004) and a trend in the same direction for the 36°C group (-0.17 ± 0.09; z = -1.87; p = 0.06). There was no difference in sex ratio of offspring depending on whether mothers or fathers were exposed to heatwave ($\chi^2 = 0.26$, df = 1, p = 0.61), or interaction effect ($\chi^2 = 0.89$, df = 2, p = 0.64).

There was also a small but significant increase in offspring CT_{max} if either parent had undergone a heatwave of 36°C compared to the control group ($\chi^2 = 7.03$, df = 2, p = 0.03; Supplementary Table 4.9; Fig. 4.6D), and a marginally non-significant trend of 38°C parental exposure increasing offspring CT_{max}, to a lesser degree. There was no difference between mother and father heatwave on offspring CT_{max} (Fig. 4.6D; $\chi^2 = 0.002$, df = 1, p = 0.96) or interaction effect (Fig. 4.6D; $\chi^2 = 1.35$, df = 2, p = 0.51). As anticipated, larger flies had greater CT_{max} (Chapter 3; $\chi^2 = 21.06$, df = 1, $p < 0.0001$). Abortions were relatively rare, so sample size was too small for formal analysis. Instead, findings are presented in Supplementary Table 4.10.

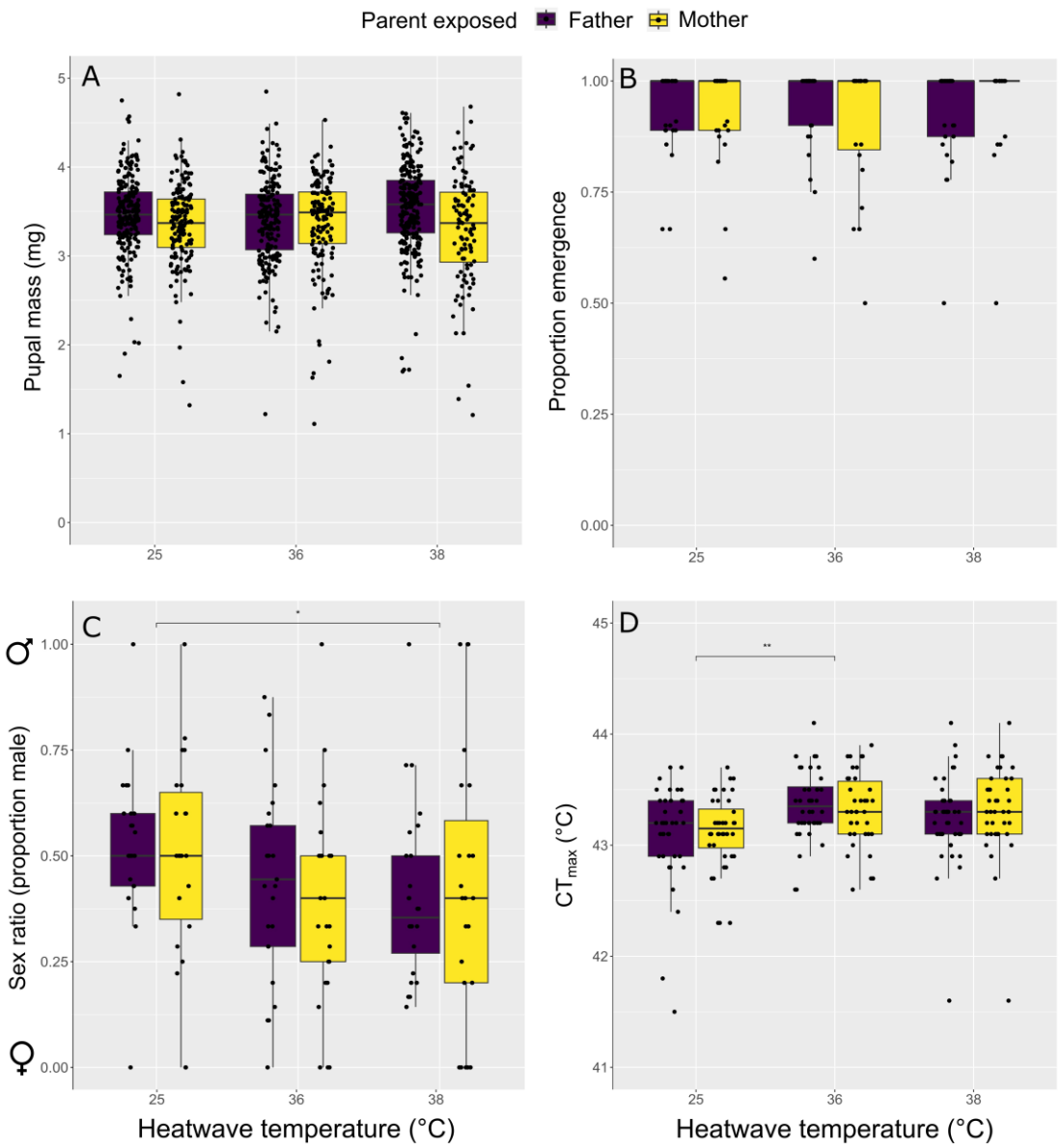


Figure 4.6 Transgenerational effects on offspring of tsetse (*G. pallidipes*) after parents (fathers or mothers) experienced a heatwave. Cages of 5 flies (three female and two male) were allowed six weeks to produce offspring. A. Pupal mass the week of deposition (n = 1049). B. Proportion emergence per cage (n = 144) and C. sex ratio (1 = complete male (♂) bias, 0 = complete female (♀)) were determined per cage. D. Critical Thermal Maximum (CT_{max}) was measured in a random subset (n = 240). Significant values are 0 '***' 0.001 '**' 0.01 '*'.

4.6 Discussion

Here, I exposed male and female tsetse to simulated three-day long heatwave peaking at 36, 38 and 40°C. The three key results are that, first, Thermal Fertility Limits occur below lethal limits, second, female TFLs appear more vulnerable to heatwave than male TFLs, and third, transgenerational effects of heatwave cause a female-biased sex ratio and can prime offspring CT_{max} for future extreme heat (although note that the difference is <0.5°C).

Infertility at sublethal temperatures occurs in many endothermic and ectothermic species (David et al., 1971; Hansen, 2009; Karaca et al., 2002; Perez-Crespo et al., 2008), as in the present study. Counter to general assumptions, female fertility was more sensitive to heatwave than male fertility (Iossa, 2019; Sales et al., 2018; Walsh et al., 2019b, 2019a). Female heatwave exposure reduced overall reproductive output and resulted in temporary infertility, delaying the production of offspring by one week. Recovery after regaining fertility was complete as once flies produced offspring again, reproductive output was no different from the control. In contrast, male exposure caused no obvious reproductive delay, infertility, or decrease in reproductive output.

Unlike effects on reproduction, the effect of heatwave on acute mortality did not depend on which sex was exposed. Strikingly, a heatwave peaking at 40°C killed 100% of individuals, but only a 2°C reduction at 38°C allowed 92% survival. At 36°C, there was no difference in mortality from the control. Although my sample size was low for the 40°C heatwave treatment (n = 5, approximately 10 flies per group), other studies have found that survival probability falls almost to zero at 1, 2, or 3 hour exposures at 40°C (Terblanche et al., 2008). Despite high survival in the 38°C group soon after the heatwave, mortality risk doubled compared to the control over the six-week experiment. Overall, the population size including births, continued to decrease over time if individuals had been exposed to heatwave peaking at either 36°C or 38°C. Therefore, I argue that effects of heatwave on mortality of tsetse are likely more important for population viability than transient effects on female fertility.

Despite similarities between sexes in CT_{max} found in Chapter 3, tsetse female fertility may be particularly vulnerable to high temperature due to high maternal investment in reproduction (English et al., 2023; Hargrove, 2022; Lord et al., 2021; Riordan, 1986). Tsetse face a trade-off between allocating energy for either current reproduction or survival, to enable future reproduction (Barreaux et al., 2022). Therefore, if conditions are unfavourable an adaptive strategy for female tsetse is to delay reproduction either through suspended ovulation or early abortion. Abortion may occur at the egg or early larval stage in response to adverse conditions to avoid high costs of producing a larva (English et al., 2023; Hargrove, 2022; Lord et al., 2021; Riordan, 1986). Indeed, field studies have noted a higher rate of abortions in hotter months of the year (Hargrove, 2022). I found no difference in

abortion rates between treatment groups in the present study (Supplementary Table 4.10), but egg stage abortions may not have been detected as microscopy is necessary (English et al., 2023). Damage to reproductive organs may have also caused a reproductive delay. Abnormal ovaries were found in *Glossina fuscipes fuscipes* after exposure to constant 30°C (Mellanby, 1937). Other studies too have shown that infertility can be temporary in *Drosophila melanogaster* and *T. castaneum*, indicating that physiological repair can occur over time (Canal Domenech and Fricke, 2022; Sales et al., 2021).

Generally, studies find that male gametogenesis is more temperature sensitive than female gametogenesis. Thermal stress can lead to atypical spermatogenesis, producing abnormal sperm form and reduced motility (David et al., 2005; Rohmer et al., 2004). Mature sperm are less temperature sensitive than spermatogenesis and, as a result, the effect of high temperature on male fertility can be delayed (Hirano et al., 2022; Meyerhoeffer et al., 1985; Parratt et al., 2021). In *Drosophila*, this delay results in a weeklong period where there is no apparent effect of heat shock on male fertility until the mature sperm stock is used up (Walsh et al., 2021). In contrast, tsetse sperm production occurs during development and ceases upon emergence (Leak, 1998), meaning that males have their entire mature sperm stock as teneral adults. I suggest this is why tsetse male fertility appears more temperature tolerant than female in the present study. I argue that the stage at which spermatogenesis occurs is an important distinction when assessing the difference between males and females in thermal fertility limits. Instead, male tsetse will likely suffer from the effects of heatwave during the pupal stage. Future work should confirm this hypothesis by examining how the provision of virgin females to heatwave treated males at regular time intervals post-heatwave affects male fertility in tsetse. Additionally, the effect of heatwave in the pupal stage on male fertility of tsetse should be examined.

This study highlights that female fertility should not be overlooked. In sexually reproducing species, often female reproductive output is more important than that of males for population viability (Caswell, 2012), especially in polyandrous species where fertile males can mate with multiple females. Indeed, tsetse population models are often based on female mortality and fertility, assuming that there will be enough males to inseminate the population (Are and Hargrove, 2020). These modelling studies show that small changes in mortality are more important than small changes in fertility for population persistence (Hargrove, 1988), supporting my findings that increases in mortality due to heatwave are likely more important than effects on fertility. However, the success of the sterile insect technique in eradicating tsetse from the island of Zanzibar should be noted, where thousands of sterile males were released to control the population (Vreysen et al., 2014). Similarities can be drawn between this technique and the thermal sterility of males (Walsh et al., 2019a).

Transgenerational effects in response to parental heatwave on offspring sex ratio and thermal tolerance were found, and did not depend on whether mothers or fathers were exposed. Offspring sex ratio was biased towards females when either parent experienced a 38°C heatwave. Sex determination in tsetse might be based on the ratio of X chromosomes to autosomes as they exhibit chromosome aneuploidy (females are XX, XXY, or XXXY, and males are XY, XYY, or XO), similar to *Drosophila* (Cline and Meyer, 1996). It is unknown, however, how temperature could affect this mechanism. From an evolutionary perspective, a female-biased sex ratio may be adaptive as females tend to be larger and longer lived than males (Hargrove et al., 2019, 2011), therefore producing more females may have fitness benefits in times of stress (English et al., 2023). Female biased sex ratio under high temperature has also been identified in moths (Traut et al., 2008), but in this study the authors argue that more females are produced due to favourable conditions as females are more expensive to produce. I also found that if either parent experienced 36°C, their offspring had marginally higher CT_{max} (less than 0.5°C difference), and similarly there was a trend for higher CT_{max} for offspring from parents exposed to 38°C. This could indicate a fitness benefit of parents experiencing a short mild high temperature (transgenerational plasticity), or that those parents which survive this stressful event produce offspring with a higher thermal tolerance (selection). Previous studies have found similar results on other insect species. CT_{max} of *D. melanogaster* offspring was significantly higher when both parents were reared in a variable thermal environment (Cavieres et al., 2020), and another study found similar results if mothers were raised at high temperature (Crill et al., 1996).

Finally, in general, more females died than males over the six-week experiment across all the temperature treatments, and males that underwent water bath treatment gave rise to larger offspring than exposed females. This is surprising as females tend to be larger and survive for longer in the laboratory (Pagabeleguem et al., 2016). I suggest that females may have suffered negative effects from the water baths treatments more than males, such as from excessive handling. Alternatively, females may have experienced male harassment as other studies have suggested a ratio of three to four females per one male (Clutton-Brock and Langley, 1997; Opiyo et al., 2006). However, a 25°C water bath control was used for both sexes with which comparisons are made to account for this difference. In future, researchers should be aware that handling may affect female tsetse more than males, and the importance of sex ratio in cages.

Here, the response of male and female fertility to heatwave in a tsetse is investigated. Female Thermal Fertility Limits are found to occur below temperatures which are fatal. However, thermal effects on fertility are transient whereas those on survival have a greater impact on population viability. Ultimately, female fertility limits should not be overlooked, but I emphasise the importance of recovery in fertility after heatwave exposure.

5. Chapter 5 - No evidence for direct thermal carryover effects on adult survival in the obligate blood-feeder, *Glossina morsitans morsitans*

5.1 Statement

This chapter is published in Ecology and Evolution with authors: Hester Weaving, Jennifer Lord, Lee Haines, and Sinead English. I, Hester Weaving, completed the analysis, wrote the code, and led the writing of the manuscript and reviews. SE supervised the work. All authors contributed to conceptualisation and the development of drafts. Find here: Weaving, H., Lord, J., Haines, L., and English, S. (2023) No evidence of direct thermal carryover effects on starvation tolerance in the obligate blood-feeder, *Glossina morsitans morsitans*. *Ecology and Evolution,* **13**, e10652. 10.1002/ece3.10652.

5.2 Abstract

Thermal acclimation during development can prime animals to cope better with similar conditions in later life. Alternatively, negative effects of temperature can persist across life stages and result in poorer quality adults (negative carryover effects). As mean temperatures increase due to climate change, evidence for such effects across diverse taxa is required. Using *Glossina morsitans morsitans*, a species of tsetse fly and vector of trypanosomiasis, we asked whether i) adaptive developmental plasticity allows flies to survive for longer under blood deprivation when pupal and adult temperatures are matched; or ii) temperature stress during development persists into adulthood, resulting in a greater risk of death. No advantage of matched pupal and adult temperature in terms of improved adult survival was found, and no direct negative carryover effects were observed. There was some evidence for indirect carryover effects - high pupal temperature produced flies of lower body mass, which, in turn, resulted in greater adult mortality. However, adult temperature had the largest impact on survival by far: flies died 60% faster at 31°C than those experiencing 25°C, consequently reducing survival time from a median of 8 (interquartile range (IQR) 7 – 9) to 5 (IQR 5 – 5.25) days. This highlights differences in temperature sensitivity between life stages, as there was no direct effect of pupal temperature on adult survival. Overall, for some regions of sub-Saharan Africa, climate change may result in a higher mortality rate in emerging tsetse due to either desiccation or starvation whilst they search for their first blood meal. This study reinforces existing evidence that responses to temperature are life stage specific and that plasticity may have limited capacity to buffer the effects of climate change.

5.3 Introduction

Many animals can modify their phenotypes in response to the environment experienced during development, often in a way that provides a fitness benefit in adulthood (Fawcett and Frankenhuis, 2015; West-Eberhard, 2002). This adaptive developmental plasticity may be an important response to heat stress given rising mean temperatures due to climate change, especially as research indicates that insects tend to be most plastic during developmental stages, rather than during adulthood (Chapter 2; Pottier et al., 2022; Weaving et al., 2022). Phenotypic changes can be adaptive when the offspring and adult thermal environments are matched, or when variability is predictable, e.g. seasonal (Monaghan, 2008). However, these anticipatory phenotypic changes can have a deleterious effect if the environment becomes mismatched, for example as seasons advance due to climate change, or as conditions become more unpredictable and extreme (Parmesan, 2006). Thermal stress during development can also be harmful, where deleterious effects extend from juvenile stages into adulthood, which can result in the emergence of poor quality adults (Kingsolver and Huey, 2008).

Insects are a good model for the study of developmental plasticity due to their life histories with multiple distinct stages, and trackable, short life spans (English and Barreaux, 2020). Adaptive developmental plasticity, where matching juvenile and adult temperatures provide a fitness benefit, has been previously observed in insects. For example, high developmental temperature leads to lighter adult pigmentation in the harlequin bug (*Murgantia histrionica*), and fewer dark spots in ladybirds (*Harmonia axyridis*), which subsequently reduces the risk of overheating (Michie et al., 2011; Sibilia et al., 2018). Similarly, heat stress during development in red flour beetles, *Tribolium castaneum,* improves heat shock tolerance in adults (Scharf et al., 2014). In contrast, if juvenile and adult environments are mismatched, plasticity can become deleterious. For example, the tropical butterfly, *Bicyclus anynana*, is seasonally polymorphic with camouflage suited to either the warm, wet season or the dry, cool season. When the conspicuous, wet season form is released in the dry season, these butterflies have lower rates of survival compared to the resident form (Brakefield & Reitsma, 1991; Brakefield & French, 1999). Unseasonable temperatures caused by climate change could therefore trigger a mismatch of wing pattern to season, thus resulting in greater predation (De Jong et al., 2010).

Deleterious plasticity can also occur when unfavourable conditions during development result in reduced fitness in the adult stage, defined here as negative carryover effects (although note that carryover effects can be defined as effects which increase fitness too, see O'connor et al. (2014)). Warmer developmental temperatures often result in smaller adult body size, known as the temperature-size rule, which tends to be related to lower fecundity, mating success and survival (Kingsolver and Huey, 2008). Heat stress during development can additionally cause direct damage to

reproduction, as demonstrated in several *Drosophila* species. When larvae develop at their upper thermal limit, emerging adult males have reduced sperm mobility and smaller testis length, and females have fewer ovarioles and reduced fertility (Porcelli et al., 2017; Sisodia & Singh, 2009).

Studies have typically focused on how heat stress during development affects later adult thermal traits, but some have considered other fitness-related traits such as starvation tolerance and desiccation resistance. These measures are useful fitness proxies because many organisms face periods without food or water, and these resources are likely to become more unpredictable as climate change progresses (IPCC, 2021). This cross-tolerance, where exposure to one environmental stressor results in resistance to another, has been demonstrated for starvation tolerance, desiccation resistance and thermal tolerance in several insect species (Bauerfeind et al., 2014; Bubliy et al., 2012; Gotcha et al., 2018; Kalra et al., 2017). For example, in the red flour beetle, *T. castaneum*, adults raised at high developmental temperature have better tolerance to starvation as adults, as well as improved heat and cold tolerance (Scharf et al., 2014). Similarly, in the butterfly *B. anynana*, high developmental temperature increased starvation tolerance in adults, but this result was dependant on polyphenic morph and sex (Pijpe et al., 2007). For *Drosophila melanogaster*, flies reared at cool temperatures had improved cold tolerance, desiccation, and starvation resistance (Bauerfeind et al., 2014). However, cross-tolerance is not universal to all combinations of stressors as some studies find no association or a negative association (Bubliy et al., 2012; Kalra et al., 2017). Clearly, thermal developmental plasticity of among these traits is complex and further studies are needed, particularly using a greater diversity of species with varying life histories.

Starvation tolerance and desiccation resistance are relevant to blood-feeding vectors as vertebrate blood is a temporally and spatially unpredictable food source. This is particularly true for obligate blood feeders, where the time between blood meals is critical to survival as they cannot rely on other sources of sustenance or hydration. As temperatures increase due to climate change, host availability may shift (Dawe and Boutin, 2016; Simon et al., 2014), and higher metabolic demands and increased water loss in ectothermic blood feeders may lead to behavioural changes, such as increased bite rate (Rogers and Packer, 1993). Studies have also shown that a period of blood deprivation can increase competency to infection in vectors such as mosquitoes and tsetse flies (Gingrich, et al., 1982; Gooding, 1988; Herd et al., 2021). Therefore, research into how vectors respond to blood deprivation under high temperature can have important epidemiological implications.

Tsetse (*Glossina* spp.) are insect vectors of human and animal African trypanosomiasis, which are prevalent diseases throughout sub-Saharan Africa (Buxton, 1955). Both male and female flies feed exclusively on vertebrate blood so may undergo extended periods without taking a bloodmeal due to

the spatial and temporal unpredictability of vertebrate animals (Lehane, 2005; Lord et al., 2017). Newly emerged teneral flies are at risk of mortality as they rely solely on reserves provided by the mother until they locate their first blood meal. This is due to adenotrophic viviparity, where mothers nourish one offspring at a time *in utero* with a milk-like secretion (Benoit et al., 2015; Haines et al., 2020). The offspring is deposited as a late-stage larva, which rapidly burrows below ground to pupate, and does not feed until emergence around a month later (Buxton, 1955). Teneral flies must rapidly locate their first blood meal or risk starvation and desiccation (Hargrove, 2004; Lord et al., 2017; Phelps & Clarke, 1974).

In the laboratory, relationships between temperature and development rates, body size, lipid consumption, water loss and pupal mortality have been well documented for *Glossina* species (Bursell, 1960; Hargrove and Vale, 2020; Kleynhans and Terblanche, 2011; Phelps, 1973; Phelps and Burrows, 1969). As pupal temperature increases, development times become shorter and resulting adults are smaller (Bursell, 1960). The total lipid consumed during pupation is lowest at 25°C, and thus the reserves available to a teneral fly decrease above this temperature. At temperatures above 32°C, increased pupal mortality rates result from direct effects of temperature, rather than from lipid consumption alone (Hargrove & Vale, 2020). Developmental plasticity has also been investigated in tsetse, where changes to development temperature result in plasticity of lower thermal limits, but not upper thermal limits (Terblanche and Chown, 2006). In adult tsetse, water and lipid loss has been measured in several species subject to a range of temperatures and humidities. Results show that water loss rates were affected by temperature and humidity, but body water, lipid content, and body mass, less so. Overall, high adult temperature led to shorter predicted survival times (Kleynhans and Terblanche, 2011). Despite the importance of temperature on the quality of emerging teneral flies, the effect of developmental temperature on adult survival under blood deprivation remains unknown in tsetse.

Here, I investigated whether pupal and adult temperature stress influences adult survival when tsetse (*G. m. morsitans*) are deprived of blood. The aim of the study was to test whether adaptive developmental plasticity allows flies to survive for longer when pupal and adult temperatures are matched, or if negative carryover effects from high temperature during development reduces survival in emerging adults. I also measured effects of developmental temperature on adult size, and the relationship between size and adult survival, as a potential mechanism linking pupal temperature to adult survival.

5.4 Methods

5.4.1 Colony maintenance

Glossina morsitans morsitans pupae were collected from an established colony at the Liverpool School of Tropical Medicine (LSTM) on 14th and 15th November 2019. The colony is maintained at 25 ± 2°C, 68–79% relative humidity (RH) and a 12:12 h light:dark cycle, and fed on defibrinated horse blood (TCS Biosciences) three times a week. Pupae were held 5–48 h after being deposited under the above conditions until transport to the University of Bristol. For each pupa, maternal age was known ± one week, ranging from three to nine weeks old. Misshapen and undersized pupae were not included in the study.

5.4.2 Experimental design

Pupae (n = 440) were divided between four treatment groups (Fig. 5.1). Pupae from each collection date and maternal age group were divided equally between the groups as there is a relationship between pupal mass and maternal age (Lord et al., 2021; Supplementary Table 5.1). Each pupa was transferred into a 50 ml falcon tube with conical base (Fisher Scientific, Loughborough) drilled with 2 mm holes in the centre of each lid to allow for gas exchange. Treatment groups followed a full factorial design. Half of the pupae were maintained at 25°C and half at 31°C. Upon adult emergence, half of the flies were moved to the alternate treatment, and the other half remained in the same temperature. This gave four treatments in total: two where the pupal and adult stages were matched (25°C / 25°C or 31°C / 31°C) and two where the pupal and adult stages were mismatched (25°C / 31°C or 31°C / 25°C). The control treatment of 25°C was chosen as a baseline as the minimum quantity of lipid is used at this temperature during the pupal stage, and as this is the temperature at which the tsetse colony at LSTM is maintained. A temperature of 31°C was used as a heat treatment as it is near to the upper temperature at which pupation will be completed (32°C), but few deaths occur due to the direct effects of heat stress (Hargrove & Vale, 2020). Tsetse regularly experience these temperatures in the field. For example, see daily temperature data collected at Rekomitjie Research Station in Mana Pools National Park in Supplementary data for Lord et al. (2018).

The 25°C treatment was maintained in a Reftech room (Reftech, Sassenheim, The Netherlands), and the 31 °C treatment in an incubator (Snijders Labs, Tilburg, The Netherlands). Flies were maintained at 60% RH, in a 8:16 h light:dark cycle. Temperature and humidity were recorded using iButton data loggers (Mouser Electronics, High Wycombe) placed inside an empty falcon tube. iButtons recorded actual mean temperatures (± SD) of 24.9 ± 0.8°C, and 31.3 ± 0.4°C. The 25°C and 31°C treatments were 56.1 RH ± 1.4% and 55.9 RH ± 0.9%, respectively. Relative humidity differed by 8 to 19% from the LSTM colony conditions due to other experiments running in the communal Reftec room concurrently. Approximately 10% reduction in emergence has been found between 80% RH and 60% RH (Bursell,

1958). Light:dark cycle was altered from colony conditions from 9am – 9pm to 9am – 5pm. Altering dusk does not affect flight patterns in *G. m. morsitans* so this alteration should not affect tsetse activity (Brady and Crump, 1978). Humidity and light:dark cycle were matched between treatments to eliminate potential confounding variables in each group.

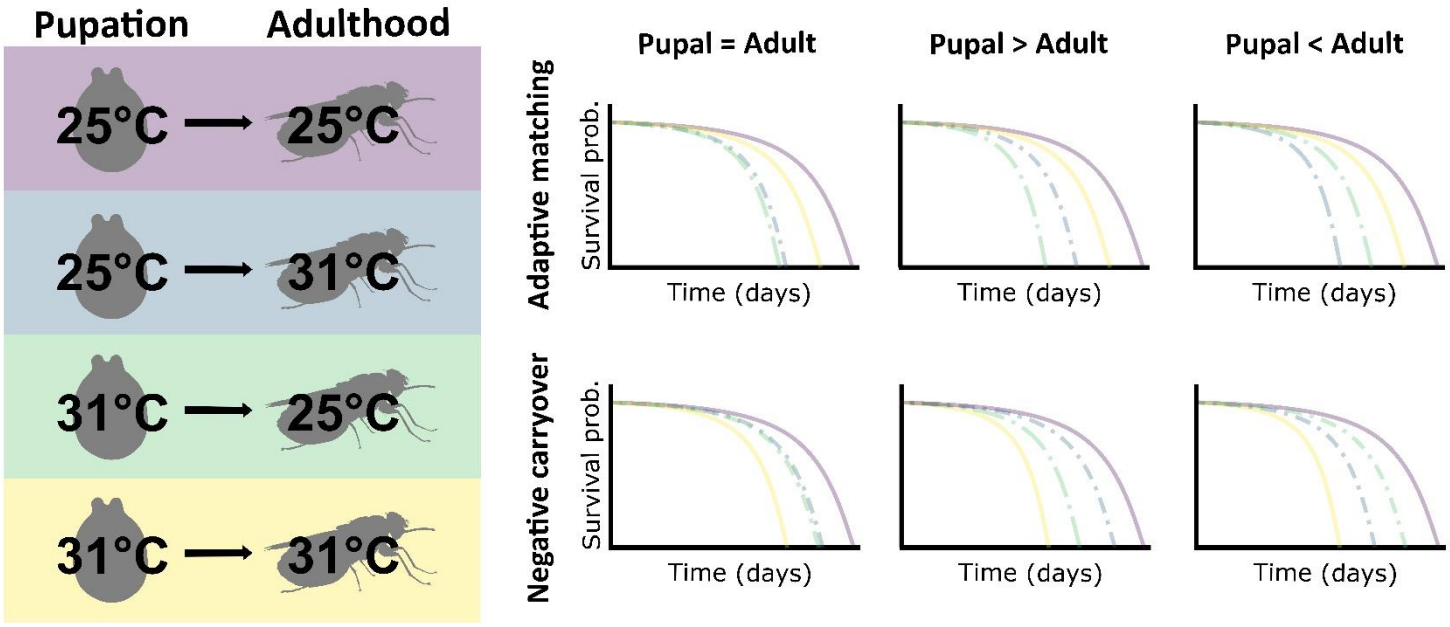


Figure 5.1 Overview of the experimental design and predicted outcomes. Pupae were kept at either constant 25°C or 31°C for development and transferred to either a matched (solid) or unmatched (dot-dashed) temperature upon emergence for adulthood. The time until death was recorded in absence of blood meals. Predictions are given for the relationship between the probability of survival and time (days) under the hypotheses of either adaptive matching or negative carryover effects. Predictions are given for: an equal effect of pupal and adult temperature on survival, or an unequal effect i.e. Pupal > Adult or Pupal < Adult.

5.4.3 **Data collection**

Pupae were checked daily between 09:00 and 10:00 for adult emergence. Pupal development time was recorded in days, and emerging flies were either returned to the original temperature treatment or moved to the contrasting treatment. Flies were sexed on emergence. Pupae were assumed inviable if they had not emerged by the end of the 47 day experiment as development takes approximately 30 days at 25°C degrees (Phelps and Burrows, 1969).

Both mass and size were measured to account for direct effects of temperature on body mass or size, and to ascertain whether they affect survival. Both mass and size measurements were taken, as mass indicates resource use throughout the experiment, but adult size is influenced only by developmental temperature, becoming fixed at emergence. Mass measurements were taken at three stages: pupal mass (approx. 60 h after larviposition), adult mass at emergence (within 24 h, including puparium mass), and mass at death (within 24 h, including puparium mass). Both adult masses were taken within the falcon tube, with the tube mass later subtracted. Pupal mass indicates the reserves provided maternally at the start of the experiment, adult mass is indicative of the effect of pupal temperature on reserves until adult emergence, and mass at death indicates the effect of pupal and adult temperature on reserves. Mass loss was calculated for the pupal and adult stage to give a rough estimate of energy reserve use and water loss (Brady, 1975). For pupal mass loss, teneral adult mass was subtracted from pupal start mass. Adult mass loss was calculated by subtracting adult mass at death from teneral adult mass. A Sartorius CPA26P balance (Göttingen, Germany), accurate to 2/10000 mg, was used to take the mass measurements. Adult flies were kept at room temperature (~20°C) when weighed, and the process lasted no longer than 25 min.

Wing length – a proxy for adult size – was measured by removal of the left wing after flies had died. The hatchet cell section of the fourth longitudinal vein was selected for measurement (Supplementary Figure 5.1; Jackson, 1946). Images were taken with a Leica MZ21 camera microscope (Wetzlar, Germany) and LAS V3.8. The image (calibrated using a 1 mm graticule) was later analysed using ImageJ (Version 1.52a) to measure the wing vein in mm. Wing measurements could not be obtained for 43/428 adults because the emerged adult flies were unable to unfurl their wings (see Results section for details on how these were distributed across the temperature treatments).

Teneral adult fly mortality was recorded daily between 09:00 and 10:00 h by flicking the tube in which the fly was held to test for movement. If no movement was observed, the fly was considered dead and the number of days since emergence recorded as the time until death. Here, we consider the number of days between adult emergence until death as an indicator of starvation tolerance and desiccation resistance, but since tsetse consume only blood, death may have been due to either

dehydration or starvation. Note that tsetse can drink water in a highly artificial laboratory situation from a membrane, but evidence that this behaviour occurs in the field is sparse (Solano et al., 2015).

5.4.4 **Data analysis**

All data analyses were completed in R Studio (Version 3.5.1). Data and code are deposited at: https://osf.io/t4r5s/. The effect of pupal temperature, sex and mass (and the two-way interactions between these variables) on development time was determined by Generalised Least Squares due to unequal variances across treatment groups. The nlme package (Version 3.1-162) was used to fit the model by the residual maximum likelihood method. Development time was log transformed so that the model residuals were normally distributed. The final model was determined by comparing AIC and deviance between models and examining residuals for normality. The 'drop one' method was used whereby, starting from a maximal model, each term is excluded and removed if it does not significantly improve model fit (Bradburn et al., 2003). Posthoc analyses were completed by Tukey Honest Significant Differences using the emmeans package (Version 1.8.5). Adults that did not emerge from the puparium were not included in the models (n = 12; see Results for breakdown depending on temperature treatment).

The effect of pupal temperature and sex on adult size (either using wet mass or wing vein length) was determined by a linear model, controlling for maternal age as a covariate (as it has been shown to affect offspring size (Lord et al., 2021)). Variables with no significant effect were removed using the 'drop one' method. Posthoc analysis was completed as above. Mass loss through pupation and adulthood (until death) was also investigated by two generalised linear models. Pupal mass loss data were not normally distributed, so the log link function was used. The effects of temperature, sex, and their interaction, on mass loss were tested using the 'drop one' method. All model residuals were normally distributed.

A Cox proportional hazards model was used to determine how adult survival differed between the four temperature treatments, and whether pupal mass or sex had an effect. Packages 'survivalAnalysis' (Version 0.3.0), 'survival' (Version 3.2.13) and 'survminer' (Version 0.4.9) were used (Therneau and Grambsch, 2000). Temperature, sex and pupal mass, and interactions between these variables were investigated. Variables with no significant effect were removed using the 'drop one' method. Hazard ratios refer to the risk of death where ratios of greater than 1 suggest higher risk of death, and below 1 lower risk of death compared to the reference group. Pupal mass was used in these models as it is independent of the temperature treatments so represents differences in size before the experiment began. Three individuals were not included in the analysis as they died within 24h of emergence, i.e. before transfer into the adult temperature (due to failure to leave the pupal

case). Posthoc analyses were completed by Tukey Honest Significant Differences using the emmeans package.

Finally, a path analysis was run to ascertain whether pupal temperature indirectly affected adult survival through its effect on adult mass, in addition to the direct effects of pupal and adult temperature. The model was run using packages lavaan (Version 0.6-15) and semplot (Version 1.1.6) for data visualisation. The effect of pupal temperature and pupal mass on adult mass and the effect of pupal temperature, adult temperature, pupal mass and adult mass on adult survival was tested.

5.5 Results

5.5.1 Development time is shorter at high temperatures and in females

Pupal development time was 10.8 days shorter when pupae were kept at 31°C compared to 25°C (gls: $F = 4160$, $df = 1$, $P < 0.001$), and females developed 1.6 days more quickly than males, as expected based on previous studies (gls: $F = 515$, $df = 1$, $P < 0.001$, Supplementary Table 5.2, 5.3; Fig. 5.2). An interaction between sex and temperature showed that the difference in development time between males and females was slightly greater at higher temperatures (gls: $F = 7.7$, $df = 1$, $P < 0.001$; Supplementary Table 5.3, 5.4). Initial pupal mass had no effect on development time (gls: $F = 3.3$, $df = 1$, $P = 0.07$; Table 5.2; Supplementary Table 5.2, 5.3). Only two individuals failed to emerge in the 25°C treatment, whereas there were ten inviable pupae in the 31°C treatment. Of the 10% (n = 43/428) of individuals that emerged with deformed wings, almost all (n = 42/43) belonged to the high temperature treatment, and most were male (female:male, 15:28). All individuals (n = 3/428) that emerged but failed to leave the puparium were from the 31°C treatment.

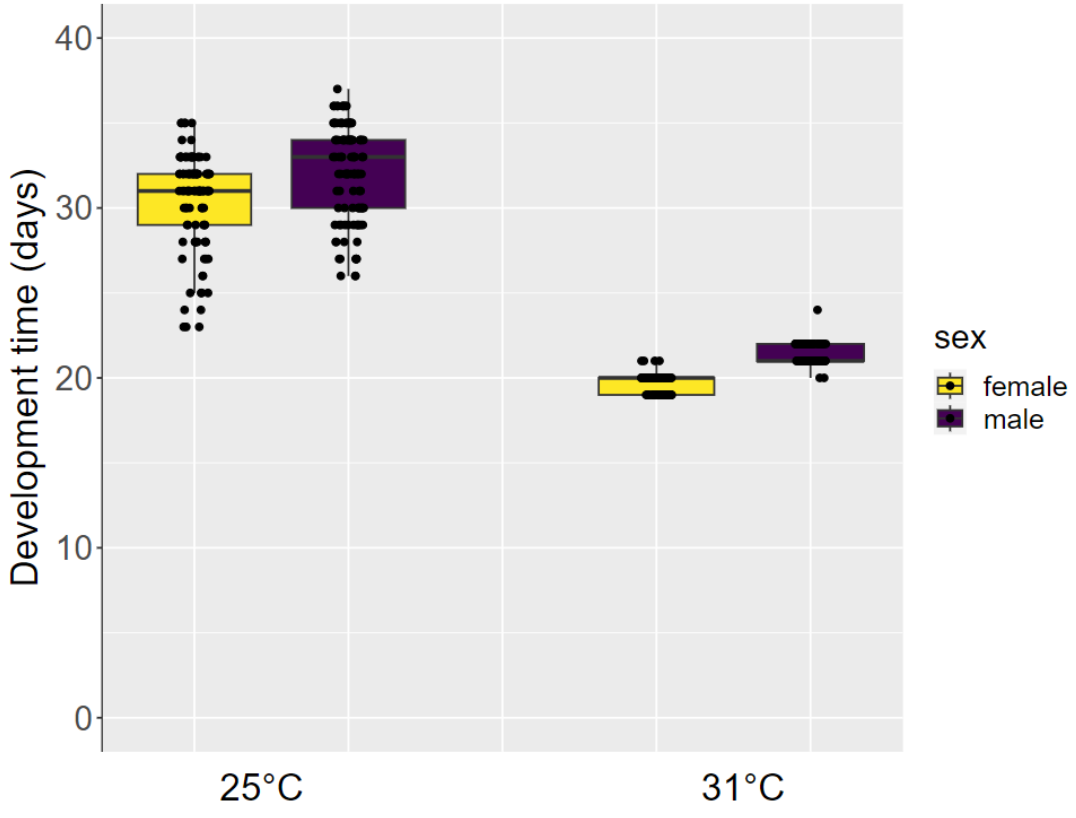


Figure 5.2 Pupal development time (days) for tsetse (*G. m. morsitans*) maintained at constant temperatures of 25°C or 31°C. Median values and interquartile ranges are displayed with data split into females and males.

5.5.2 **High pupal temperature produces smaller adults**

As expected, initial pupal mass before temperature treatment did not differ between treatments or sexes. Controlling for maternal age and sex, emerging adults were smaller when pupae developed at 31°C as evidenced by teneral adult mass (lm: SS = 150, df = 1, $p < 0.001$) and wing vein length (lm: SS = 0.004, df = 1, $p < 0.001$; Supplementary Table 5.5). Individuals maintained at 31°C during pupation, compared to those maintained at 25°C, weighed on average 1.1 mg less (5 %) at emergence and their wing veins were 0.006 mm shorter (0.4 %). Across both temperature treatments, adult males weighed 0.80 mg less than females (3 %) and had 0.16 mm shorter wing veins (10 %).

5.5.3 **Mass loss is greater at high temperature and during adulthood**

Mass loss during pupation (glm: $\chi^2 = 65.5$, df = 1, $p < 0.0001$) and adulthood (glm: $\chi^2 = 56.7$, df = 3, $p < 0.001$) exposed to 31°C was greater than those kept at 25°C (Supplementary Table 5.6, 5.7; Fig. 5.3). Rate of adult mass loss was approximately 10-fold greater than the rate of pupal mass loss (Supplementary Table 5.6). Across both temperature treatments, males lost more mass than females during pupal development (glm: $\chi^2 = 21.6$, df = 1, $p < 0.001$), but the opposite was true for adulthood, where female mass loss was greater than male mass loss (glm: $\chi^2 = 6.1$, df = 1, $p = 0.01$; Supplementary Table 5.6, 5.7). Post-hoc analyses examining pairwise comparisons between temperature treatment groups are given in Supplementary Table 5.8.

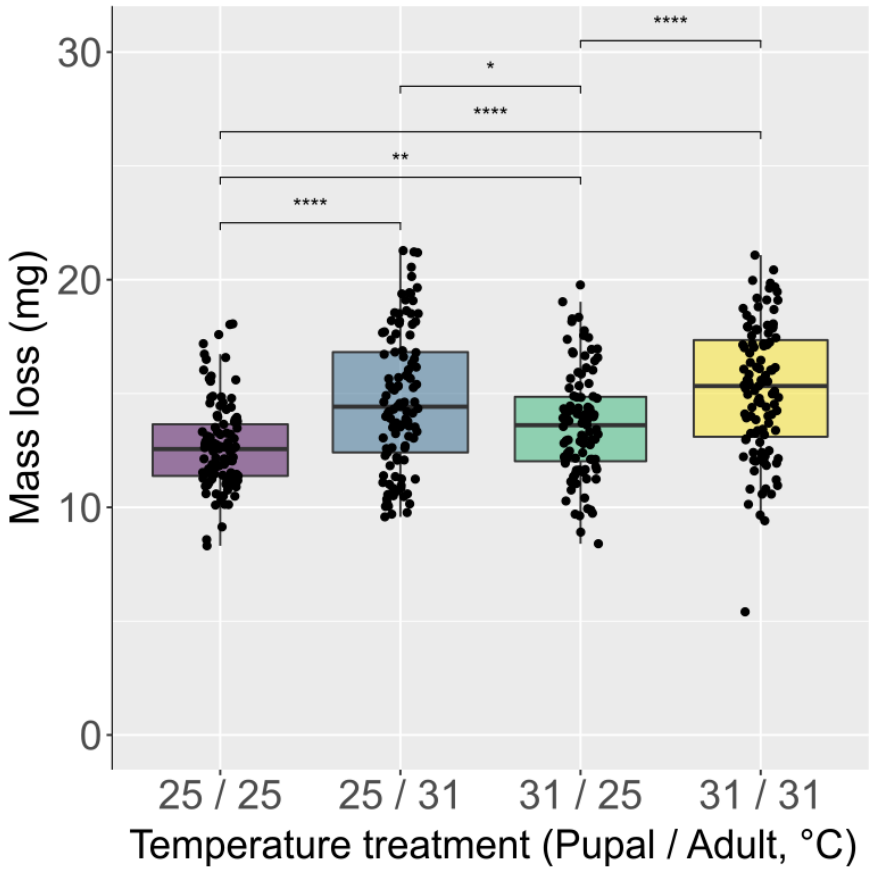


Figure 5.3 Total mass loss (mg) of tsetse (*G. m. morsitans*) under four temperature regimes (pupal / adult) of constant 25°C or 31°C. Shown are the median, interquartile range and confidence interval (boxes and lines) as well as the individual data (black points). Colours indicate treatment groups as described in Figure 5.1.

* = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$ **** = $p < 0.0001$.

5.5.4 **High adult temperature and low body mass reduce adult survival**

Adult survival was primarily influenced by adult temperature, with 40% greater mortality risk in flies maintained at 31°C in comparison to those at 25°C, consequently reducing median survival time from 8 (IQR 7 – 9) to 5 (IQR 5 – 5.25) days ($\chi^2 = 279$, df = 3, $p < 0.001$; Table 5.1; Fig. 5.4). There was no effect of matched pupal and adult temperature on survival, or evidence for negative carryover effects (Table 5.1). Pupae with greater mass at the start of the experiment (before experiencing any temperature treatment) had greater adult survival; for every additional 1 mg body weight, mortality risk decreased by 16% ($\chi^2 = 46.5$, df = 1, $p < 0.001$; Table 5.1). We found a significant interaction between sex and temperature ($\chi^2 = 31.7$, df = 3, $p < 0.001$; Table 5.1; Fig. 5.5; Supplementary Figure 5.2a, b) whereby male tsetse took longer to die than females when pupae were maintained at 31°C and adults at 25°C, yet died more quickly than females when kept at 31°C for their entire lives. This is suggestive of a priming effect of being maintained at 31°C, for males only. Post hoc analysis examining the interaction between treatment temperature and sex is given in Supplementary Table 5.9.

Path analysis indicated that the largest effect on adult survival was adult temperature, in accordance with the survival analysis (Table 5.2; Fig. 5.6). The analysis also illustrated an indirect effect of pupal temperature on adult survival through its effect on teneral adult mass. High pupal temperature produced flies with smaller teneral adult mass which, in turn, show reduced adult survival. Initial pupal mass was strongly related to teneral adult mass illustrating the importance of maternal effects on adult survival.

Table 5.1 Multivariate Cox Proportional-Hazards Models examining adult survival risk of unfed tsetse (*G. m. morsitans*) under four constant temperature regimes, given as pupal / adult temperature (°C) (n = 428). Hazard ratios of greater than 1 suggest higher risk of death, and below 1 lower risk of death compared to the reference group. Significant results are in bold font.

Variable	Treatment	Hazard ratio	z-score	p-value
Temperature	25 / 31	**45.97 (26.17 - 80.74)**	**13.32**	**<0.001**
	31 / 25	1.30 (0.80 - 1.91)	1.36	0.17
	31 / 31	**29.71 (17.39 - 50.75)**	**12.41**	**<0.001**
Pupal mass		**0.85 (0.81 - 0.89)**	**-6.72**	**<0.001**
Sex	Male	**1.23 (0.84 - 1.80)**	**1.09**	0.28
Temperature x	25 / 31 x Male	0.84 (0.50 - 1.44)	-0.62	0.54
sex	31 / 25 x Male	**0.35 (0.20 -** 0.61**)**	**-3.70**	**<0.001**
	31 / 31 x Male	**1.71 (0.99 - 2.94)**	**1.94**	**0.05**

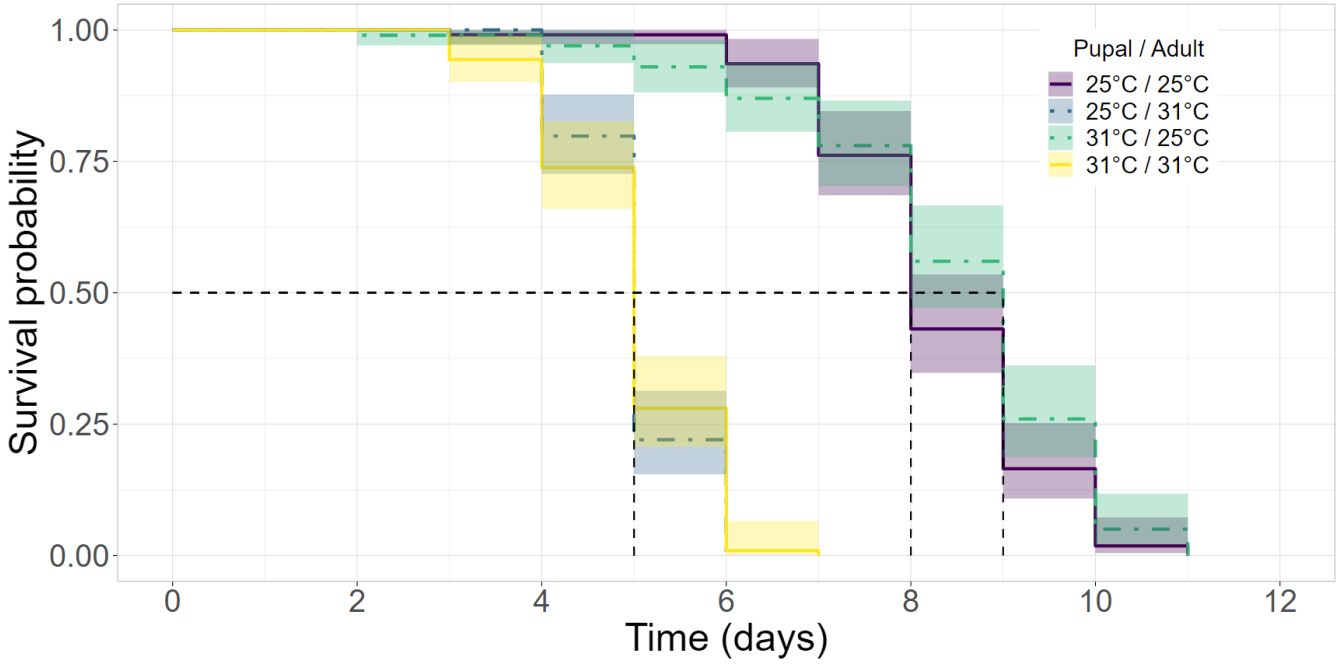


Figure 5.4 Probability of survival for unfed adult tsetse (*G. m. morsitans*) over time (days) under four temperature regimes throughout pupation and adulthood. Temperature was maintained at constant 25°C or 31°C and is given as pupal temperature / adult temperature. Matched pupal and adult temperature is depicted by solid lines and unmatched temperatures are depicted by dot-dash lines. 95% confidence intervals are shaded and black dotted lines mark where 50% of the population have survived.

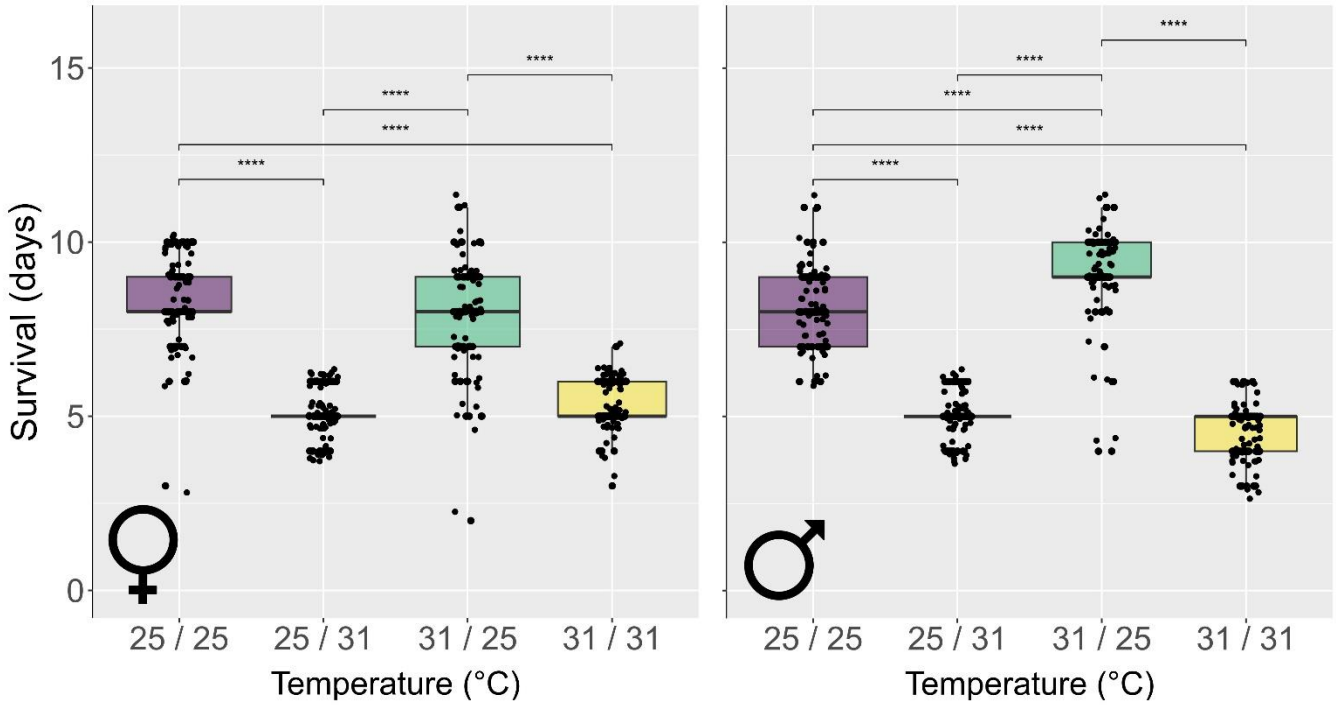


Figure 5.5 Female (♀) and male (♂) tsetse (*G. m. morsitans*) median survival (days) over pupation and adulthood, under blood deprivation, under four constant temperature regimes (pupal / adult) of 25°C or 31°C. Shown are the median, interquartile range and confidence interval (boxes and lines) as well as the individual data points. Colours indicate treatment groups as described in Figure 5.1.

* = p < 0.05 ** = p < 0.01 *** = p < 0.001 **** = p < 0.0001.

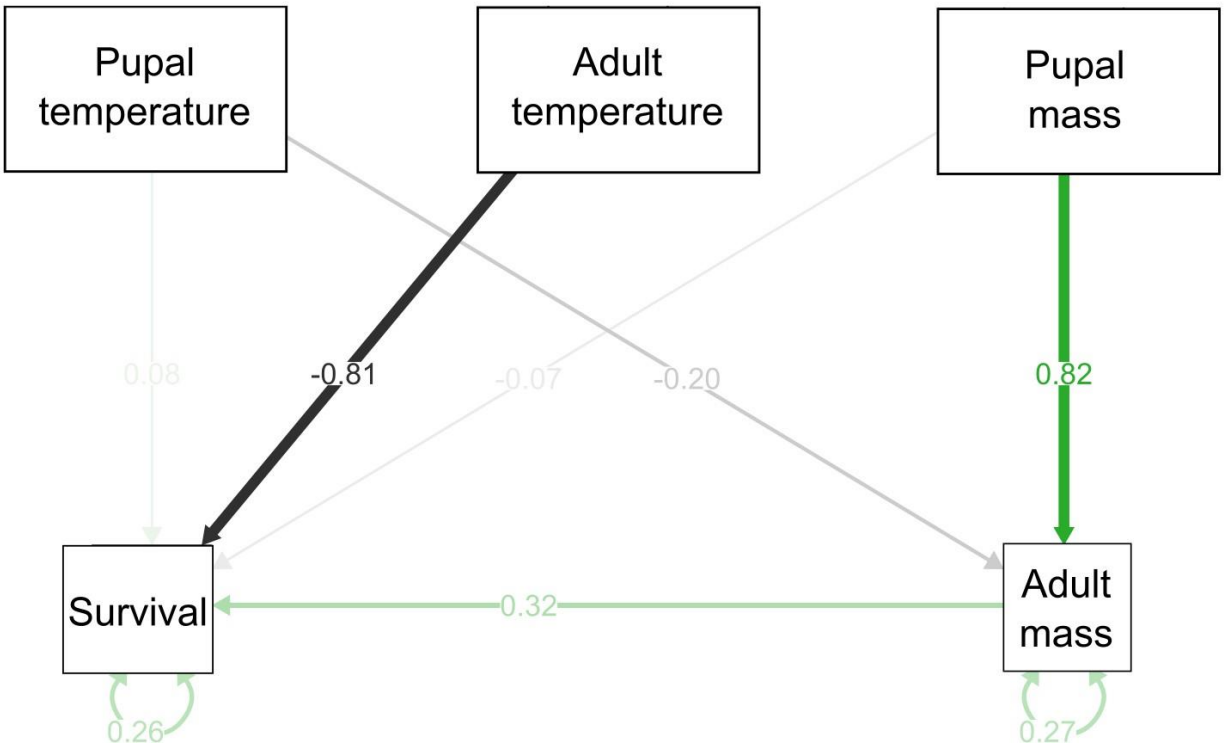


Figure 5.6 Path analysis showing the effect of mass and pupal or adult temperature on teneral adult survival in tsetse (*G. m. morsitans*). Straight arrows indicate a causal relationship. Variance is indicated by double-ended curved arrows. The path coefficient is overlayed on each arrow and indicates the strength of each causal relationship. Higher colour intensity indicates stronger relationship weights, green for positive associations while black denotes negative associations.

Table 5.2 Results from path analysis investigating the effect of pupal temperature and mass on adult mass, as well as the effect of pupal and adult temperature and mass on teneral adult survival (days) in tsetse (*G. m. morsitans*). Significant results are in bold font. SE = standard error.

	Estimate	SE	z-value	P value	Standardised latent coef.	Standardised coef.	R^2
Regressions:							
Survival ~							
Pupal temp	**0.05**	**0.02**	**2.9**	**0.004**	**0.05**	**0.08**	0.74
Adult temp	**-0.55**	**0.02**	**-32.5**	**<0.001**	**-0.55**	**-0.81**	
Pupal mass	-0.07	0.04	-1.6	0.11	-0.07	-0.08	
Adult mass	**0.27**	**0.04**	**6.7**	**<0.001**	**0.27**	**0.32**	
Adult mass ~							
Pupal temp	**-0.16**	**0.02**	**-7.8**	**<0.001**	**-0.16**	**-0.20**	0.73
Pupal mass	**0.93**	**0.03**	**32.6**	**<0.001**	**0.93**	**0.83**	
Variances:							
Survival	**1.09**	**0.08**	**14.6**	**<0.001**	**1.09**	**0.26**	
Adult mass	**1.61**	**0.11**	**14.6**	**<0.001**	**1.61**	**0.27**	

5.6 Discussion

Here, adult survival in tsetse was largely dependent on the temperature to which newly emerged (teneral) adults were exposed; teneral flies died three days earlier if kept at 31°C compared to 25°C. In contrast, pupal temperature played a minor or non-existent role on adult survival. Thus, in contrast to predictions, no strong evidence for adaptive developmental plasticity was found, as there was no effect of matching pupal and adult temperatures on teneral adult survival. Similarly, there was no evidence for direct carryover effects - overall, a 6°C difference in pupal temperature did not affect adult survival.

The largest effect was that of adult temperature on teneral fly survival when deprived of blood rather than pupal temperature, as found in other similar studies (Pijpe et al., 2007; Scharf et al., 2014). High temperatures elevate metabolic rate, thus energy reserves burn more rapidly and water loss rate increases due to cuticular loss and increased respiration (Chown et al., 2011). The pupal stage can mitigate some of the impacts of elevated temperatures due to its inactivity and protective cuticle, as evidenced by a tenfold reduction in the rate of mass loss. A considerable proportion of mass loss is likely due to higher water loss rates under high temperature (Kleynhans and Terblanche, 2011). Additionally, some mass loss will result from higher rates of lipid consumption; a positive log-linear relationship has been demonstrated in various tsetse species where adult metabolic rate increases between 20°C and 32°C (Terblanche et al., 2005). It is unclear whether flies died due to starvation or desiccation in the present study as body lipid and water measurements were not taken, but according to other studies on tsetse, it seems more likely that the flies desiccated rather than starved (Bursell, 1959; Kleynhans and Terblanche, 2011).

In response to high temperature, fly bite rate is predicted to double with a 5°C temperature increase (Terblanche and Chown, 2007). Therefore, as conditions become warmer, emerging tsetse will need to locate hosts rapidly, or risk starvation and desiccation. Additionally, feeding is a high-risk activity, so a higher bite rate is likely to result in increased mortality of tsetse through predation as temperature increases (Hargrove & Williams, 1995; Randolf et al., 1992). Indeed, mark recapture studies conducted in Zimbabwe and Tanzania find that adult tsetse survival probability decreases with increasing temperature (Hargrove, 2001), and that mortalities in the field are particularly high in the hot season (see references within Hargrove, 2004). In Zimbabwe, high temperatures may already be causing declines in tsetse abundance. At Rekomitjie research station, tsetse numbers have been continually recorded since the 1960s and these data show a drastic decline in the numbers of adult flies collected (Mangwiro et al., 1999; Thomson, 1987; Torr, Holloway, & Vale, 1992). Mechanistic modelling suggests that the decline can be explained by a 0.9°C increase in temperature since 1975 (Lord et al., 2018).

Some evidence of indirect carryover effects were found through the effect of development temperature on adult body mass. When pupae were maintained at 31°C, the size and mass of emerging adult flies was reduced. This result is found generally in insects (the temperature-size rule (e.g., Kingsolver and Huey, 2008), and in tsetse specifically (Bursell, 1960). Our path analysis also indicated that flies with lower mass had reduced adult survival under blood deprivation. Although not measured specifically in this experiment, this is likely due to reduced lipid and/or water reserves in smaller flies (Kleynhans and Terblanche, 2011). The relationship between larger body size and improved starvation tolerance and desiccation resistance has been found in other insect species, such as aquatic bugs, beetles and mosquitoes (Gergs and Jager, 2014; Lehmann et al., 2006; Nervo et al., 2021; Renault et al., 2003). Selective elimination of small tsetse has also been observed in field populations (Bursell & Glasgow, 1960; Jackson, 1948). A study by Phelps & Clarke (1974) found that field-caught flies were significantly larger than adults from field-collected pupae reared to adulthood in a laboratory setting. This discrepancy in size suggests there was selective elimination of small-bodied flies in the hot season, when the mortality of small flies was up to 76% (Phelps and Clarke, 1974). In this way, pupal temperature is indirectly related to adult survival under blood deprivation: high development temperatures produce small, light flies, and flies of lower mass die more quickly under these conditions. However, it is important to note that, overall, this effect did not translate into greater survival when pupae were developed at 31°C.

In contrast, there was also some evidence of an adaptive priming effect - there was greater adult survival when males were maintained at 31°C for development, but this was only apparent when these males were transferred to 25°C for adulthood. This improved ability to survive longer without a bloodmeal when exposed to high developmental temperature could be due to plasticity, or acclimation, causing changes to cell stress responses and repair processes (Sørensen et al., 2003). A similar effect was found in a study on red flour beetles where a high development temperature allowed adults to survive for longer when starved (Scharf et al., 2014). For the beetles, this effect was present at both high and low adult temperatures, and in both sexes, but was more pronounced when adult starvation was tolerated at low temperature. Another study on *B. anynana* also found that butterflies reared at high temperatures were more starvation resistant, particularly when adults were starved at low temperature (Pijpe et al., 2007). The authors found starvation tolerance was higher in female butterflies. These findings could indicate an adaptive response of developmental stages to high temperature, where effects are only apparent when adults are kept at a benign temperature. Alternatively, in the present study, there may have been selective disappearance of less tolerant individuals in the 31°C treatment. Of the 3% of flies which failed to emerge successfully overall, more belonged to the 31°C treatment (n = 10/220: 4.5%) compared to 25°C (n = 2/220: 0.9%), so the removal

of these less tolerant individuals could have caused a higher average survival time in the remainder. Assuming a 1:1 sex ratio, it is likely that these individuals were largely male, as of the emerged adult flies, 222 were female and 206 were male – explaining why this effect may have only been apparent in males.

Adaptive developmental plasticity can evolve where there is environmental variability, high environmental predictability and low cost of plastic response (Monaghan, 2008). As pupal and adult tsetse ecology are so different – highly mobile, flying adults versus immobile pupae, deposited in sheltered microclimates (Buxton, 1955) – pupal temperature may not adequately predict adult conditions. These distinctive ecologies could be why we found no effect of matching temperature conditions in this experiment. Future work could consider the effect of *in utero* exposure to higher temperatures instead (during the nine days when larvae are developing within their mother), which may be more predictive of adult temperature conditions. Indeed, higher pupal mass improved adult survival indicating the importance of maternal provisioning *in utero*. Additionally, the high temperature treatment in this experiment may have killed symbionts of tsetse, which are important for maintenance of homeostasis (Michalkova, et al., 2014). *Wigglesworthia glossinidia* (present in all flies) and *Sodalis glossinidius* (not obligate) are maternally transferred endosymbionts that colonise tsetse tissues (Wang et al., 2013). Studies have shown that heat tolerance, longevity and/or fecundity are reduced when these bacterial symbionts are pharmaceutically eliminated (Dale & Welburn, 2001; Pais et al., 2008). Moreover, *Sodalis glossinidius* does not grow at temperatures above 31°C, and is unable to survive for more than 48 hours at 30°C (Roma et al., 2019). These bacteria were likely killed in the 31°C treatments and may have influenced the ability of tsetse to respond adaptively. Future investigations into how symbiont populations are affected by temperature in mother and offspring flies would give insight into their role in mediating survival and fecundity responses to high temperatures.

Overall, we find that adult temperature had the most pronounced effect on teneral adult survival under blood deprivation in tsetse. Pupal temperature had non-existent or contrasting effects, showing that responses to temperature are life stage specific. We found no convincing evidence of adaptive developmental plasticity or negative carryover effects in tsetse. Therefore, rising temperatures due to climate change are likely to exert the greatest effect on emerging adult tsetse, causing metabolic rate to increase, and forcing flies to locate their first blood meal more rapidly.

6. Chapter 6 - General discussion

Thermal tolerance, and its plasticity, is important for describing species responses to climate change. In this thesis, I investigated the response of insects – significant as ecosystem service providers, vectors of disease, and crop pests – to warming temperatures using a multi-faceted approach, by means of comparative meta-analyses across over 100 insect species, and detailed experimental work on tsetse flies.

I found, first, that plasticity of insects' thermal tolerance was pervasive but generally weak, providing limited ability to buffer insects from the effects of climate change (Chapter 2). Responses in tsetse mirrored this finding; changes to upper thermal tolerance in response to both adult hardening (Chapter 3) and transgenerational plasticity (Chapter 4) were limited. There was considerable variation in the level of thermal plasticity among insects generally (ARR = ~ -1.0 – 1.5) and among tsetse species (ARR = ~ 0.01 – 0.12), but patterns in thermal tolerance plasticity remained obscure, likely due to heterogeneous, interacting, and conflicting selection pressures on plasticity. Tsetse were an interesting case study for thermal tolerance as they deviated from trends found more broadly in animals and plants. Unlike *Drosophila* species, for example, female fertility was just as sensitive to heatwave as male fertility. Finally, I found that body size was important in shaping thermal tolerance limits, with high developmental temperature leading to small adult body size and, in consequence, reduced upper thermal tolerance and adult survival. Below, I discuss these findings in the context of (1) insect thermal tolerance plasticity; (2) broad scale patterns and assumptions and (3) a detailed look at tsetse thermal tolerance.

6.1 Plasticity of insect thermal tolerance is generally weak

Overall, I show that the upper critical thermal limits of insects increase by a mean of 0.09°C per 1°C acclimation, whereas lower critical thermal limits decrease by 0.15°C (Chapter 2). Weak thermal tolerance plasticity is mirrored in the tsetse species I tested which show limited or non-existent adult hardening (Chapter 3) and transgenerational plasticity (Fig. 6.1; Chapter 4) of upper thermal limits.

Weak plasticity of upper rather than lower thermal limits reflects physiological and evolutionary differences between the two measures (Bennett et al., 2021; Clarke, 2014). A strong phylogenetic signal for upper thermal limits in my meta-analysis suggests that related species have similar upper thermal tolerances, which may reflect constraints on evolution (Kellermann et al., 2012). In support, an analysis of over 2000 species through deep-time indicates that evolution of upper thermal tolerance appears to occur towards a particular value, consistent either with stabilising selection to an optimum, or indicating a physiological boundary (Bennett et al., 2021). This physiological boundary

or “concrete ceiling” where upper thermal tolerance can no longer evolve or change via plasticity (Sandblom et al., 2016), appears to be conserved across kingdoms. For example, eukaryotes have upper thermal tolerance within tight boundaries; they are unable to complete their lifecycles above 60°C, with most species requiring below 40°C (Clarke, 2014). Conversely, some Archaea and Bacteria living in hydrothermal vents can tolerate temperatures above 120°C, with an optimal growth temperature of 80°C due to the high thermal tolerance of macromolecular structures, such as proteins (Brininger et al., 2018; Clarke, 2014; Hoffmann et al., 2013). Modification to these proteins for high thermal tolerance requires substantial genetic changes, which are unlikely to be achieved in eukaryotes through evolution (Barik, 2020). Therefore, it appears that limited plasticity and slow evolution of upper thermal limits is likely to constrain the capacity of animals, including insects, to respond adaptively to warming temperatures.

The high basal thermal tolerance of tsetse may explain why three of the five species did not show any response to adult acclimation (Chapter 3) and demonstrated limited transgenerational plasticity (Chapter 4). If upper thermal tolerance is phylogenetically and physiologically constrained, then tsetse may already be near this concrete ceiling. In agreement with this hypothesis, the two species of tsetse which were the most plastic had the lowest basal tolerance. If plasticity and evolution are constrained in the most thermally tolerant species, then animals around the equator with the highest thermal tolerances, such as tsetse, may be the most vulnerable to climate warming (van Heerwaarden and Kellermann, 2020). Evidence for a trade-off between plasticity and thermal tolerance has also been found in species comparisons between crustaceans (Faulkner et al., 2014; Stillman, 2003; Vinagre et al., 2018), frogs (Simon et al., 2015), flies (Kellermann et al., 2017; van Heerwaarden et al., 2016), nudibranchs (Armstrong et al., 2019), lizards (Gilbert and Miles, 2019; Phillips et al., 2016), and fish (Comte and Olden, 2017; Vinagre et al., 2018). However, whether thermal tolerance trades off with plasticity is an open question under active debate due to artifacts of experimental design and statistical analysis issues preventing accurate testing of this hypothesis (Barley et al., 2021; Gunderson, 2023; Gunderson and Revell, 2022; Roff and Fairbairn, 2007; van Heerwaarden and Kellermann, 2020). Future research should focus on overcoming these challenges to determine if high thermal tolerance indicates that species will have limited plasticity (reviewed in van Heerwaarden and Kellermann, 2020). For example, rather than correlating tolerance to plasticity, studies can instead compare tolerance and plasticity across independent variables (e.g., latitude) to overcome statistical bias (e.g., Stillman, 2003). Additionally, plasticity should be assessed across a range of acclimation temperatures and lengths as there is evidence that animals with higher tolerance require longer acclimation treatments to induce maximum plastic responses (van Heerwaarden et al., 2024).

6.2 Plasticity is highly variable among insect species and broad scale patterns and assumptions may not apply

There was considerable variation in the level of plastic response among insects (Chapter 2), Diptera (Chapter 3), and tsetse species (Chapter 3). Among insects generally (Chapter 2), and Diptera specifically (Chapter 3), only a small proportion of variation was explained by ontogeny, sex, and methodological end points, indicating that broad scale trends are difficult to identify. In tsetse, I was unable to explain the variation in CT_{max} plasticity by body size or sex differences (Chapter 3). Overall, patterns in thermal tolerance plasticity remained obscure. Broad scale trends may be difficult to capture in a meta-analysis due to heterogeneous, interacting, and conflicting selection pressures on thermal plasticity. In tsetse, thermal history could be important for determining interspecies differences in plasticity. The thermal tolerance plasticity of further tsetse species, ideally from field-caught populations, should be measured so that a phylogenetically controlled comparison can made, using a variable such as seasonality, latitude, or microclimate temperature variation to explain species differences.

Data on the thermal tolerance of insects were highly taxon biased, with 43% of effect sizes from my meta-analysis derived from studies on Drosophilidae. Concerningly, my research on tsetse shows that some patterns found in animals and plants, and particularly *Drosophila*, may not apply to all insect species. Female fertility of tsetse was just as sensitive to heatwave as male fertility, in opposition to trends found across animals and plants (David et al., 2005; Hansen, 2009; Porcelli et al., 2017; Sales et al., 2018; van Heerwaarden and Sgrò, 2021; Walsh et al., 2019b; Zinn et al., 2010). Female exposure to a three-day heatwave peaking at 38°C delayed offspring production for a week, but male exposure to heatwave had no effect on fertility. In contrast, *Drosophila* male fertility is more heat sensitive than female fertility (van Heerwaarden and Sgrò, 2021). This difference is likely due to the timing of spermatogenesis, which is continuous throughout adulthood in *Drosophila*, but for tsetse occurs during pupation and ceases at emergence (Leak, 1998). Therefore, I predict that the timing of a heatwave during an insect's lifecycle could give rise to sex differences in thermal fertility limits. For example, heatwave during the pupal stage of tsetse on subsequent male fertility would give insights on this prediction. However, I note that the TFL experiment on tsetse should be modified by pairing males with several sets of virgin females over time to test whether there is a decline in male fertility across repeated breeding attempts, as in *Drosophila* (Parratt et al., 2021; Walsh et al., 2021). My study highlights potentially important differences between tsetse and *Drosophila,* indicating that a range of species should be examined to ensure generalisations and broadscale patterns are relevant across species.

Additionally, thermal fertility limits of *G. pallidipes* were near to lethal temperatures; a 40°C heatwave killed 100% of tsetse, but a 38°C heatwave prevented offspring production for just a week and only if female flies were exposed. Therefore, in response to adult heatwave in *G. pallidipes*, direct mortality and reduced longevity were the most important for population viability, rather than effects on fertility (Chapter 4). Here, my TFL research was limited to *G. pallidipes* so future work should determine critical thermal limits and TFLs in further species of tsetse, to confirm my finding that lethal limits are likely more important for population viability. Updated distribution maps are currently being compiled for tsetse across sub-Saharan African countries (Cecchi et al., 2015; de Gier et al., 2020; Gebre et al., 2022; Percoma et al., 2022; Shereni et al., 2021). Therefore, a comparative analysis on tsetse species should be completed, relating thermal tolerance data to tsetse distribution, as in Parratt *et al.* (2021) with *Drosophila*. Whether TFLs or critical thermal limits best describe distribution, and if this differs between species, could be determined. I anticipate, unlike *Drosophila* (see Parratt *et al.*, 2021; van Heerwaarden and Sgrò, 2021), tsetse critical thermal maximums would be most important in defining current distributions. Again, this would be an important finding as it would indicate patterns found in *Drosophila* may not be indicative of all insects.

6.3 A detailed examination of upper thermal tolerance in tsetse

Upper critical thermal limits of tsetse were high compared to *Drosophila*, which rarely have CT_{max} values greater than 39°C (Kellermann et al., 2012). In comparison, all five tsetse species I tested had CT_{max} of greater than 41°C, and the most heat tolerant species (*G. f. fuscipes*) had a CT_{max} of 43.1°C. These CT_{max} values lie beyond the lethal limits I found in Chapter 4 (100% mortality at 40°C for *G. pallidipes*), and beyond those found in the field, which are also around 40°C (Hargrove, 2004). Differences between lethal limits and CT_{max} are likely due to accumulation of thermal injury the longer an animal is kept under stressful conditions (e.g. discussed in, Cossins and Bowler, 1987; Loeschcke and Hoffmann, 2002).

Despite having high lethal limits and CT_{max}, tsetse live in environments which approach or surpass these limits, i.e., small warming tolerances and thermal safety margins. For example, in Zimbabwe where *G. m. morsitans* and *G. pallidipes* reside, maximum daily air temperatures at Rekomitje research station have reached a maximum of 43 to 44°C seven times between 2009 and 2019 (Lord et al., 2018; Shereni et al., 2021). Based on my research, these temperatures would directly kill tsetse, as these species have an acclimated CT_{max} of 42.9°C and 42.8°C, respectively. However, tsetse use microclimates to shelter from high temperature, which can be 2 – 6°C below ambient (Hargrove and Coates, 1990). Use of such microclimates is, therefore, likely to be an important strategy for survival of tsetse in the future. These results have implications for insects generally, as, in future, studies will

need to include microclimatic temperature data for accurate estimates of climate change vulnerability (Pincebourde and Woods, 2020).

Finally, there was a clear trend in my experimental studies on tsetse that 'bigger is better' (i.e., in terms of larger flies having improved survival and thermal tolerance) and 'hotter is smaller' (i.e., smaller individuals are produced during warm conditions) (Fig. 6.1), a trend apparent in many ectothermic species (Kingsolver and Huey, 2008). I found that larger body size was associated with both greater survival (Chapter 5) and with higher CT_{max} values (Chapter 3 and Chapter 4). Conversely, I found that high temperature during development produced smaller adults (Chapter 5). Therefore, tsetse could suffer in addition to greater mortality and fertility losses by becoming smaller, and as a result, have lower CT_{max} and reduced tolerance to starvation or desiccation (Fig. 6.1). These effects may be additive on top of increasing levels of mortality. These findings could have implications for insects more broadly as the temperature-size rule is common among insects (Kingsolver and Huey, 2008).

6.4 Conclusion

The limited plasticity of upper thermal limits may mean that insects, including tsetse, need to use other means to escape high temperature, such as behavioural thermoregulation or range shifts (Pincebourde and Woods, 2020; Suggitt et al., 2011). The greater plasticity of lower thermal limits may aid range shifts to higher elevations and latitudes where temperatures are more variable (Ma et al., 2021; Parmesan, 2006). Overall, insects are likely to be vulnerable to climate change, which is concerning as they provide essential ecosystem services such as pollination and waste disposal. As trends across insects in thermal tolerance plasticity are difficult to identify, I conclude that detailed experiments are needed across a range of insect species to determine which will be most vulnerable. Particularly, studies are lacking in the Global South and on hemimetabolous insects so these areas should be the focus of future research and funding. Due to the general pattern of weak plasticity in thermal tolerance of insects, city planners can aid insects by providing suitable microclimates as refugia. For example, maintaining a network of foliage such as bushes and trees throughout cities will be important so that insects can shelter during heatwaves.

Overall, tsetse exposed to high temperatures, such as heatwave events, will suffer from greater mortality, reduced longevity, and delayed reproduction. The average body size of tsetse populations is likely to decline in response to high temperature, leading to a reduction in adult survival and thermal tolerance in some species. These data support modelling studies which predict range contractions of tsetse, and current data indicating tsetse numbers are diminishing at range boundaries. This work provides further description of the thermal tolerance and plasticity of several species of tsetse,

potentially explaining these population declines. Plasticity and thermal fertility limits can be used to further refine models to predict future tsetse distribution and abundance. Additionally, thermal traits are important when making decisions for tsetse control including the most appropriate techniques for control and where they should be focused. For example, using techniques which target tsetse searching for cool refugia are effective. Tsetse are attracted to blue and black colours, possibly due the blue/black colours of day time shadows and cost-effective “tiny-target” traps using these colours have proven successful (Steverding and Troscianko, 2004; Tanekou et al., 2023). Control efforts should be focused temporally and spatially where tsetse numbers are likely to be high, i.e. outside of the hot season, and where trypanosomiasis is most prevent or tsetse are most abundant (FAO WHO, 2022). In this way distribution modelling using thermal tolerance limits can aid the deployment of traps or other control techniques (Dicko et al., 2014). Continued monitoring, such as at Rekomitje Research Station in Zimbabwe, will be an important tool in assessing tsetse responses to high temperature, and if responses are in line with current predictions.

Ultimately, I use both comparative tools and detailed experiments to show how a range of thermal exposures (both heatwaves and longer-term acclimation) at different life stages can impact survival, reproduction, and plasticity of thermal tolerance. More broadly, my findings highlight the grave consequences of warming climates for insect populations and the need for detailed experimental work on further under-studied groups of insects.

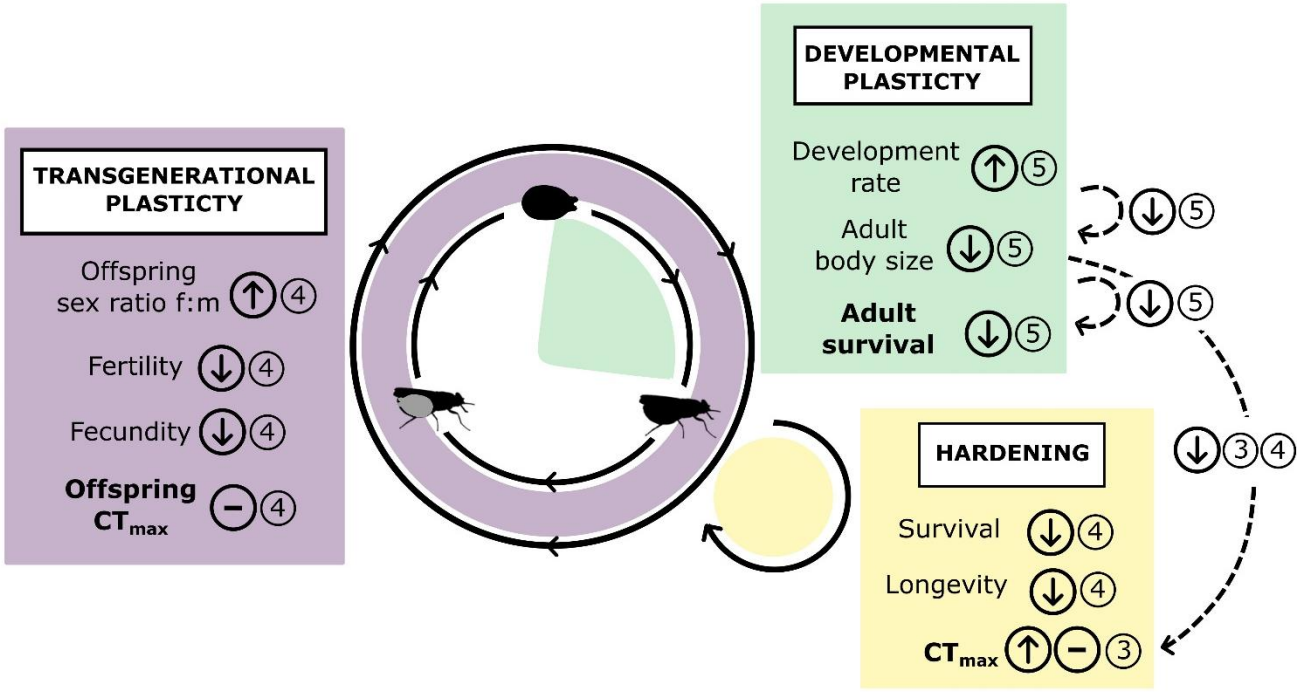


Figure 6.1 Schematic representation of tsetse (*Glossina* spp.) responses to heat acclimation. Arrows indicate the direction of change. Bold font indicates the thermal tolerance limit that was tested. Numbers in circles reference the thesis chapter. Chapter 3 refers to a three-day adult acclimation of 30°C in five tsetse species. Plastic responses in CT_{max} were dependant on which species was tested. Chapter 4 responses are for the three-day 38°C adult heatwave on *G. pallidipes*, but note that, in contrast, a 36°C heatwave increased offspring CT_{max}. Chapter 5 refers to constant acclimation at 31°C during the pupal stage of *G. m. morsitans*. I include both positive and deleterious plastic responses to acclimation.

7. Reference list

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. B Biol. Sci. 267, 739–745. https://doi.org/10.1098/rspb.2000.1065

Allen, J.L., Chown, S.L., Janion-Scheepers, C., Clusella-Trullas, S., 2016. Interactions between rates of temperature change and acclimation affect latitudinal patterns of warming tolerance. Conserv. Physiol. 4. https://doi.org/10.1093/conphys/cow053

Allen, J.L., Clusella-Trullas, S., Chown, S.L., 2012. The effects of acclimation and rates of temperature change on critical thermal limits in Tenebrio molitor (Tenebrionidae) and Cyrtobagous salviniae (Curculionidae). J. Insect Physiol. 58, 669–678. https://doi.org/10.1016/j.jinsphys.2012.01.016

Angilletta, M.J., 2009. Thermal adaptation: a theoretical and empirical synthesis, Oxford University Press. Elsevier, Oxford.

Araújo, M.B., Whittaker, R.J., Ladle, R.J., Erhard, M., 2005. Reducing uncertainty in projections of extinction risk from climate change. Glob. Ecol. Biogeogr. 14, 529–538. https://doi.org/10.1111/j.1466-822X.2005.00182.x

Are, E.B., Hargrove, J.W., 2020. Extinction probabilities as a function of temperature for populations of tsetse (Glossina spp.). PLoS Negl. Trop. Dis. 14, 1–19. https://doi.org/10.1371/journal.pntd.0007769

Armstrong, E.J., Tanner, R.L., Stillman, J.H., 2019. High heat tolerance is negatively correlated with heat tolerance plasticity in nudibranch mollusks. Physiol. Biochem. Zool. 92, 430–444. https://doi.org/10.1086/704519

Arneth, A., Shin, Y.J., Leadley, P., Rondinini, C., Bukvareva, E., Kolb, M., Midgley, G.F., Oberdorff, T., Palomo, I., Saito, O., 2020. Post-2020 biodiversity targets need to embrace climate change. Proc. Natl. Acad. Sci. U. S. A. 117, 30882–30891. https://doi.org/10.1073/pnas.2009584117

Auld, J.R., Agrawal, A.A., Relyea, R.A., 2010. Re-evaluating the costs and limits of adaptive phenotypic plasticity. Proc. R. Soc. B Biol. Sci. 277, 503–511. https://doi.org/10.1098/rspb.2009.1355

Bak, C.W., Bahrndorff, S., Noer, N.K., Jørgensen, L.B., Overgaard, J., Kristensen, T.N., 2020. Comparison of static and dynamic assays when quantifying thermal plasticity of drosophilids. Insects 11, 1–11. https://doi.org/10.3390/insects11080537

Barik, S., 2020. Evolution of protein structure and stability in global warming. Int. J. Mol. Sci. 21, 1–22.

https://doi.org/10.3390/ijms21249662

Barley, J.M., Cheng, B.S., Sasaki, M., Gignoux-Wolfsohn, S., Hays, C.G., Putnam, A.B., Sheth, S., Villeneuve, A.R., Kelly, M., 2021. Limited plasticity in thermally tolerant ectotherm populations: Evidence for a trade-off. Proc. R. Soc. B Biol. Sci. 288, 20210765. https://doi.org/10.1098/rspb.2021.0765

Barreaux, A.M.G., Higginson, A.D., Bonsall, M.B., English, S., 2022. Incorporating effects of age on energy dynamics predicts nonlinear maternal allocation patterns in iteroparous animals. Proc. R. Soc. B Biol. Sci. 289, 20211884. https://doi.org/10.1098/rspb.2021.1884

Barton, K., 2020. MuMIn: Multi-Model Inference.

Bates, D., Machler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67, 1–48.

Baudier, K., O'Donnell, S., 2018. Complex body size differences in thermal tolerance among army ant workers (Eciton burchellii parvispinum). J. Therm. Biol. 78, 277–280. https://doi.org/10.1016/j.jtherbio.2018.10.011

Bauerfeind, S.S., Kellermann, V., Moghadam, N.N., Loeschcke, V., Fischer, K., 2014. Temperature and photoperiod affect stress resistance traits in Drosophila melanogaster. Physiol. Entomol. 39, 237–246. https://doi.org/10.1111/phen.12068

Baur, J., Jagusch, D., Michalak, P., Koppik, M., Berger, D., 2022. The mating system affects the temperature sensitivity of male and female fertility. Funct. Ecol. 36, 92–106. https://doi.org/10.1111/1365-2435.13952

Belliard, S.A., De La Vega, G.J., Schilman, P.E., 2019. Thermal Tolerance Plasticity in Chagas Disease Vectors Rhodnius prolixus (Hemiptera: Reduviidae) and Triatoma infestans. J. Med. Entomol. 56, 997–1003. https://doi.org/10.1093/jme/tjz022

Bennett, J.M., Calosi, P., Clusella-Trullas, S., Martínez, B., Sunday, J., Algar, A.C., Araújo, M.B., Hawkins, B.A., Keith, S., Kühn, I., Rahbek, C., Rodríguez, L., Singer, A., Villalobos, F., Ángel Olalla-Tárraga, M., Morales-Castilla, I., 2018. GlobTherm, a global database on thermal tolerances for aquatic and terrestrial organisms. Sci. Data 5. https://doi.org/10.1038/sdata.2018.22

Bennett, J.M., Sunday, J., Calosi, P., Villalobos, F., Martínez, B., Molina-Venegas, R., Araújo, M.B., Algar, A.C., Clusella-Trullas, S., Hawkins, B.A., Keith, S.A., Kühn, I., Rahbek, C., Rodríguez, L., Singer, A., Morales-Castilla, I., Olalla-Tárraga, M.Á., 2021. The evolution of critical thermal limits of life on Earth. Nat. Commun. 12, 1–9. https://doi.org/10.1038/s41467-021-21263-8

Benoit, J.B., Attardo, G.M., Baumann, A.A., Michalkova, V., Aksoy, S., 2015. Adenotrophic Viviparity in Tsetse Flies: Potential for Population Control and as an Insect Model for Lactation. Annu. Rev. Entomol. 60, 351–371. https://doi.org/10.1146/annurev-ento-010814-020834

Bergmann, C., 1847. Ueber die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Gottingerstudien 3, 595–708.

Berkeley, S.A., Chapman, C., Sogard, S.M., 2004. Maternal age as a determinant of larval growth and survival in a marine fish, Sebastes melanops. Ecology 85, 1258–1264. https://doi.org/10.1890/03-0706

Bernardo, J., Ossola, R.J., Spotila, J., Crandall, K.A., 2007. Interspecies physiological variation as a tool for cross-species assessments of global warming-induced endangerment: Validation of an intrinsic determinant of macroecological and phylogeographic structure. Biol. Lett. 3, 695–699. https://doi.org/10.1098/rsbl.2007.0259

Beynon, S.A., Wainwright, W.A., Christie, M., 2015. The application of an ecosystem services framework to estimate the economic value of dung beetles to the U.K. cattle industry. Ecol. Entomol. 40, 124–135. https://doi.org/10.1111/een.12240

Blackburn, S., Van Heerwaarden, B., Kellermann, V., Sgro, C.M., 2014. Evolutionary capacity of upper thermal limits: Beyond single trait assessments. J. Exp. Biol. 217, 1918–1924. https://doi.org/10.1242/jeb.099184

Boffelli, D., Takayama, S., Martin, D.I.K., 2014. Now you see it: Genome methylation makes a comeback in drosophila. BioEssays 36, 1138–1144. https://doi.org/10.1002/bies.201400097

Bowler, K., 2018. Heat death in poikilotherms: Is there a common cause? J. Therm. Biol. 76, 77–79. https://doi.org/10.1016/j.jtherbio.2018.06.007

Bowler, K., Terblanche, J.S., 2008. Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? Biol. Rev. 83, 339–355. https://doi.org/10.1111/j.1469-185X.2008.00046.x

Bozinovic, F., Calosi, P., Spicer, J.I., 2011. Physiological Correlates of Geographic Range in Animals. Annu. Rev. Ecol. Evol. Syst. 42, 155–179. https://doi.org/10.1146/annurev-ecolsys-102710-145055

Bradburn, M.J., Clark, T.G., Love, S.B., Altman, D.G., 2003. Survival Analysis Part II: Multivariate data analysis- An introduction to concepts and methods. Br. J. Cancer 89, 431–436. https://doi.org/10.1038/sj.bjc.6601119

Brady, J., 1975. “Hunger” in the tsetse fly: The nutritional correlates of behaviour. J. Insect Physiol. 21, 807–829. https://doi.org/10.1016/0022-1910(75)90011-6

Brady, J., Crump, A.J., 1978. The control of circadian activity rhythms in tsetse flies : Physiol. Entomol. 3, 177–190.

Brakefield, P.M., French, V., 1999. Butterfly wings: The evolution of development of colour patterns. BioEssays 21, 391–401. https://doi.org/10.1002/(SICI)1521-1878(199905)21:5<391::AID-BIES6>3.0.CO;2-Q

Brakefield, P.M., Reitsma, N., 1991. Phenotypic plasticity, seasonal climate and the population biology of Bicyclus butterflies (Satyridae) in Malawi. Ecol. Entomol. 16, 291–303. https://doi.org/10.1111/j.1365-2311.1991.tb00220.x

Brininger, C., Spradlin, S., Cobani, L., Evilia, C., 2018. The more adaptive to change, the more likely you are to survive: Protein adaptation in extremophiles. Semin. Cell Dev. Biol. 84, 158–169. https://doi.org/10.1016/j.semcdb.2017.12.016

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9, 378–400. https://doi.org/10.32614/rj-2017-066

Brown, J., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G., 2004. Toward a Metabolic Theory of Ecology. Ecology 85, 1771–1789.

Bubliy, O.A., Kristensen, T.N., Kellermann, V., Loeschcke, V., 2012. Plastic responses to four environmental stresses and cross-resistance in a laboratory population of Drosophila melanogaster. Funct. Ecol. 26, 245–253. https://doi.org/10.1111/j.1365-2435.2011.01928.x

Buckley, L.B., Huey, R.B., 2016. Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities. Glob. Chang. Biol. 22, 3829–3842. https://doi.org/10.1111/gcb.13313

Budd, L., 1999. DFID-funded Tsetse and Trypanosomosis research and development since 1980, Economic A. ed. Natural Resources International Limited, Aylesford, UK.

Bujan, J., Roeder, K.A., Yanoviak, S.P., Kaspari, M., 2020. Seasonal plasticity of thermal tolerance in ants. Ecology 101. https://doi.org/10.1002/ecy.3051

Bulté, G., Blouin-Demers, G., 2010. Implications of extreme sexual size dimorphism for

thermoregulation in a freshwater turtle. Oecologia 162, 313–322. https://doi.org/10.1007/s00442-009-1469-8

Burger, R., Lynch, M., 1995. Evolution and extinction in a changing environment: A quantitative-genetic analysis. Evolution (N. Y). 49, 151–163. https://doi.org/10.1111/j.1558-5646.1995.tb05967.x

Burgess, S.C., Marshall, D.J., 2011. Temperature-induced maternal effects and environmental predictability. J. Exp. Biol. 214, 2329–2336. https://doi.org/10.1242/jeb.054718

Bursell, E., 1960. The Measurement of Size in tsetse flies (Glossina). Bull. Entomol. Res. 51, 33–37. https://doi.org/10.1017/S0007485300054924

Bursell, E., 1959. The water balance of tsetse flies. Trans. R. Entomol. Soc. London 111, 205–235. https://doi.org/10.1111/j.1365-2311.1959.tb02282.x

Bursell, E., 1958. The water balance of tsetse pupae. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 241, 179–210. https://doi.org/10.1098/rstb.1958.0002

Bursell, E., Glasgow, J.P., 1960. Further observations on lake-side and riverine communities of Glossina palpalis fuscipes Newstead. Bull. Entomol. Res. 51, 47–56. https://doi.org/10.1017/S0007485300054948

Buxton, P.A., 1955. The Natural History of Tsetse Flies. An Account of the Biology of the Genus Glossina (Diptera), London School of Hygiene and Tropical Medicine. H. K. Lewis & Co Ltd. https://doi.org/10.2307/3564834

Calderone, N.W., 2012. Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992-2009. PLoS One 7, e37235. https://doi.org/10.1371/journal.pone.0037235

Calosi, Piero, Bilton, D.T., Spicer, J.I., 2008. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. Biol. Lett. 4, 99–102. https://doi.org/10.1098/rsbl.2007.0408

Calosi, P., Bilton, D.T., Spicer, J.I., Atfield, A., 2008. Thermal tolerance and geographical range size in the Agabus brunneus group of European diving beetles (Coleoptera: Dytiscidae). J. Biogeogr. 35, 295–305. https://doi.org/10.1111/j.1365-2699.2007.01787.x

Caminade, C., Turner, J., Metelmann, S., Hesson, J.C., Blagrove, M.S.C., Solomon, T., Morse, A.P., Baylis, M., 2017. Global risk model for vector-borne transmission of Zika virus reveals the role of El Niño 2015. Proc. Natl. Acad. Sci. U. S. A. 114, 119–124.

https://doi.org/10.1073/pnas.1614303114

Canal Domenech, B., Fricke, C., 2022. Recovery from heat-induced infertility—A study of reproductive tissue responses and fitness consequences in male Drosophila melanogaster. Ecol. Evol. 12. https://doi.org/10.1002/ece3.9563

Caswell, H., 2012. Matrix Population Models, in: Encyclopedia of Environmetrics. John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470057339.vam006m

Cavieres, G., Rezende, E.L., Clavijo-Baquet, S., Alruiz, J.M., Rivera-Rebella, C., Boher, F., Bozinovic, F., 2020. Rapid within- and transgenerational changes in thermal tolerance and fitness in variable thermal landscapes. Ecol. Evol. 10, 8105–8113. https://doi.org/10.1002/ece3.6496

Cecchi, G., Paone, M., Argilés Herrero, R., Vreysen, M.J.B., Mattioli, R.C., 2015. Developing a continental atlas of the distribution and trypanosomal infection of tsetse flies (Glossina species). Parasites and Vectors 8. https://doi.org/10.1186/s13071-015-0898-y

Chang, S.L., Lee, W.S., Munch, S.B., 2021. Separating Paternal and Maternal Contributions to Thermal Transgenerational Plasticity. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.734318

Chapelle, G., Peck, L.S., 1999. Polar gigantism dictated by oxygen availability. Nature 398, 114–115. https://doi.org/10.1038/20099

Chen, I.C., Hill, J.K., Ohlemüller, R., Roy, D.B., Thomas, C.D., 2011. Rapid range shifts of species associated with high levels of climate warming. Science (80-.). 333, 1024–1026. https://doi.org/10.1126/science.1206432

Chown, S.L., Gaston, K.J., Robinson, D., 2004. Macrophysiology: Large-scale patterns in physiological traits and their ecological implications. Funct. Ecol. 18, 159–167. https://doi.org/10.1111/j.0269-8463.2004.00825.x

Chown, S.L., Jumbam, K.R., Sørensen, J.G., Terblanche, J.S., 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. Funct. Ecol. 23, 133–140. https://doi.org/10.1111/j.1365-2435.2008.01481.x

Chown, S. L., Sørensen, J.G., Terblanche, J.S., 2011. Water loss in insects: An environmental change perspective. J. Insect Physiol. https://doi.org/10.1016/j.jinsphys.2011.05.004

Christidis, N., Jones, G.S., Stott, P.A., 2015. Dramatically increasing chance of extremely hot summers since the 2003 European heatwave. Nat. Clim. Chang. 5, 46–50. https://doi.org/10.1038/nclimate2468

Clarke, A., 2014. The thermal limits to life on Earth. Int. J. Astrobiol. 13, 141–154. https://doi.org/10.1017/S1473550413000438

Cline, T.W., Meyer, B.J., 1996. Vive la différence: Males vs Females in Flies vs Worms. Annu. Rev. Genet. 30, 637–702. https://doi.org/10.1146/annurev.genet.30.1.637

Clusella-Trullas, S., Chown, S.L., 2014. Lizard thermal trait variation at multiple scales: A review. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 184, 5–21. https://doi.org/10.1007/s00360-013-0776-x

Clusella-Trullas, S., Garcia, R.A., Terblanche, J.S., Hoffmann, A.A., 2021. How useful are thermal vulnerability indices? Trends Ecol. Evol. 36, 1000–1010. https://doi.org/10.1016/j.tree.2021.07.001

Clutton-Brock, T., Langley, P., 1997. Persistent courtship reduces male and female longevity in captive tsetse flies Glossina morsitans morsitans westwood (Diptera: Glossinidae). Behav. Ecol. 8, 392–395. https://doi.org/10.1093/beheco/8.4.392

GBD 2016 DALYs and HALE Collaborators 2017. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1859–1922. https://doi.org/10.1016/S0140-6736(17)32648-X

Comte, L., Olden, J.D., 2017. Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. Glob. Chang. Biol. 23, 728–736. https://doi.org/10.1111/gcb.13427

Cossins, A.R., Bowler, K., 1987. Temperature Biology of Animals. Chapman and Hall. https://doi.org/10.2307/2403720

Couet, J., Marjakangas, E.L., Santangeli, A., Kålås, J.A., Lindström, Å., Lehikoinen, A., 2022. Short-lived species move uphill faster under climate change. Oecologia 198, 877–888. https://doi.org/10.1007/s00442-021-05094-4

Couper, L.I., Farner, J.E., Caldwell, J.M., Childs, M.L., Harris, M.J., Kirk, D.G., Nova, N., Shocket, M., Skinner, E.B., Uricchio, L.H., Exposito-Alonso, M., Mordecai, E.A., 2021. How will mosquitoes adapt to climate warming? Elife 10. https://doi.org/10.7554/eLife.69630

Courtin, F., Jamonneau, V., Duvallet, G., Garcia, A., Coulibaly, B., Doumenge, J.P., Cuny, G., Solano, P., 2008. Sleeping sickness in West Africa (1906-2006): Changes in spatial repartition and lessons from the past. Trop. Med. Int. Heal. 13, 334–344. https://doi.org/10.1111/j.1365-3156.2008.02007.x

Courtin, F., Rayaissé, J.B., Tamboura, I., Serdébéogo, O., Koudougou, Z., Solano, P., Sidibé, I., 2010. Updating the northern tsetse limit in Burkina Faso (1949-2009): Impact of global change. Int. J. Environ. Res. Public Health 7, 1708–1719. https://doi.org/10.3390/ijerph7041708

Crill, W.D., Huey, R.B., Gilchrist, G.W., 1996. Within- and between-generation effects of temperature on the morphology and physiology of Drosophila melanogaster. Evolution (N. Y). 50, 1205–1218. https://doi.org/10.1111/j.1558-5646.1996.tb02361.x

Dai, A., 2011. Drought under global warming: A review. Wiley Interdiscip. Rev. Clim. Chang. 2, 45–65. https://doi.org/10.1002/wcc.81

Dale, C., Welburn, S.C., 2001. The endosymbionts of tsetse flies: Manipulating host-parasite interactions. Int. J. Parasitol. 31, 628–631. https://doi.org/10.1016/S0020-7519(01)00151-5

David, J.R., Araripe, L.O., Chakir, M., Legout, H., Lemos, B., Pétavy, G., Rohmer, C., Joly, D., Moreteau, B., 2005. Male sterility at extreme temperatures: A significant but neglected phenomenon for understanding Drosophila climatic adaptations. J. Evol. Biol. 18, 838–846. https://doi.org/10.1111/j.1420-9101.2005.00914.x

David, J.R., Arens, M.F., Cohet, Y., 1971. Sterilite male a haute temperature chez Drosophila melanogaster: nature, progressivite et reversibilite des effets de la chaleur. Comptes rendus l'Académie des Sci. 272, 1007–1010.

Dawe, K.L., Boutin, S., 2016. Climate change is the primary driver of white-tailed deer (Odocoileus virginianus) range expansion at the northern extent of its range; land use is secondary. Ecol. Evol. 6, 6435–6451. https://doi.org/10.1002/ece3.2316

de Gier, J., Cecchi, G., Paone, M., Dede, P., Zhao, W., 2020. The continental atlas of tsetse and African animal trypanosomosis in Nigeria. Acta Trop. 204, 105328. https://doi.org/10.1016/j.actatropica.2020.105328

De Jong, M.A., Kesbeke, F.M.N.H., Brakefield, P.M., Zwaan, B.J., 2010. Geographic variation in thermal plasticity of life history and wing pattern in Bicyclus anynana. Clim. Res. 43, 91–102. https://doi.org/10.3354/cr00881

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. U. S. A. 105, 6668–6672. https://doi.org/10.1073/pnas.0709472105

Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B., Naylor, R.L., 2018. Increase in crop losses to insect pests in a warming climate. Science (80-.). 361, 916–919.

https://doi.org/10.1126/science.aat3466

DeWitt, T.J., Sih, A., Wilson, D.S., 1998. Costs and limits of phenotypic plasticity. Trends Ecol. Evol. 13, 77–81. https://doi.org/10.1016/S0169-5347(97)01274-3

Díaz, S., Settele, J., Brondízio, E.S., Ngo, H.T., Agard, J., Arneth, A., Balvanera, P., Brauman, K.A., Butchart, S.H.M., Chan, K.M.A., Lucas, A.G., Ichii, K., Liu, J., Subramanian, S.M., Midgley, G.F., Miloslavich, P., Molnár, Z., Obura, D., Pfaff, A., Polasky, S., Purvis, A., Razzaque, J., Reyers, B., Chowdhury, R.R., Shin, Y.J., Visseren-Hamakers, I., Willis, K.J., Zayas, C.N., 2019. Pervasive human-driven decline of life on Earth points to the need for transformative change. Science (80-.). 366. https://doi.org/10.1126/science.aax3100

Dicko, A.H., Lancelot, R., Seck, M.T., Guerrini, L., Sall, B., Lo, M., Vreysen, M.J.B., Lefrançois, T., Fonta, W.M., Peck, S.L., Bouyer, J., 2014. Using species distribution models to optimize vector control in the framework of the tsetse eradication campaign in Senegal. Proc. Natl. Acad. Sci. U. S. A. 111, 10149–10154. https://doi.org/10.1073/pnas.1407773111

Donelson, J.M., Salinas, S., Munday, P.L., Shama, L.N.S., 2018. Transgenerational plasticity and climate change experiments: Where do we go from here? Glob. Chang. Biol. 24, 13–34. https://doi.org/10.1111/gcb.13903

Egeru, A., Opio, J., Siya, A., Barasa, B., Magaya, J.P., Namaalwa, J.J., 2020. Tsetse invasion as an emerging threat to socioecological resilience of pastoral communities in Karamoja, Uganda. Sustain. 12. https://doi.org/10.3390/su12041599

Elphick, M.J., Shine, R., 1998. Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (Bassiana duperreyi, Scincidae). Biol. J. Linn. Soc. 63, 429–447. https://doi.org/10.1006/bijl.1997.0198

English, S., Barreaux, A.M. 2020. The evolution of sensitive periods in development: insights from insects. Curr. Opin. Behav. Sci. 36, 71–78. https://doi.org/10.1016/j.cobeha.2020.07.009

English, S., Barreaux, A.M.G., Leyland, R., Lord, J.S., Hargrove, J.W., Vale, G.A., Haines, L.R., 2023. Investigating the unaccounted ones: insights on age-dependent reproductive loss in a viviparous fly. Front. Ecol. Evol. 11, 1057474. https://doi.org/10.3389/FEVO.2023.1057474

Enriquez, T., Colinet, H., 2017. Basal tolerance to heat and cold exposure of the spotted wing drosophila, drosophila suzukii. PeerJ 2017. https://doi.org/10.7717/peerj.3112

FAO, PAAT, 2008. On target against poverty: the Programme against African Trypanosomiasis (PAAT) 1997-2007. PAAT Inf. Serv. Publ.

Faulkner, K.T., Clusella-Trullas, S., Peck, L.S., Chown, S.L., 2014. Lack of coherence in the warming responses of marine crustaceans. Funct. Ecol. 28, 895–903. https://doi.org/10.1111/1365-2435.12219

Fawcett, T.W., Frankenhuis, W.E., 2015. Adaptive explanations for sensitive windows in development. Front. Zool. 12. https://doi.org/10.1186/1742-9994-12-S1-S3

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 243–282. https://doi.org/10.1146/annurev.physiol.61.1.243

Fossheim, M., Primicerio, R., Johannesen, E., Ingvaldsen, R.B., Aschan, M.M., Dolgov, A. V., 2015. Recent warming leads to a rapid borealization of fish communities in the Arctic. Nat. Clim. Chang. 5, 673–677. https://doi.org/10.1038/nclimate2647

Foster, W.A., 1976. Male sexual maturation of the tsetse flies glossina morsitans westwood and g. austeni Newstead (Dipt., Glossinidae) in relation to blood feeding. Bull. Entomol. Res. 66, 389–399. https://doi.org/10.1017/S0007485300006787

Frölicher, T.L., Fischer, E.M., Gruber, N., 2018. Marine heatwaves under global warming. Nature 560, 360–364. https://doi.org/10.1038/s41586-018-0383-9

García-Robledo, C., Kuprewicz, E.K., Staines, C.L., Erwin, T.L., Kress, W.J., 2016. Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction. Proc. Natl. Acad. Sci. U. S. A. 113, 680–685. https://doi.org/10.1073/pnas.1507681113

Gardner, J.L., Peters, A., Kearney, M.R., Joseph, L., Heinsohn, R., 2011. Declining body size: A third universal response to warming? Trends Ecol. Evol. 26, 285–291. https://doi.org/10.1016/j.tree.2011.03.005

Gebre, T., Kapitano, B., Beyene, D., Alemu, D., Beshir, A., Worku, Z., Kifle, T., Selamu, A., Debas, E., Kalsa, A., Asfaw, N., Zhao, W., Paone, M., Cecchi, G., 2022. The national atlas of tsetse flies and African animal trypanosomosis in Ethiopia. Parasit. Vectors 15. https://doi.org/10.1186/s13071-022-05617-9

Genner, M.J., Sims, D.W., Southward, A.J., Budd, G.C., Masterson, P., Mchugh, M., Rendle, P., Southall, E.J., Wearmouth, V.J., Hawkins, S.J., 2010. Body size-dependent responses of a marine fish assemblage to climate change and fishing over a century-long scale. Glob. Chang. Biol. 16, 517–527. https://doi.org/10.1111/j.1365-2486.2009.02027.x

Gergs, A., Jager, T., 2014. Body size-mediated starvation resistance in an insect predator. J. Anim. Ecol. 83, 758–768. https://doi.org/10.1111/1365-2656.12195

Gething, M.-J., 1997. Guidebook to Molecular Chaperones and Protein-Folding Catalysts, Sambrook & Tooze Guidebook Series. Oxford University Press. https://doi.org/10.1086/393278

Gilbert, A.L., Miles, D.B., 2019. Antagonistic responses of exposure to sublethal temperatures: Adaptive phenotypic plasticity coincides with a reduction in organismal performance. Am. Nat. 194, 344–355. https://doi.org/10.1086/704208

Gingrich, J.B., Ward, R.A., Macken, L.M., Esser, K.M., 1982. African sleeping sickness: New evidence that mature tsetse flies (Glossina morsitans) can become potent vectors. Trans. R. Soc. Trop. Med. Hyg. 76, 479–481. https://doi.org/10.1016/0035-9203(82)90142-0

Githeko, A.K., Lindsay, S.W., Confalonieri, U.E., Patz, J.A., 2000. Climate change and vector-borne diseases: A regional analysis. Bull. World Health Organ. 78, 1136–1147. https://doi.org/10.1590/S0042-96862000000900009

Gooding, R.H., 1988. Infection of post-teneral tsetse flies (Glossina morsitans morsitans and Glossina morsitans centralis) with Trypanosoma brucei brucei. Can. J. Zool. 66, 1289–1292. https://doi.org/10.1139/z88-188

Gotcha, N., Terblanche, J.S., Nyamukondiwa, C., 2018. Plasticity and cross-tolerance to heterogeneous environments: divergent stress responses co-evolved in an African fruit fly. J. Evol. Biol. 31, 98–110. https://doi.org/10.1111/jeb.13201

Gratz, N.G., 2004. Critical review of the vector status of Aedes albopictus. Med. Vet. Entomol. 18, 215–227. https://doi.org/10.1111/j.0269-283X.2004.00513.x

Gunderson, A.R., 2023. Trade-offs between baseline thermal tolerance and thermal tolerance plasticity are much less common than it appears. Glob. Chang. Biol. 29, 3519–3524. https://doi.org/10.1111/gcb.16710

Gunderson, A.R., Dillon, M.E., Stillman, J.H., 2017. Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. Funct. Ecol. 31, 1529–1539. https://doi.org/10.1111/1365-2435.12874

Gunderson, A.R., Revell, L.J., 2022. Testing for genetic assimilation with phylogenetic comparative analysis: Conceptual, methodological, and statistical considerations. Evolution (N. Y). 76, 1942–1952. https://doi.org/10.1111/evo.14570

Gunderson, A.R., Stillman, J.H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proceeding R. Soc. B Biol. Sci. 282. https://doi.org/10.1098/rspb.2015.0401

Hadley, N.F., 1994. Water relations of terrestrial arthropods, 1st ed. Academic Press.

Haines, L.R., Vale, G.A., Barreaux, A.M.G., Ellstrand, N.C., Hargrove, J.W., English, S., 2020. Big Baby, Little Mother: Tsetse Flies Are Exceptions to the Juvenile Small Size Principle. BioEssays 42, 2000049. https://doi.org/10.1002/bies.202000049

Halsch, C.A., Shapiro, A.M., Fordyce, J.A., Nice, C.C., Thorne, J.H., Waetjen, D.P., Forister, M.L., 2021. Insects and recent climate change. Proc. Natl. Acad. Sci. U. S. A. 118, 2002543117. https://doi.org/10.1073/PNAS.2002543117

Hampe, A., Petit, R.J., 2005. Conserving biodiversity under climate change: the rear edge matters. Ecol. Lett. 8, 461–467. https://doi.org/Genetic Considerations in Ecological Restoration

Hansen, P.J., 2009. Effects of heat stress on mammalian reproduction. Philos. Trans. R. Soc. B Biol. Sci. 364, 3341–3350. https://doi.org/10.1098/rstb.2009.0131

Hargrove, J., English, S., Torr, S.J., Lord, J., Haines, L.R., Van Schalkwyk, C., Patterson, J., Vale, G., 2019. Wing length and host location in tsetse (Glossina spp.): Implications for control using stationary baits. Parasites and Vectors 12. https://doi.org/10.1186/s13071-018-3274-x

Hargrove, J.W., 2022. Improved estimates of abortion rates in tsetse (Glossina spp.). Med. Vet. Entomol. 1–16. https://doi.org/10.1111/mve.12659

Hargrove, J.W., 2004. Tsetse population dynamics, in: The Trypanosomiases. CABI, pp. 113–137. https://doi.org/10.1079/9780851994758.0113

Hargrove, J.W., 2001. The effect of temperature and saturation deficit on mortality in populations of male Glossina m. morsitans (Diptera: Glossinidae) in Zimbabwe and Tanzania. Bull. Entomol. Res. 91, 79–86. https://doi.org/10.1079/BER200074

Hargrove, J.W., 1999. Reproductive abnormalities in field tsetse flies in Zimbabwe. Entomol. Exp. Appl. 92, 89–99.

Hargrove, J.W., 1994. Reproductive rates of tsetse flies in the field in Zimbabwe. Physiol. Entomol. 19, 307–318. https://doi.org/10.1111/j.1365-3032.1994.tb01057.x

Hargrove, J.W., 1993. Age-dependent sampling biases in tsetse flies (Glossina). Problems associated with estimating mortality from sample age distributions, in: Management of Insect Pests:

Nuclear and Related Molecular and Genetic Techniques. International Atomic Energy Agency, Vienna, pp. 549–556.

Hargrove, J.W., 1988. Tsetse: the limits to population growth. Med. Vet. Entomol. 2, 203–217. https://doi.org/10.1111/j.1365-2915.1988.tb00184.x

Hargrove, J.W., Coates, T.W., 1990. Metabolic rates of tsetse flies in the field as measured by the excretion of injected caesium. Physiol. Entomol. 15, 157–166. https://doi.org/10.1111/j.1365-3032.1990.tb00503.x

Hargrove, J.W., Ouifki, R., Ameh, J.E., 2011. A general model for mortality in adult tsetse (Glossina spp.). Med. Vet. Entomol. 25, 385–394. https://doi.org/10.1111/j.1365-2915.2011.00953.x

Hargrove, J.W., Vale, G.A., 2020. Models for the rates of pupal development, fat consumption and mortality in tsetse (Glossina spp). Bull. Entomol. Res. 110, 44–56. https://doi.org/10.1017/S0007485319000233

Hargrove, J.W., Williams, B.G., 1995. A cost-benefit analysis of feeding in female tsetse. Med. Vet. Entomol. 9, 109–119. https://doi.org/10.1111/j.1365-2915.1995.tb00166.x

Hay, S.I., Guerra, C.A., Tatem, A.J., Noor, A.M., Snow, R.W., 2004. The global distribution and population at risk of malaria: Past, present, and future. Lancet Infect. Dis. 4, 327–336. https://doi.org/10.1016/S1473-3099(04)01043-6

Helmuth, B., Kingsolver, J.G., Carrington, E., 2005. Biophysics, physiological ecology, and climate change: Does mechanism matter? Annu. Rev. Physiol. 67, 177–201. https://doi.org/10.1146/annurev.physiol.67.040403.105027

Herd, C.S., Grant, D.G., Lin, J., Franz, A.W.E., 2021. Starvation at the larval stage increases the vector competence of Aedes aegypti females for Zika virus. PLoS Negl. Trop. Dis. 15, e0010003. https://doi.org/10.1371/journal.pntd.0010003

Hinchliff, C.E., Smith, S.A., Allman, J.F., Burleigh, J.G., Chaudhary, R., Coghill, L.M., Crandall, K.A., Deng, J., Drew, B.T., Gazis, R., Gude, K., Hibbett, D.S., Katz, L.A., Dail Laughinghouse, H., McTavish, E.J., Midford, P.E., Owen, C.L., Ree, R.H., Rees, J.A., Soltisc, D.E., Williams, T., Cranston, K.A., 2015. Synthesis of phylogeny and taxonomy into a comprehensive tree of life. Proc. Natl. Acad. Sci. U. S. A. 112, 12764–12769. https://doi.org/10.1073/pnas.1423041112

Hirano, K., Nonami, Y., Nakamura, Y., Sato, Toshiyuki, Sato, Takuya, Ishiguro, K. ichiro, Ogawa, T., Yoshida, S., 2022. Temperature sensitivity of DNA double-strand break repair underpins heat-induced meiotic failure in mouse spermatogenesis. Commun. Biol. 5.

https://doi.org/10.1038/s42003-022-03449-y

Hoffmann, A.A., Chown, S.L., Clusella-Trullas, S., 2013. Upper thermal limits in terrestrial ectotherms: How constrained are they? Funct. Ecol. 27, 934–949. https://doi.org/10.1111/j.1365-2435.2012.02036.x

Hoffmann, A.A., Sørensen, J.G., Loeschcke, V., 2003. Adaptation of Drosophila to temperature extremes: Bringing together quantitative and molecular approaches. J. Therm. Biol. 28, 175–216. https://doi.org/10.1016/S0306-4565(02)00057-8

Honěk, A., 1993. Intraspecific Variation in Body Size and Fecundity in Insects: A General Relationship. Oikos 66, 483. https://doi.org/10.2307/3544943

Iossa, G., 2019. Sex-Specific Differences in Thermal Fertility Limits. Trends Ecol. Evol. 34, 490–492. https://doi.org/10.1016/j.tree.2019.02.016

IPCC, 2021. Assessment Report 6 Climate Change 2021: The Physical Science Basis.

Jackson, C.H., 1948. The analysis of a tsetse-fly population. III. Ann. Eugen. 14, 91–108. https://doi.org/10.1111/j.1469-1809.1947.tb02381.x

Jackson, C.H.N., 1946. An artificially isolated generation of tsetse flies (Diptera). Bull. Entomol. Res. 37, 291–299. https://doi.org/10.1017/S0007485300022203

Jiang, L., Zhang, Jing, Wang, J.J., Wang, L., Zhang, L., Li, G., Yang, X., Ma, X., Sun, X., Cai, J., Zhang, Jun, Huang, X., Yu, M., Wang, X., Liu, F., Wu, C.I., He, C., Zhang, B., Ci, W., Liu, J., 2013. Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. Cell 153, 773–784. https://doi.org/10.1016/j.cell.2013.04.041

Jørgensen, L.B., Robertson, R.M., Overgaard, J., 2020. Neural dysfunction correlates with heat coma and CTmax in Drosophila but does not set the boundaries for heat stress survival. J. Exp. Biol. 223. https://doi.org/10.1242/jeb.218750

Kalra, B., Tamang, A.M., Parkash, R., 2017. Cross-tolerance effects due to adult heat hardening, desiccation and starvation acclimation of tropical drosophilid-Zaprionus indianus. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 209, 65–73. https://doi.org/10.1016/j.cbpa.2017.04.014

Karaca, A.G., Parker, H.M., Yeatman, J.B., McDaniel, C.D., 2002. The effects of heat stress and sperm quality classification on broiler breeder male fertility and semen ion concentrations. Br. Poult. Sci. 43, 621–628. https://doi.org/10.1080/0007166022000004552

Kellermann, V., Overgaard, J., Hoffmann, A.A., Fljøgaard, C., Svenning, J.C., Loeschcke, V., 2012. Upper thermal limits of Drosophila are linked to species distributions and strongly constrained phylogenetically. Proc. Natl. Acad. Sci. U. S. A. 109, 16228–16233. https://doi.org/10.1073/pnas.1207553109

Kellermann, V., van Heerwaarden, B., 2019. Terrestrial insects and climate change: adaptive responses in key traits. Physiol. Entomol. 44, 99–115. https://doi.org/10.1111/phen.12282

Kellermann, V., van Heerwaardenf, B., Sgrò, C.M., 2017. How important is thermal history? Evidence for lasting effects of developmental temperature on upper thermal limits in Drosophila melanogaster. Proc. R. Soc. B Biol. Sci. 284, 14–16. https://doi.org/10.1098/rspb.2017.0447

Kelly, A.E., Goulden, M.L., 2008. Rapid shifts in plant distribution with recent climate change. Proc. Natl. Acad. Sci. U. S. A. 105, 11823–11826. https://doi.org/10.1073/pnas.0802891105

Kingsolver, J.G., Huey, R.B., 2008. Size, temperature, and fitness: three rules. Evol. Ecol. Res. 10, 251–268.

Kinzner, M.C., Gamisch, A., Hoffmann, A.A., Seifert, B., Haider, M., Arthofer, W., Schlick-Steiner, B.C., Steiner, F.M., 2019. Major range loss predicted from lack of heat adaptability in an alpine Drosophila species. Sci. Total Environ. 695, 133753. https://doi.org/10.1016/j.scitotenv.2019.133753

Kleynhans, E., Terblanche, J.S., 2011. Complex interactions between temperature and relative humidity on water balance of adult tsetse (Glossinidae, Diptera): implications for climate change. Front. Physiol. 2, 14379. https://doi.org/10.3389/fphys.2011.00074

Knobler, S., Mahmoud, A., Lemon, S., Pray, L., 2006. Exploring the Consequences and Opportunities: Workshop Summary, The Impact of Globalization on Infectious Disease Emergence and Control. https://doi.org/10.17226/11588

Koricheva, J., Gurevitch, J., Mengersen, K., 2013. Handbook of meta-analysis in ecology and evolution, Handbook of Meta-analysis in Ecology and Evolution. Princeton University Press. https://doi.org/10.23943/princeton/9780691137285.001.0001

Kosmidis, I., Kenne Pagui, E.C., Sartori, N., 2020. Mean and median bias reduction in generalized linear models. Stat. Comput. 30, 43–59.

Koštál, V., Tollarová-Borovanská, M., 2009. The 70 kDa heat shock protein assists during the repair of chilling injury in the insect, Pyrrhocoris apterus. PLoS One 4. https://doi.org/10.1371/journal.pone.0004546

Kristensen, T.N., Hoffmann, A.A., Overgaard, J., Sørensen, J.G., Hallas, R., Loeschcke, V., 2008. Costs and benefits of cold acclimation in field-released Drosophila. Proc. Natl. Acad. Sci. U. S. A. 105, 216–221. https://doi.org/10.1073/pnas.0708074105

Kuehn, L., McCormick, S., 2017. Heat exposure and maternal health in the face of climate change. Int. J. Environ. Res. Public Health 14, 853. https://doi.org/10.3390/ijerph14080853

Laland, K.N., Uller, T., Feldman, M.W., Sterelny, K., Müller, G.B., Moczek, A., Jablonka, E., Odling-Smee, J., 2015. The extended evolutionary synthesis: Its structure, assumptions and predictions. Proc. R. Soc. B Biol. Sci. 282. https://doi.org/10.1098/rspb.2015.1019

Lancaster, L.T., Humphreys, A.M., 2020. Global variation in the thermal tolerances of plants. Proc. Natl. Acad. Sci. U. S. A. 1–8. https://doi.org/10.1073/pnas.1918162117

Leak, S.G.A., 1998. Tsetse biology and ecology: their role in the epidemiology and control of trypanosomosis. CABI Publishing, Oxford and New York.

Leclère, D., Obersteiner, M., Barrett, M., Butchart, S.H.M., Chaudhary, A., De Palma, A., DeClerck, F.A.J., Di Marco, M., Doelman, J.C., Dürauer, M., Freeman, R., Harfoot, M., Hasegawa, T., Hellweg, S., Hilbers, J.P., Hill, S.L.L., Humpenöder, F., Jennings, N., Krisztin, T., Mace, G.M., Ohashi, H., Popp, A., Purvis, A., Schipper, A.M., Tabeau, A., Valin, H., van Meijl, H., van Zeist, W.J., Visconti, P., Alkemade, R., Almond, R., Bunting, G., Burgess, N.D., Cornell, S.E., Di Fulvio, F., Ferrier, S., Fritz, S., Fujimori, S., Grooten, M., Harwood, T., Havlík, P., Herrero, M., Hoskins, A.J., Jung, M., Kram, T., Lotze-Campen, H., Matsui, T., Meyer, C., Nel, D., Newbold, T., Schmidt-Traub, G., Stehfest, E., Strassburg, B.B.N., van Vuuren, D.P., Ware, C., Watson, J.E.M., Wu, W., Young, L., 2020. Bending the curve of terrestrial biodiversity needs an integrated strategy. Nature 585, 551–556. https://doi.org/10.1038/s41586-020-2705-y

Lehane, M.J., 2005. The biology of blood-sucking in insects, second edition, The Biology of Blood-Sucking in Insects, Second Edition. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511610493

Lehmann, T., Dalton, R., Kim, E.H., Dahl, E., Diabate, A., Dabire, R., Dujardin, J.P., 2006. Genetic contribution to variation in larval development time, adult size, and longevity of starved adults of Anopheles gambiae. Infect. Genet. Evol. 6, 410–416. https://doi.org/10.1016/j.meegid.2006.01.007

Lenth, R. V., 2016. Least-Squares Means: The R Package lsmeans. J. Stat. Softw. 69, 1–33. https://doi.org/10.18637/jss.v069.i01

Leonard, A.M., Lancaster, L.T., 2020. Maladaptive plasticity facilitates evolution of thermal tolerance during an experimental range shift. BMC Evol. Biol. 20, 1–11. https://doi.org/10.1186/s12862-020-1589-7

Leong, C.M., Tsang, T.P.N., Guenard, B., 2022. Testing the reliability and ecological implications of ramping rates in the measurement of Critical Thermal maximum. PLoS One 17, e0265361. https://doi.org/10.1371/journal.pone.0265361

Li, Z., Srivastava, P., 2003. Heat-Shock Proteins. Curr. Protoc. Immunol. 58, A.1T.1-A.1T.6. https://doi.org/10.1002/0471142735.ima01ts58

Liu, P.R., Raftery, A.E., 2021. Country-based rate of emissions reductions should increase by 80% beyond nationally determined contributions to meet the 2 °C target. Commun. Earth Environ. 2, 1–10. https://doi.org/10.1038/s43247-021-00097-8

Loeschcke, V., Hoffmann, A.A., 2002. The detrimental acclimation hypothesis. Trends Ecol. Evol. 17, 407–408. https://doi.org/10.1016/S0169-5347(02)02558-2

Longbottom, J., Caminade, C., Gibson, H.S., Weiss, D.J., Torr, S., Lord, J.S., 2020. Modelling the impact of climate change on the distribution and abundance of tsetse in Northern Zimbabwe. Parasites and Vectors 13, 1–11. https://doi.org/10.1186/s13071-020-04398-3

Lord, J.S., Hargrove, J.W., Torr, S.J., Vale, G.A., 2018. Climate change and African trypanosomiasis vector populations in Zimbabwe's Zambezi Valley: A mathematical modelling study. PLoS Med. 15. https://doi.org/10.1371/journal.pmed.1002675

Lord, J.S., Leyland, R., Haines, L.R., Barreaux, A.M.G., Bonsall, M.B., Torr, S.J., English, S., 2021. Effects of maternal age and stress on offspring quality in a viviparous fly. Ecol. Lett. 24, 2113–2122. https://doi.org/10.1111/ele.13839

Lord, J.S., Mthombothi, Z., Lagat, V.K., Atuhaire, F., Hargrove, J.W., 2017. Host-seeking efficiency can explain population dynamics of the tsetse fly Glossina morsitans morsitans in response to host density decline. PLoS Negl. Trop. Dis. 11. https://doi.org/10.1371/journal.pntd.0005730

Losey, J.E., Vaughan, M., 2006. The economic value of ecological services provided by insects. Bioscience 56, 311–323. https://doi.org/10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2

Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: History and critique. Can. J. Zool. 75, 1561–1574. https://doi.org/10.1139/z97-783

Ma, C. Sen, Ma, G., Pincebourde, S., 2021. Survive a Warming Climate: Insect Responses to Extreme

High Temperatures. Annu. Rev. Entomol. 66, 163–184. https://doi.org/10.1146/annurev-ento-041520-074454

Macartney, E.L., Crean, A.J., Nakagawa, S., Bonduriansky, R., 2019. Effects of nutrient limitation on sperm and seminal fluid: a systematic review and meta-analysis. Biol. Rev. 94, 1722–1739. https://doi.org/10.1111/brv.12524

Maclean, H.J., Sørensen, J.G., Kristensen, T.N., Loeschcke, V., Beedholm, K., Kellermann, V., Overgaard, J., Maclean, H.J., 2019. Evolution and plasticity of thermal performance: An analysis of variation in thermal tolerance and fitness in 22 Drosophila species. Philos. Trans. R. Soc. B Biol. Sci. 374. https://doi.org/10.1098/rstb.2018.0548

MacMillan, H.A., Nørgård, M., MacLean, H.J., Overgaard, J., Williams, C.J.A., 2017. A critical test of Drosophila anaesthetics: Isoflurane and sevoflurane are benign alternatives to cold and CO2. J. Insect Physiol. 101, 97–106. https://doi.org/10.1016/j.jinsphys.2017.07.005

MacMillan, H.A., Sinclair, B.J., 2011. Mechanisms underlying insect chill-coma. J. Insect Physiol. 57, 12–20. https://doi.org/10.1016/j.jinsphys.2010.10.004

Mangwiro, T.N.C., Torr, S.J., Cox, J.R., Holloway, M.T.P., 1999. The efficacy of various pyrethroid insecticides for use on odour-baited targets to control tsetse. Med. Vet. Entomol. 13, 315–323. https://doi.org/10.1046/j.1365-2915.1999.00165.x

Maxwell, S.L., Fuller, R.A., Brooks, T.M., Watson, J.E.M., 2016. Biodiversity: The ravages of guns, nets and bulldozers. Nature 536, 143–145. https://doi.org/10.1038/536143a

Meehl, G.A., Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science (80-.). 305, 994–997. https://doi.org/10.1126/science.1098704

Mellanby, H., 1937. Experimental work on reproduction in the tsetse fly, glossina palpalis. Parasitology 29, 131–141. https://doi.org/10.1017/S0031182000026263

Memmott, J., Craze, P.G., Waser, N.M., Price, M. V, 2007. Global warming and the disruption of plant-pollinator interactions. Ecol. Lett. 10, 710–717. https://doi.org/10.1111/j.1461-0248.2007.01061.x

Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L.F., Backeljau, T., Bonte, D., Brans, K.I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J.M.T., Fontaneto, D., Gianuca, A.T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E., Sablon, R., Schön, I., Van Doninck, K., De Meester, L., Van Dyck, H., 2018. Body-size shifts in aquatic and terrestrial urban communities. Nature 558, 113–116. https://doi.org/10.1038/s41586-018-

0140-0

Meyerhoeffer, D.C., Wettemann, R.P., Coleman, S.W., Wells, M.E., 1985. Reproductive criteria of beef bulls during and after exposure to increased ambient temperature. J. Anim. Sci. 60, 352–357. https://doi.org/10.2527/jas1985.602352x

Michalkova, V., Benoit, J.B., Weiss, B.L., Attardo, G.M., Aksoy, S., 2014. Vitamin B6 generated by obligate symbionts is critical for maintaining proline homeostasis and fecundity in tsetse flies. Appl. Environ. Microbiol. 80, 5844–5853. https://doi.org/10.1128/AEM.01150-14

Michie, L.J., Masson, A., Ware, R.L., Jiggins, F.M., 2011. Seasonal phenotypic plasticity: Wild ladybirds are darker at cold temperatures. Evol. Ecol. 25, 1259–1268. https://doi.org/10.1007/s10682-011-9476-8

Mockett, R.J., Matsumoto, Y., 2014. Effect of prolonged coldness on survival and fertility of Drosophila melanogaster. PLoS One 9, e92228. https://doi.org/10.1371/journal.pone.0092228

Moczek, A.P., 2010. Phenotypic plasticity and diversity in insects. Philos. Trans. R. Soc. B 365, 593–603. https://doi.org/10.1098/rstb.2009.0263

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., Altman, D., Antes, G., Atkins, D., Barbour, V., Barrowman, N., Berlin, J.A., Clark, J., Clarke, M., Cook, D., D'Amico, R., Deeks, J.J., Devereaux, P.J., Dickersin, K., Egger, M., Ernst, E., Gøtzsche, P.C., Grimshaw, J., Guyatt, G., Higgins, J., Ioannidis, J.P.A., Kleijnen, J., Lang, T., Magrini, N., McNamee, D., Moja, L., Mulrow, C., Napoli, M., Oxman, A., Pham, B., Rennie, D., Sampson, M., Schulz, K.F., Shekelle, P.G., Tovey, D., Tugwell, P., 2009. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 6. https://doi.org/10.1371/journal.pmed.1000097

Monaghan, P., 2008. Early growth conditions, phenotypic development and environmental change. Philos. Trans. R. Soc. B 363, 1635–1645. https://doi.org/10.1098/rstb.2007.0011

Moore, S.M., Eisen, R.J., Monaghan, A., Mead, P., 2014. Meteorological influences on the seasonality of lyme disease in the United States. Am. J. Trop. Med. Hyg. 90, 486–496. https://doi.org/10.4269/ajtmh.13-0180

Morin, C.W., Comrie, A.C., Ernst, K., 2013. Climate and dengue transmission: Evidence and implications. Environ. Health Perspect. 121, 1264–1272. https://doi.org/10.1289/ehp.1306556

Morley, S.A., Peck, L.S., Sunday, J.M., Heiser, S., Bates, A.E., 2019. Physiological acclimation and persistence of ectothermic species under extreme heat events. Glob. Ecol. Biogeogr. 28, 1018–1037. https://doi.org/10.1111/geb.12911

Mousseau, T.A., Fox, C.W., 1998. The adaptive significance of maternal effects. Trends Ecol. Evol. 13, 403–407. https://doi.org/10.1016/S0169-5347(98)01472-4

Mugenyi, A.W., 2015. The spatial distribution of Tsetse (Diptera: Glossinidae) within the Trypanosoma brucei rhodesiense focus of Uganda. University of Edinburgh.

Nakagawa, S., Lagisz, M., Jennions, M.D., Koricheva, J., Noble, D.W.A., Parker, T.H., Sánchez-Tójar, A., Yang, Y., O'Dea, R., 2022. Methods for testing publication bias in ecological and evolutionary meta-analyses. Methods Ecol. Evol. 13, 4–21. https://doi.org/10.32942/OSF.IO/K7PMZ

Nash, T.A.M., 1936. The Relationship between the Maximum Temperature and the Seasonal Longevity of Glossina submorsitans, Newst., and G. tachinoides, Westw., in Northern Nigeria. Bull. Entomol. Res. 27, 273–279. https://doi.org/10.1017/S000748530005848X

Nati, J.J.H., Svendsen, M.B.S., Marras, S., Killen, S.S., Steffensen, J.F., McKenzie, D.J., Domenici, P., 2021. Intraspecific variation in thermal tolerance differs between tropical and temperate fishes. Sci. Rep. 11, 1–8. https://doi.org/10.1038/s41598-021-00695-8

Nervo, B., Roggero, A., Chamberlain, D., Caprio, E., Rolando, A., Palestrini, C., 2021. Physiological, morphological and ecological traits drive desiccation resistance in north temperate dung beetles. BMC Zool. 6, 1–13. https://doi.org/10.1186/s40850-021-00089-3

Nguyen, T.M., Bressac, C., Chevrier, C., 2013. Heat stress affects male reproduction in a parasitoid wasp. J. Insect Physiol. 59, 248–254. https://doi.org/10.1016/j.jinsphys.2012.12.001

Nijhout, H.F., 2003. Development and evolution of adaptive polyphenisms. Evol. Dev. 5, 9–18. https://doi.org/10.1046/j.1525-142X.2003.03003.x

Nik Abdull Halim, N.M.H., Che Dom, N., Dapari, R., Salim, H., Precha, N., 2022. A systematic review and meta-analysis of the effects of temperature on the development and survival of the Aedes mosquito. Front. Public Heal. 10, 1074028. https://doi.org/10.3389/fpubh.2022.1074028

O'connor, C.M., Norris, D.R., Crossin, G.T., Cooke, S.J., 2014. Biological carryover effects: Linking common concepts and mechanisms in ecology and evolution. Ecosphere 5, 1–11. https://doi.org/10.1890/ES13-00388.1

Odhiambo, T.R., Kokwaro, E.D., Sequeira, L.M., 1983. Histochemical and ultrastructural studies of the male accessory reproductive glands and spermatophore of the tsetse, Glossina morsitans morsitans Westwood. Int. J. Trop. Insect Sci. 4, 227–236. https://doi.org/10.1017/s1742758400001193

Oliveira, B.F., Yogo, W.I.G., Hahn, D.A., Yongxing, J., Scheffers, B.R., 2021. Community-wide seasonal shifts in thermal tolerances of mosquitoes. Ecology 102. https://doi.org/10.1002/ecy.3368

Oostra, V., Saastamoinen, M., Zwaan, B.J., Wheat, C.W., 2018. Strong phenotypic plasticity limits potential for evolutionary responses to climate change. Nat. Commun. 9. https://doi.org/10.1038/s41467-018-03384-9

Opiyo, E., Parker, A.G., Mohammed, A.H., 2006. Standard Operating Procedures for Mass-Rearing Tsetse Flies.

Ørsted, M., Jørgensen, L.B., Overgaard, J., 2022. Finding the right thermal limit: a framework to reconcile ecological, physiological and methodological aspects of CTmax in ectotherms. J. Exp. Biol. 225. https://doi.org/10.1242/jeb.244514

Overgaard, J., Gerber, L., Andersen, M.K., 2021. Osmoregulatory capacity at low temperature is critical for insect cold tolerance. Curr. Opin. Insect Sci. https://doi.org/10.1016/j.cois.2021.02.015

Overgaard, J., Hoffmann, A.A., Kristensen, T.N., 2011. Assessing population and environmental effects on thermal resistance in Drosophila melanogaster using ecologically relevant assays. J. Therm. Biol. 36, 409–416. https://doi.org/10.1016/j.jtherbio.2011.07.005

Overgaard, J., Kearney, M.R., Hoffmann, A.A., 2014. Sensitivity to thermal extremes in Australian Drosophila implies similar impacts of climate change on the distribution of widespread and tropical species. Glob. Chang. Biol. 20, 1738–1750. https://doi.org/10.1111/gcb.12521

Overgaard, J., Kristensen, T.N., Sørensen, J.G., 2012. Validity of thermal ramping assays used to assess thermal tolerance in arthropods. PLoS One 7, e32758. https://doi.org/10.1371/journal.pone.0032758

Overgaard, J., Macmillan, H.A., 2017. The Integrative Physiology of Insect Chill Tolerance. Annu. Rev. Physiol. https://doi.org/10.1146/annurev-physiol-022516-034142

Overgaard, J., Sørensen, J.G., Petersen, S.O., Loeschcke, V., Holmstrup, M., 2006. Reorganization of membrane lipids during fast and slow cold hardening in Drosophila melanogaster. Physiol. Entomol. 31, 328–335. https://doi.org/10.1111/j.1365-3032.2006.00522.x

Overgaard, J., Sørensen, J.G., Petersen, S.O., Loeschcke, V., Holmstrup, M., 2005. Changes in membrane lipid composition following rapid cold hardening in Drosophila melanogaster. J. Insect Physiol. 51, 1173–1182. https://doi.org/10.1016/j.jinsphys.2005.06.007

Oyen, K.J., Dillon, M.E., 2018. Critical thermal limits of bumblebees (Bombus impatiens) are marked

by stereotypical behaviors and are unchanged by acclimation, age or feeding status. J. Exp. Biol. 221. https://doi.org/10.1242/jeb.165589

Pacifici, M., Foden, W.B., Visconti, P., Watson, J.E.M., Butchart, S.H., Kovacs, K.M., Scheffers, B.R., Hole, D.G., Martin, T.G., Akçakaya, H.R., Corlett, R.T., Huntley, B., Bickford, D., Carr, J.A., Hoffmann, A.A., Midgley, G.F., Pearce-Kelly, P., Pearson, R.G., Williams, S.E., Willis, S.G., Young, B., Rondinini, C., 2015. Assessing species vulnerability to climate change. Nat. Clim. Chang. 5, 215–225. https://doi.org/10.1038/nclimate2448

Pagabeleguem, S., Ravel, S., Dicko, A.H., Vreysen, M.J.B., Parker, A., Takac, P., Huber, K., Sidibé, I., Gimonneau, G., Bouyer, J., 2016. Influence of temperature and relative humidity on survival and fecundity of three tsetse strains. Parasites and Vectors 9, 520. https://doi.org/10.1186/s13071-016-1805-x

Pais, R., Lohs, C., Wu, Y., Wang, J., Aksoy, S., 2008. The obligate mutualist Wigglesworthia glossinidia influences reproduction, digestion, and immunity processes of its host, the tsetse fly. Appl. Environ. Microbiol. 74, 5965–5974. https://doi.org/10.1128/AEM.00741-08

Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. 37, 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Parmesan, C., Root, T.L., Willig, M.R., 2000. Impacts of extreme weather and climate on terrestrial biota. Bull. Am. Meteorol. Soc. 81, 443–450. https://doi.org/10.1175/1520-0477(2000)081<0443:IOEWAC>2.3.CO;2

Parratt, S.R., Walsh, B.S., Metelmann, S., White, N., Manser, A., Bretman, A.J., Hoffmann, A.A., Snook, R.R., Price, T.A.R., 2021. Temperatures that sterilize males better match global species distributions than lethal temperatures. Nat. Clim. Chang. 11, 481–484. https://doi.org/10.1038/s41558-021-01047-0

Peck, L.S., Clark, M.S., Morley, S.A., Massey, A., Rossetti, H., 2009. Animal temperature limits and ecological relevance: Effects of size, activity and rates of change. Funct. Ecol. 23, 248–256. https://doi.org/10.1111/j.1365-2435.2008.01537.x

Peralta-Maraver, I., Rezende, E.L., 2021. Heat tolerance in ectotherms scales predictably with body size. Nat. Clim. Chang. 11, 58–63. https://doi.org/10.1038/s41558-020-00938-y

Percoma, L., Rayaissé, J.B., Gimonneau, G., Bengaly, Z., Pooda, S.H., Pagabeleguem, S., Ganaba, R., Sow, A., Argilés, R., Bouyer, J., Ouedraogo, M., Zhao, W., Paone, M., Sidibé, I., Gisele, O. /S, Cecchi, G., 2022. An atlas to support the progressive control of tsetse-transmitted animal

trypanosomosis in Burkina Faso. Parasites and Vectors 15, 1–13. https://doi.org/10.1186/s13071-021-05131-4

Perez-Crespo, M., Pintado, B., Gutierrez-Adan, A., 2008. Scrotal Heat Stress Effects on Sperm Viability, Sperm DNA Integrity, and the Offspring Sex Ratio in Mice. Mol. Reprod. Dev. 75, 40–47. https://doi.org/10.1002/mrd

Perkins-Kirkpatrick, S.E., Lewis, S.C., 2020. Increasing trends in regional heatwaves. Nat. Commun. 11, 1–8. https://doi.org/10.1038/s41467-020-16970-7

Perkins, S.E., Alexander, L. V., Nairn, J.R., 2012. Increasing frequency, intensity and duration of observed global heatwaves and warm spells. Geophys. Res. Lett. 39. https://doi.org/10.1029/2012GL053361

Phelps, R.J., 1973. The effect of temperature on fat consumption during the puparial stages of Glossina morsitans morsitans Westw. (Dipt., Glossinidae) under laboratory conditions, and its implication in the field. Bull. Entomol. Res. 62, 423–438. https://doi.org/10.1017/S0007485300003953

Phelps, R. J., Burrows, P.M., 1969. Lethal temperatures for puparia of Glossina morsitans orientalis. Entomol. Exp. Appl. 12, 23–32. https://doi.org/10.1111/j.1570-7458.1969.tb02493.x

Phelps, R J, Burrows, P.M., 1969. Prediction of the Pupal Duration of Glossina morsitans orientalis Vanderplank under Field Conditions. J. Appl. Ecol. 6, 323–337. https://doi.org/10.2307/2401546

Phelps, R.J., Clarke, G.P.Y., 1974. Seasonal elimination of some size classes in males of Glossina morsitans morsitans Westw. (Diptera, Glossinidae). Bull. Entomol. Res. 64, 313–324. https://doi.org/10.1017/S0007485300031205

Phillips, B.L., Muñoz, M.M., Hatcher, A., Macdonald, S.L., Llewelyn, J., Lucy, V., Moritz, C., 2016. Heat hardening in a tropical lizard: geographic variation explained by the predictability and variance in environmental temperatures. Funct. Ecol. 30, 1161–1168. https://doi.org/10.1111/1365-2435.12609

Pijpe, J., Brakefield, P.M., Zwaan, B.J., 2007. Phenotypic plasticity of starvation resistance in the butterfly Bicyclus anynana. Evol. Ecol. 21, 589–600. https://doi.org/10.1007/s10682-006-9137-5

Pincebourde, S., Dillon, M.E., Woods, H.A., 2021. Body size determines the thermal coupling between insects and plant surfaces. Funct. Ecol. 35, 1424–1436. https://doi.org/10.1111/1365-2435.13801

Pincebourde, S., Woods, H.A., 2020. There is plenty of room at the bottom: microclimates drive insect

vulnerability to climate change. Curr. Opin. Insect Sci. 41, 63–70. https://doi.org/10.1016/j.cois.2020.07.001

Pinsky, M.L., Eikeset, A.M., McCauley, D.J., Payne, J.L., Sunday, J.M., 2019. Greater vulnerability to warming of marine versus terrestrial ectotherms. Nature 569, 108–111. https://doi.org/10.1038/s41586-019-1132-4

Pintor, A.F.V., Schwarzkopf, L., Krockenberger, A.K., 2016. Extensive acclimation in ectotherms conceals interspecific variation in thermal tolerance limits. PLoS One 11, e0150408. https://doi.org/10.1371/journal.pone.0150408

Porcelli, D., Gaston, K.J., Butlin, R.K., Snook, R.R., 2017. Local adaptation of reproductive performance during thermal stress. J. Evol. Biol. 30, 422–429. https://doi.org/10.1111/jeb.13018

Pörtner, H.O., 2010. Oxygen- And capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881–893. https://doi.org/10.1242/jeb.037523

Pottier, P., Burke, S., Drobniak, S.M., Lagisz, M., Nakagawa, S., 2021. Sexual (in)equality? A meta-analysis of sex differences in thermal acclimation capacity across ectotherms. Funct. Ecol. 35, 2663–2678. https://doi.org/10.1111/1365-2435.13899

Pottier, P., Burke, S., Zhang, R.Y., Noble, D.W.A., Schwanz, L.E., Drobniak, S.M., Nakagawa, S., 2022. Developmental plasticity in thermal tolerance: Ontogenetic variation, persistence, and future directions. Ecol. Lett. 25, 2245–2268. https://doi.org/10.1111/ele.14083

Potts, W.H., 1933. Observations on Glossina morsitans, Westw., in East Africa. Bull. Entomol. Res. 24, 293–300. https://doi.org/10.1017/S000748530003145X

Price, T.D., Qvarnström, A., Irwin, D.E., 2003. The role of phenotypic plasticity in driving genetic evolution. Proc. R. Soc. B Biol. Sci. 270, 1433–1440. https://doi.org/10.1098/rspb.2003.2372

Purse, B. V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C., Matthew, B., Abstract, 2005. Climate change and the recent emergence of bluetongue in Europe. Nat. Rev. 3, 171–182.

R Core Team, 2023. R: A language and environment for statistical computing.

“R Core Team,” 2021. R: A language and environment for statistical computing. R Found. Stat. Comput.

Randolf, S.E., Williams, B.G., Rogers, D.J., Connor, H., 1992. Modelling the effect of feeding-related mortality on the feeding strategy of tsetse (Diptera: Glossinidae). Med. Vet. Entomol. 6, 231–240. https://doi.org/10.1111/j.1365-2915.1992.tb00611.x

Reading, C.J., 2007. Linking global warming to amphibian declines through its effects on female body condition and survivorship. Oecologia 151, 125–131. https://doi.org/10.1007/s00442-006-0558-1

Recsetar, M.S., Zeigler, M.P., Ward, D.L., Bonar, S.A., Caldwell, C.A., 2012. Relationship between fish size and upper thermal tolerance. Trans. Am. Fish. Soc. 141, 1433–1438. https://doi.org/10.1080/00028487.2012.694830

Renault, D., Hance, T., Vannier, G., Vernon, P., 2003. Is body size an influential parameter in determining the duration of survival at low temperatures in Alphitobius diaperinus Panzer (Coleoptera: Tenebrionidae)? J. Zool. 259, 381–388. https://doi.org/10.1017/S0952836902003382

Rezende, E.L., Tejedo, M., Santos, M., 2011. Estimating the adaptive potential of critical thermal limits: Methodological problems and evolutionary implications. Funct. Ecol. 25, 111–121. https://doi.org/10.1111/j.1365-2435.2010.01778.x

Riordan, E.K., 1986. Abortion by the tsetse fly Glossina palpalis palpalis after treatment with insecticides. Entomol. Exp. Appl. 41, 193–200. https://doi.org/10.1111/j.1570-7458.1986.tb00528.x

Robine, J.M., Cheung, S.L.K., Le Roy, S., Van Oyen, H., Griffiths, C., Michel, J.P., Herrmann, F.R., 2008. Death toll exceeded 70,000 in Europe during the summer of 2003. Comptes Rendus - Biol. 331, 171–178. https://doi.org/10.1016/j.crvi.2007.12.001

Rocklöv, J., Tozan, Y., Ramadona, A., Sewe, M.O., Sudre, B., Garrido, J., De Saint Lary, C.B., Lohr, W., Semenza, J.C., 2019. Using big data to monitor the introduction and spread of Chikungunya, Europe, 2017. Emerg. Infect. Dis. 25, 1041–1049. https://doi.org/10.3201/eid2506.180138

Rodrigues, Y.K., Beldade, P., 2020. Thermal Plasticity in Insects' Response to Climate Change and to Multifactorial Environments. Front. Ecol. Evol. 8, 271. https://doi.org/10.3389/fevo.2020.00271

Roff, D.A., Fairbairn, D.J., 2007. The evolution of trade-offs: Where are we? J. Evol. Biol. 20, 433–447. https://doi.org/10.1111/j.1420-9101.2006.01255.x

Rogers, D.., Randolph, S.., 1993. Simple Multi-variate Analysis of Vector Distributions. Parasitol. Today 9, 266–271.

Rogers, D.J., Packer, M.J., 1993. Vector-borne diseases, models, and global change. Lancet 342, 1282–1284. https://doi.org/10.1016/0140-6736(93)92367-3

Rohatgi, A., 2010. WebPlotDigitizer - extract data from plots, images, and maps. Arohatgi.

Rohmer, C., David, J.R., Moreteau, B., Joly, D., 2004. Heat induced male sterility in Drosophila melanogaster: adaptive genetic variations among geographic populations and role of the Y chromosome. J. Exp. Biol. 207, 2735–2743.

Rohr, J.R., Civitello, D.J., Cohen, J.M., Roznik, E.A., Sinervo, B., Dell, A.I., 2018. The complex drivers of thermal acclimation and breadth in ectotherms. Ecol. Lett. 21, 1425–1439. https://doi.org/10.1111/ele.13107

Roma, J.S., D'Souza, S., Somers, P.J., Cabo, L.F., Farsin, R., Aksoy, S., Runyen-Janecky, L.J., Weiss, B.L., 2019. Thermal stress responses of Sodalis glossinidius, an indigenous bacterial symbiont of hematophagous tsetse flies. PLoS Negl. Trop. Dis. 13, e0007464. https://doi.org/10.1371/journal.pntd.0007464

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C., Pounds, J.A., 2003. Fingerprints of global warming on wild animals and plants. Nature 421, 57–60. https://doi.org/10.1038/nature01333

Russo, S., Dosio, A., Graversen, R.G., Sillmann, J., Carrao, H., Dunbar, M.B., Singleton, A., Montagna, P., Barbola, P., Vogt, J. V, 2014. Magnitude of extreme heat waves in present climate and their projection in a warming world. J. Geophys. Res. Atmos. 119, 12,500-12,512. https://doi.org/10.1002/2014JD022098

Sage, T.L., Bagha, S., Lundsgaard-Nielsen, V., Branch, H.A., Sultmanis, S., Sage, R.F., 2015. The effect of high temperature stress on male and female reproduction in plants. F. Crop. Res. 182, 30–42. https://doi.org/10.1016/j.fcr.2015.06.011

Sales, K., Vasudeva, R., Dickinson, M.E., Godwin, J.L., Lumley, A.J., Michalczyk, Ł., Hebberecht, L., Thomas, P., Franco, A., Gage, M.J.G., 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. Nat. Commun. 9, 1–11. https://doi.org/10.1038/s41467-018-07273-z

Sales, K., Vasudeva, R., Gage, M.J.G., 2021. Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. R. Soc. Open Sci. 8. https://doi.org/10.1098/rsos.201717

Sánchez-Bayo, F., Wyckhuys, K.A.G., 2019. Worldwide decline of the entomofauna: A review of its drivers. Biol. Conserv. 232, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Sandblom, E., Clark, T.D., Gräns, A., Ekström, A., Brijs, J., Sundström, L.F., Odelström, A., Adill, A., Aho,

T., Jutfelt, F., 2016. Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. Nat. Commun. 7, 1–8. https://doi.org/10.1038/ncomms11447

Santos, M.A., Antunes, M.A., Grandela, A., Quina, A.S., Santos, M., Matos, M., Simões, P., 2023. Slow and population specific evolutionary response to a warming environment. Sci. Rep. 13. https://doi.org/10.1038/s41598-023-36273-3

Scharf, I., Galkin, N., Halle, S., 2014. Disentangling the Consequences of Growth Temperature and Adult Acclimation Temperature on Starvation and Thermal Tolerance in the Red Flour Beetle. Evol. Biol. 42, 54–62. https://doi.org/10.1007/s11692-014-9298-z

Schou, M.F., Kristensen, T.N., Kellermann, V., Schlötterer, C., Loeschcke, V., 2014. A Drosophila laboratory evolution experiment points to low evolutionary potential under increased temperatures likely to be experienced in the future. J. Evol. Biol. 27, 1859–1868. https://doi.org/10.1111/jeb.12436

Schou, M F., Kristensen, T.N., Pedersen, A., Göran Karlsson, B., Loeschcke, V., Malmendal, A., 2017. Metabolic and functional characterization of effects of developmental temperature in Drosophila melanogaster. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 312, 211–222. https://doi.org/10.1152/ajpregu.00268.2016

Schou, M.F., Loeschcke, V., Kristensen, T.N., 2015. Strong costs and benefits of winter acclimatization in Drosophila melanogaster. PLoS One 10, 1–13. https://doi.org/10.1371/journal.pone.0130307

Schou, M F, Mouridsen, M.B., Sørensen, J.G., Loeschcke, V., 2017. Linear reaction norms of thermal limits in Drosophila: predictable plasticity in cold but not in heat tolerance. Funct. Ecol. 31, 934–945. https://doi.org/10.1111/1365-2435.12782

Scolari, F., Benoit, J.B., Michalkova, V., Aksoy, E., Takac, P., Abd-Alla, A.M.M., Malacrida, A.R., Aksoy, S., Attardo, G.M., 2016. The Spermatophore in Glossina morsitans morsitans: Insights into Male Contributions to Reproduction. Sci. Rep. 6, 20334. https://doi.org/10.1038/srep20334

Seebacher, F., White, C.R., Franklin, C.E., 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nat. Clim. Chang. 5, 61–66. https://doi.org/10.1038/nclimate2457

Seko, T., Nakasuji, F., 2006. Adaptive significance of egg size plasticity in response to temperature in the migrant skipper, Parnara guttata guttata (Lepidoptera: Hesperiidae). Popul. Ecol. 48, 159–166. https://doi.org/10.1007/s10144-006-0253-4

Sgrò, C.M., Overgaard, J., Kristensen, T.N., Mitchell, K.A., Cockerell, F.E., Hoffmann, A.A., 2010. A

comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of Drosophila melanogaster from Eastern Australia. J. Evol. Biol. 23, 2484–2493. https://doi.org/10.1111/j.1420-9101.2010.02110.x

Sgrò, C.M., Terblanche, J.S., Hoffmann, A.A., 2016. What Can Plasticity Contribute to Insect Responses to Climate Change? Annu. Rev. Entomol. 61, 433–451. https://doi.org/10.1146/annurev-ento-010715-023859

Sheldon, K.S., Dillon, M.E., 2016. Beyond the mean: Biological impacts of cryptic temperature change. Integr. Comp. Biol. 56, 110–119. https://doi.org/10.1093/icb/icw005

Shereni, W., Neves, L., Argilés, R., Nyakupinda, L., Cecchi, G., 2021. An atlas of tsetse and animal African trypanosomiasis in Zimbabwe. Parasites and Vectors 14, 1–10. https://doi.org/10.1186/s13071-020-04555-8

Sheridan, J.A., Bickford, D., 2011. Shrinking body size as an ecological response to climate change. Nat. Clim. Chang. 1, 401–406. https://doi.org/10.1038/nclimate1259

Sibilia, C.D., Brosko, K.A., Hickling, C.J., Thompson, L.M., Grayson, K.L., Olson, J.R., 2018. Thermal physiology and developmental plasticity of pigmentation in the harlequin bug (Hemiptera: Pentatomidae). J. Insect Sci. 18. https://doi.org/10.1093/jisesa/iey066

Simarro, P.P., Cecchi, G., Franco, J.R., Paone, M., Diarra, A., Ruiz-Postigo, J.A., Fèvre, E.M., Mattioli, R.C., Jannin, J.G., 2012. Estimating and Mapping the Population at Risk of Sleeping Sickness. PLoS Negl. Trop. Dis. 6. https://doi.org/10.1371/journal.pntd.0001859

Simon, J.A., Marrotte, R.R., Desrosiers, N., Fiset, J., Gaitan, J., Gonzalez, A., Koffi, J.K., Lapointe, F.J., Leighton, P.A., Lindsay, L.R., Logan, T., Milord, F., Ogden, N.H., Rogic, A., Roy-Dufresne, E., Suter, D., Tessier, N., Millien, V., 2014. Climate change and habitat fragmentation drive the occurrence of Borrelia burgdorferi, the agent of Lyme disease, at the northeastern limit of its distribution. Evol. Appl. 7, 750–764. https://doi.org/10.1111/eva.12165

Simon, M.N., Arturo, P.L., Navas, C.A., 2015. Upper thermal tolerance plasticity in tropical amphibian species fromcontrasting habitats: Implications for warming impact prediction. J. Therm. Biol. 48, 36–44.

Sisodia, S., Singh, B.N., 2009. Variations in morphological and life-history traits under extreme temperatures in Drosophila ananassae. J. Biosci. 34, 263–274.

Slotsbo, S., Schou, M.F., Kristensen, T.N., Loeschcke, V., Sørensen, J.G., 2016. Reversibility of developmental heat and cold plasticity is asymmetric and has long-lasting consequences for

adult thermal tolerance. J. Exp. Biol. 219, 2726–2732. https://doi.org/10.1242/jeb.143750

Smith, J.J., Hasiotis, S.T., Kraus, M.J., Woody, D.T., 2009. Transient dwarfism of soil fauna during the Paleocene-Eocene Thermal Maximum. Proc. Natl. Acad. Sci. U. S. A. 106, 17655–17660. https://doi.org/10.1073/pnas.0909674106

Solano, P., Salou, E., Rayaisse, J.B., Ravel, S., Gimonneau, G., Traore, I., Bouyer, J., 2015. Do tsetse flies only feed on blood? Infect. Genet. Evol. 36, 184–189. https://doi.org/10.1016/j.meegid.2015.09.016

Sørensen, J.G., Kristensen, T.N., Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 6, 1025–1037. https://doi.org/10.1046/j.1461-0248.2003.00528.x

Sørensen, J.G., Kristensen, T.N., Overgaard, J., 2016. Evolutionary and ecological patterns of thermal acclimation capacity in Drosophila: is it important for keeping up with climate change? Curr. Opin. Insect Sci. 17, 98–104. https://doi.org/10.1016/j.cois.2016.08.003

Steelman, C.D., 1976. Effects of external and internal arthropod parasites on domestic livestock production. Annu. Rev. 21, 155–78.

Štětina, T., Koštál, V., Korbelová, J., 2015. The role of inducible Hsp70, and other heat shock proteins, in adaptive complex of cold tolerance of the fruit fly (Drosophila melanogaster). PLoS One 10, 1–22. https://doi.org/10.1371/journal.pone.0128976

Stevenson, R.D., 1985a. The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. Am. Nat. 126, 362–386. https://doi.org/10.1086/284423

Stevenson, R.D., 1985b. Body Size and Limits to the Daily Range of Body Temperature in Terrestrial Ectotherms. Am. Nat. 125, 102–117. https://doi.org/10.1086/284330

Steverding, D., Troscianko, T., 2004. On the role of blue shadows in the visual behaviour of tsetse flies. Proc. R. Soc. B Biol. Sci. 271, S16–S17. https://doi.org/10.1098/rsbl.2003.0121

Stillman, J.H., 2019. Heat waves, the new normal: Summertime temperature extremes will impact animals, ecosystems, and human communities. Physiology 34, 86–100. https://doi.org/10.1152/physiol.00040.2018

Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science (80-.). 301, 65. https://doi.org/10.1126/science.1083073

Stillman, J.H., Somero, G.N., 2000. A comparative analysis of the upper thermal tolerance limits of

eastern pacific porcelain crabs, genus Petrolisthes: Influences of latitude, vertical zonation, acclimation, and phylogeny. Physiol. Biochem. Zool. 73, 200–208. https://doi.org/10.1086/316738

Stillwell, R.C., Blanckenhorn, W.U., Teder, T., Davidowitz, G., Fox, C.W., 2010. Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: From physiology to evolution. Annu. Rev. Entomol. 55, 227–245. https://doi.org/10.1146/annurev-ento-112408-085500

Suggitt, A.J., Gillingham, P.K., Hill, J.K., Huntley, B., Kunin, W.E., Roy, D.B., Thomas, C.D., 2011. Habitat microclimates drive fine-scale variation in extreme temperatures. Oikos 120, 1–8. https://doi.org/10.1111/j.1600-0706.2010.18270.x

Sunday, J., Bennett, J.M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A.L., Leiva, F.P., Verberk, W.C.E.P., Olalla-Tárraga, M.Á., Morales-Castilla, I., 2019. Thermal tolerance patterns across latitude and elevation. Philos. Trans. R. Soc. B Biol. Sci. 374. https://doi.org/10.1098/rstb.2019.0036

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proc. R. Soc. B Biol. Sci. 278, 1823–1830. https://doi.org/10.1098/rspb.2010.1295

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., Huey, R.B., 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc. Natl. Acad. Sci. U. S. A. 111, 5610–5615. https://doi.org/10.1073/pnas.1316145111

Florida Arbovirus Surveillance Week 28: July 9-15, 2023.

Tanekou, T.T.M., Tsakeng, C.U.B., Tirados, I., Acho, A., Bigoga, J., Wondji, C.S., Njiokou, F., 2023. Impact of a small-scale tsetse fly control operation with deltamethrin impregnated “Tiny Targets” on tsetse density and trypanosomes’ circulation in the Campo sleeping sickness focus of South Cameroon. PLoS Negl. Trop. Dis. 17, e0011802. https://doi.org/10.1371/journal.pntd.0011802

Tarka, M., Guenther, A., Niemelä, P.T., Nakagawa, S., Noble, D.W.A., 2018. Sex differences in life history, behavior, and physiology along a slow-fast continuum: a meta-analysis. Behav. Ecol. Sociobiol. 72, 1–13. https://doi.org/10.1007/s00265-018-2534-2

Terblanche, J.S., Chown, S.L., 2007. The effects of temperature, body mass and feeding on metabolic rate in the tsetse fly Glossina morsitans centralis: Short communication. Physiol. Entomol. 32, 175–180. https://doi.org/10.1111/j.1365-3032.2006.00549.x

Terblanche, J.S., Chown, S.L., 2006. The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, Glossina pallidipes (Diptera, Glossinidae). J. Exp. Biol. 209, 1064–1073. https://doi.org/10.1242/jeb.02129

Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., Chown, S.L., 2008. Thermal tolerance in a south-east African population of the tsetse fly Glossina pallidipes (Diptera, Glossinidae): Implications for forecasting climate change impacts. J. Insect Physiol. 54, 114–127. https://doi.org/10.1016/j.jinsphys.2007.08.007

Terblanche, J.S., Deere, J.A., Clusella-Trullas, S., Janion, C., Chown, S.L., 2007. Critical thermal limits depend on methodological context. Proc. R. Soc. B Biol. Sci. 274, 2935–2942. https://doi.org/10.1098/rspb.2007.0985

Terblanche, J.S., Hoffmann, A., 2020. Validating measurements of acclimation for climate change adaptation. Curr. Opin. Insect Sci. 41, 7–16. https://doi.org/10.1016/j.cois.2020.04.005

Terblanche, J.S., Jaco, C.K., Krafsur, E.S., Chown, S.L., Klok, C.J., Krafsur, E.S., Chown, S.L., 2006. Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse Glossina pallidipes (Diptera: Glossinidae): Implications for distribution modelling. Am. J. Trop. Med. Hyg. 74, 786–794. https://doi.org/10.4269/ajtmh.2006.74.786

Terblanche, J.S., Klok, C.J., Chown, S.L., 2005. Temperature-dependence of metabolic rate in Glossina morsitans morsitans (Diptera, Glossinidae) does not vary with gender, age, feeding, pregnancy or acclimation. J. Insect Physiol. 51, 861–870. https://doi.org/10.1016/j.jinsphys.2005.03.017

Therneau, T., 2023. A Package for Survival Analysis in R.

Therneau, T.M., Grambsch, P.M., 2000. Modeling Survival Data: Extending the Cox Model. Springer, New York.

Thomson, M.C., 1987. The effect on tsetse flies (glossina spp.) of deltamethin applied to cattle either as a spray or incorporated into ear-tags. Trop. Pest Manag. 33, 329–335. https://doi.org/10.1080/09670878709371177

Todd, B.D., Nowakowski, A.J., 2021. Ectothermy and the macroecology of home range scaling in snakes. Glob. Ecol. Biogeogr. 30, 262–276. https://doi.org/10.1111/geb.13225

Torr, S.J., Holloway, M.T.P., Vale, G.A., 1992. Improved persistence of insecticide deposits on targets for controlling Glossina pallidipes (Diptera: Glossinidae). Bull. Entomol. Res. 82, 525–533. https://doi.org/10.1017/S0007485300042620

Traut, W., Sahara, K., Marec, F., 2008. Sex Chromosomes and Sex Determination in Lepidoptera. Sex. Dev. 1, 332–346. https://doi.org/10.1159/000111765

Tseng, M., Kaur, K.M., Soleimani Pari, S., Sarai, K., Chan, D., Yao, C.H., Porto, P., Toor, A., Toor, H.S., Fograscher, K., 2018. Decreases in beetle body size linked to climate change and warming temperatures. J. Anim. Ecol. 87, 647–659. https://doi.org/10.1111/1365-2656.12789

Tyukavina, A., Potapov, P., Hansen, M.C., Pickens, A.H., Stehman, S. V., Turubanova, S., Parker, D., Zalles, V., Lima, A., Kommareddy, I., Song, X.-P., Wang, L., Harris, N., 2022. Global Trends of Forest Loss Due to Fire From 2001 to 2019. Front. Remote Sens. 3, 1–20. https://doi.org/10.3389/frsen.2022.825190

van Heerwaarden, B., Kellermann, V., 2020. Does Plasticity Trade Off With Basal Heat Tolerance? Trends Ecol. Evol. 35, 874–885. https://doi.org/10.1016/j.tree.2020.05.006

van Heerwaarden, B., Kellermann, V., Sgrò, C.M., 2016. Limited scope for plasticity to increase upper thermal limits. Funct. Ecol. 30, 1947–1956. https://doi.org/10.1111/1365-2435.12687

van Heerwaarden, B., Sgrò, C., Kellermann, V.M., 2024. Threshold shifts and developmental temperature impact trade-offs between tolerance and plasticity. Proc. R. Soc. B Biol. Sci. 291, 20232700. https://doi.org/10.1098/rspb.2023.2700

van Heerwaarden, B., Sgrò, C.M., 2021. Male fertility thermal limits predict vulnerability to climate warming. Nat. Commun. 12, 1–11. https://doi.org/10.1038/s41467-021-22546-w

Vasudeva, R., Dickinson, M., Sutter, A., Powell, S., Sales, K., Gage, M.J.G., 2021. Facultative polyandry protects females from compromised male fertility caused by heatwave conditions. Anim. Behav. 178, 37–48. https://doi.org/10.1016/j.anbehav.2021.05.016

Verberk, W.C.E.P., Leuven, R.S.E.W., van der Velde, G., Gabel, F., 2018. Thermal limits in native and alien freshwater peracarid Crustacea: The role of habitat use and oxygen limitation. Funct. Ecol. 32, 926–936. https://doi.org/10.1111/1365-2435.13050

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor. J. Stat. Softw. 36, 1–48.

Vinagre, C., Dias, M., Cereja, R., Abreu-Afonso, F., Flores, A.A.V., Mendonça, V., 2019. Upper thermal limits and warming safety margins of coastal marine species – Indicator baseline for future reference. Ecol. Indic. 102, 644–649. https://doi.org/10.1016/j.ecolind.2019.03.030

Vinagre, C., Mendonça, V., Cereja, R., Abreu-Afonso, F., Dias, M., Mizrahi, D., Flores, A.A.V., 2018. Ecological traps in shallow coastal waters-Potential effect of heat-waves in tropical and

temperate organisms. PLoS One 13, e0192700. https://doi.org/10.1371/journal.pone.0192700

Vitasse, Y., Baumgarten, F., Zohner, C.M., Rutishauser, T., Pietragalla, B., Gehrig, R., Dai, J., Wang, H., Aono, Y., Sparks, T.H., 2022. The great acceleration of plant phenological shifts. Nat. Clim. Chang. 12, 300–302. https://doi.org/10.1038/s41558-022-01283-y

von May, R., Catenazzi, A., Santa-Cruz, R., Gutierrez, A.S., Moritz, C., Rabosky, D.L., 2019. Thermal physiological traits in tropical lowland amphibians: Vulnerability to climate warming and cooling. PLoS One 14. https://doi.org/10.1371/journal.pone.0219759

Vreysen, M.J.B., Saleh, K., Mramba, F., Parker, A., Feldmann, U., Dyck, V.A., Msangi, A., Bouyer, J., 2014. Sterile Insects to Enhance Agricultural Development: The Case of Sustainable Tsetse Eradication on Unguja Island, Zanzibar, Using an Area-Wide Integrated Pest Management Approach. PLoS Negl. Trop. Dis. 8, 1–5. https://doi.org/10.1371/journal.pntd.0002857

Walsh, B.S., Parratt, S.R., Atkinson, D., Snook, R.R., Bretman, A., Price, T.A.R., 2019a. Integrated Approaches to Studying Male and Female Thermal Fertility Limits. Trends Ecol. Evol. 34, 492–493. https://doi.org/10.1016/j.tree.2019.03.005

Walsh, B.S., Parratt, S.R., Hoffmann, A.A., Atkinson, D., Snook, R.R., Bretman, A., Price, T.A.R., 2019b. The Impact of Climate Change on Fertility. Trends Ecol. Evol. 34, 249–259. https://doi.org/10.1016/j.tree.2018.12.002

Walsh, B.S., Parratt, S.R., Mannion, N.L.M., Snook, R.R., Bretman, A., Price, T.A.R., 2021. Plastic responses of survival and fertility following heat stress in pupal and adult Drosophila virilis. Ecol. Evol. 11, 18238–18247. https://doi.org/10.1002/ece3.8418

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. Nature 416, 389–395. https://doi.org/10.1038/416389a

Wang, J., Weiss, B.L., Aksoy, S., 2013. Tsetse fly microbiota: Form and function. Front. Cell. Infect. Microbiol. 3. https://doi.org/10.3389/fcimb.2013.00069

Watts, N., Amann, M., Arnell, N., Ayeb-Karlsson, S., Belesova, K., Boykoff, M., Byass, P., Cai, W., Campbell-Lendrum, D., Capstick, S., Chambers, J., Dalin, C., Daly, M., Dasandi, N., Davies, M., Drummond, P., Dubrow, R., Ebi, K.L., Eckelman, M., Ekins, P., Escobar, L.E., Fernandez Montoya, L., Georgeson, L., Graham, H., Haggar, P., Hamilton, I., Hartinger, S., Hess, J., Kelman, I., Kiesewetter, G., Kjellstrom, T., Kniveton, D., Lemke, B., Liu, Y., Lott, M., Lowe, R., Sewe, M.O., Martinez-Urtaza, J., Maslin, M., McAllister, L., McGushin, A., Jankin Mikhaylov, S., Milner, J.,

Moradi-Lakeh, M., Morrissey, K., Murray, K., Munzert, S., Nilsson, M., Neville, T., Oreszczyn, T., Owfi, F., Pearman, O., Pencheon, D., Phung, D., Pye, S., Quinn, R., Rabbaniha, M., Robinson, E., Rocklöv, J., Semenza, J.C., Sherman, J., Shumake-Guillemot, J., Tabatabaei, M., Taylor, J., Trinanes, J., Wilkinson, P., Costello, A., Gong, P., Montgomery, H., 2019. The 2019 report of The Lancet Countdown on health and climate change: ensuring that the health of a child born today is not defined by a changing climate. Lancet 394, 1836–1878. https://doi.org/10.1016/S0140-6736(19)32596-6

Weaving, H., Lord, J.S., Haines, L., English, S., 2023a. No evidence for direct thermal carryover effects on starvation tolerance in the obligate blood-feeder, Glossina morsitans morsitans. Ecol. Evol. 13. https://doi.org/10.1002/ece3.10652

Weaving, H., Terblanche, J.S., English, S., 2024. Heatwaves are detrimental to fertility in the viviparous tsetse fly. Proceeding R. Soc. B Biol. Sci. 291, 20232710.

Weaving, H., Terblanche, J.S., English, S., 2023b. How plastic are upper thermal limits? A comparative study in tsetse (family: Glossinidae) and wider Diptera. J. Therm. Biol. 118. https://doi.org/10.1016/j.jtherbio.2023.103745

Weaving, H., Terblanche, J.S., Pottier, P., English, S., 2022. Meta-analysis reveals weak but pervasive plasticity in insect thermal limits. Nat. Commun. 13. https://doi.org/10.1038/s41467-022-32953-2

Welbergen, J.A., Booth, C., Martin, J., 2014. Killer climate: tens of thousands of flying foxes dead in a day. URL https://theconversation.com/killer-climate-tens-of-thousands-of-flying-foxes-dead-in-a-day-23227

Welbergen, J.A., Klose, S.M., Markus, N., Eby, P., 2008. Climate change and the effects of temperature extremes on Australian flying-foxes. Proc. R. Soc. B Biol. Sci. 275, 419–425. https://doi.org/10.1098/rspb.2007.1385

West-Eberhard, M.J., 2002. Developmental Plasticity and Evolution, Oxford University Press. Oxford Univeristy Press, New York. https://doi.org/10.1038/hdy.2015.14

WHO, 2023a. Number of reported cases of human African trypanosomiasis (T.b. gambiense). Glob. Heal. Obs. URL https://www.who.int/data/gho/data/indicators/indicator-details/GHO/hat-tb-gambiense (accessed 8.31.23).

WHO, 2023b. Number of reported cases of human African trypanosomiasis (T.b. rhodesiense). Glob. Heal. Obs. URL https://www.who.int/data/gho/data/indicators/indicator-details/GHO/number-

of-new-reported-cases-of-human-african-trypanosomiasis-(t-b-rhodesiense) (accessed 8.31.23).

WHO, 2020. Vector-borne diseases. URL https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases#:~:text=Key facts,than 700 000 deaths annually. (accessed 8.24.23).

WHO, 2013. Control and surveillance of human African trypanosomiasis. World Health Organ. Tech. Rep. Ser. 1–237.

WHO FAO, 2022. Vector control and the elimination of gambiense human African trypanosomiasis (HAT) - Joint FAO/WHO Virtual Expert Meeting, 5-6 October 2021, in: Vector Control and the Elimination of Gambiense Human African Trypanosomiasis (HAT) - Joint FAO/WHO Virtual Expert Meeting, 5-6 October 2021. https://doi.org/10.4060/cc0178en

Wiens, J.J., 2016. Climate-Related Local Extinctions Are Already Widespread among Plant and Animal Species. PLoS Biol. 14, 1–18. https://doi.org/10.1371/journal.pbio.2001104

Williams, C.M., Buckley, L.B., Sheldon, K.S., Vickers, M., Pörtner, H.O., Dowd, W.W., Gunderson, A.R., Marshall, K.E., Stillman, J.H., 2016. Biological impacts of thermal extremes: mechanisms and costs of functional responses matter. Integr. Comp. Biol. 56, 73–84. https://doi.org/10.1093/icb/icw013

Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A., Langham, G., 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. PLoS Biol. 6. https://doi.org/10.1371/journal.pbio.0060325

Wonglersak, R., Fenberg, P.B., Langdon, P.G., Brooks, S.J., Price, B.W., 2021. Insect body size changes under future warming projections: a case study of Chironomidae (Insecta: Diptera). Hydrobiologia 848, 2785–2796. https://doi.org/10.1007/s10750-021-04597-8

Woods, H.A., Dillon, M.E., Pincebourde, S., 2015. The roles of microclimatic diversity and of behavior in mediating the responses of ectotherms to climate change. J. Therm. Biol. 54, 86–97. https://doi.org/10.1016/j.jtherbio.2014.10.002

Zhang, Y., Kieffer, J.D., 2014. Critical thermal maximum (CTmax) and hematology of shortnose sturgeons (Acipenser brevirostrum) acclimated to three temperatures. Can. J. Zool. 92, 215–221. https://doi.org/10.1139/cjz-2013-0223

Zinn, K.E., Tunc-Ozdemir, M., Harper, J.F., 2010. Temperature stress and plant sexual reproduction: Uncovering the weakest links. J. Exp. Bot. 61, 1959–1968. https://doi.org/10.1093/jxb/erq053

8. Appendices

8.1 Appendix 1 – Glossary

Acclimated Critical Thermal Limit - Upper or lower critical thermal limit after a period of acclimation.

Acclimation – A thermal exposure which can later improve thermal tolerance.

Acclimation Response Ratio - The change in critical thermal limit per degree change in acclimation temperature, representing the slope of a thermal reaction norm.

Basal Critical Thermal Limit – Upper or lower critical thermal limit at rearing temperature.

Carryover effect – When unfavourable conditions during development or in the parent environment result in reduced fitness in a subsequent life stage.

Critical thermal maximum (CT_{max}) - A measure of upper thermal tolerance where performance declines to zero.

Critical thermal minimum (CT_{min}) – A measure of lower thermal tolerance where performance declines to zero.

Developmental plasticity – When thermal conditions experienced during development result in phenotypic changes in the adult stage.

Hardening – A within life stage plastic phenotypic response to temperature occurring on an hourly, daily or seasonal scale.

Operative temperature (T_e) - The body temperature of an organism at equilibrium.

Optimum temperature (T_{opt}) – The temperature at which an animal is performing at its peak.

Phenotypic plasticity – The ability of an organism to react to the environment with a change in phenotype.

Thermal Fertility Limit – The temperature at which an animal stops producing offspring. This may be a temporary or permanent loss of fertility.

Thermal plasticity - The ability of an organism to react to an environmental temperature with a change in phenotype.

Thermal Safety Margin (TSM) - The temperature difference between the optimum performance and an animal's habitat or operative temperature.

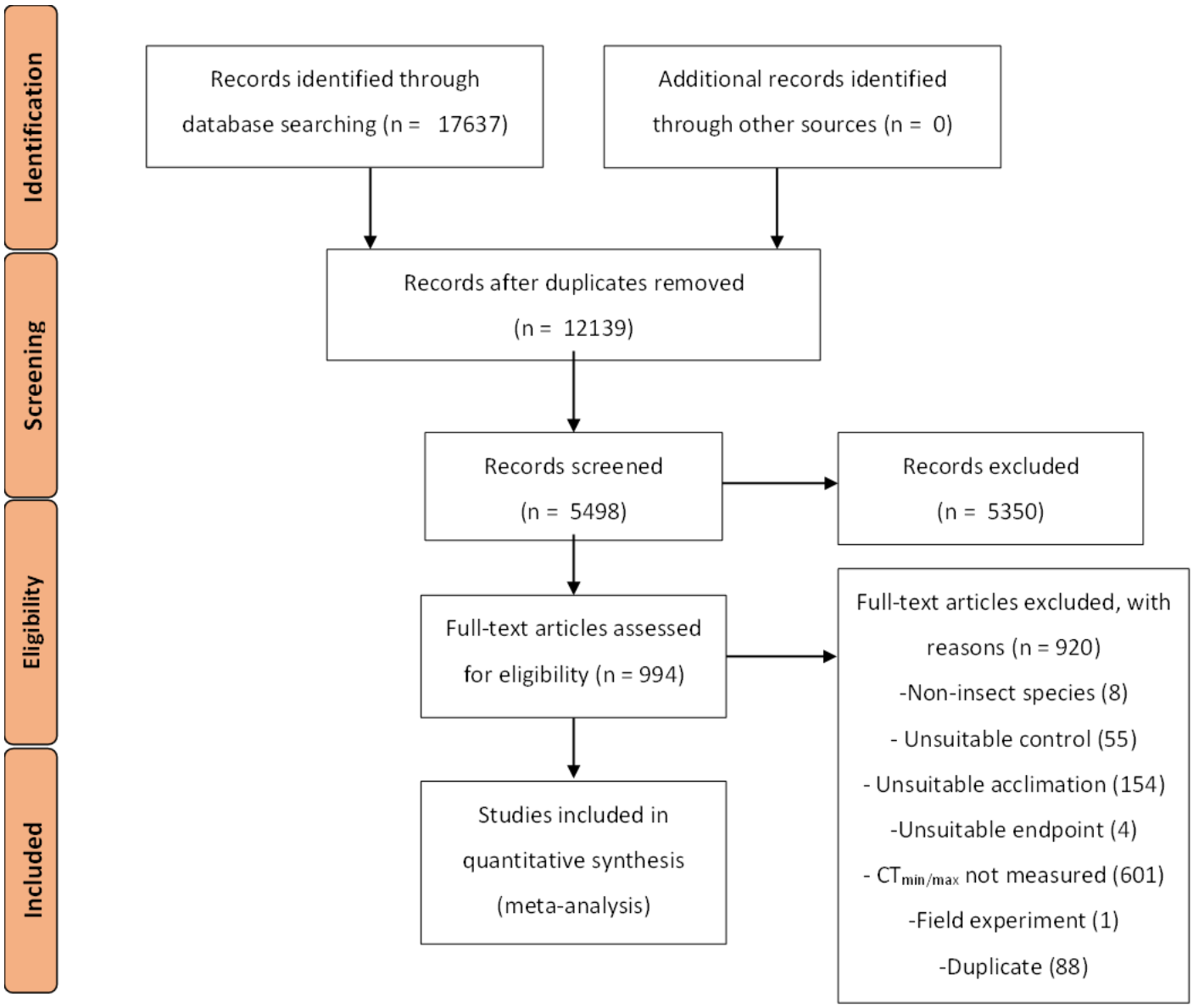
Thermal Sensitivity of Fertilty – The number of viable offspring produced at a given temperature.

Thermal tolerance breadth - The range of temperatures at which an organism can function with at least 80% performance.

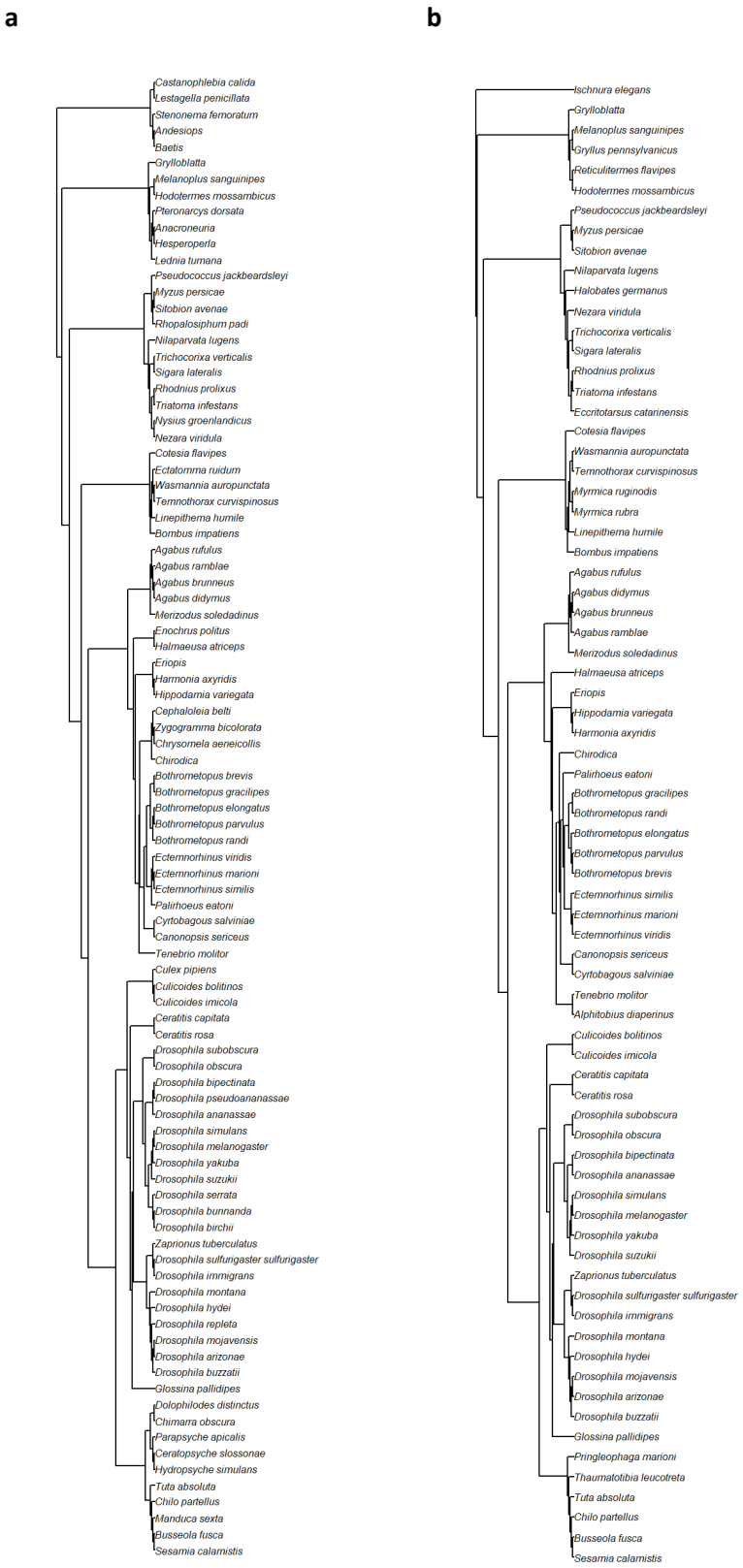
Transgenerational plasticity – Where the environment of the parent or grandparent results in a phenotypic change in offspring.

Warming tolerance - The temperature difference between CT_{max} and the animal's habitat or operative temperature.

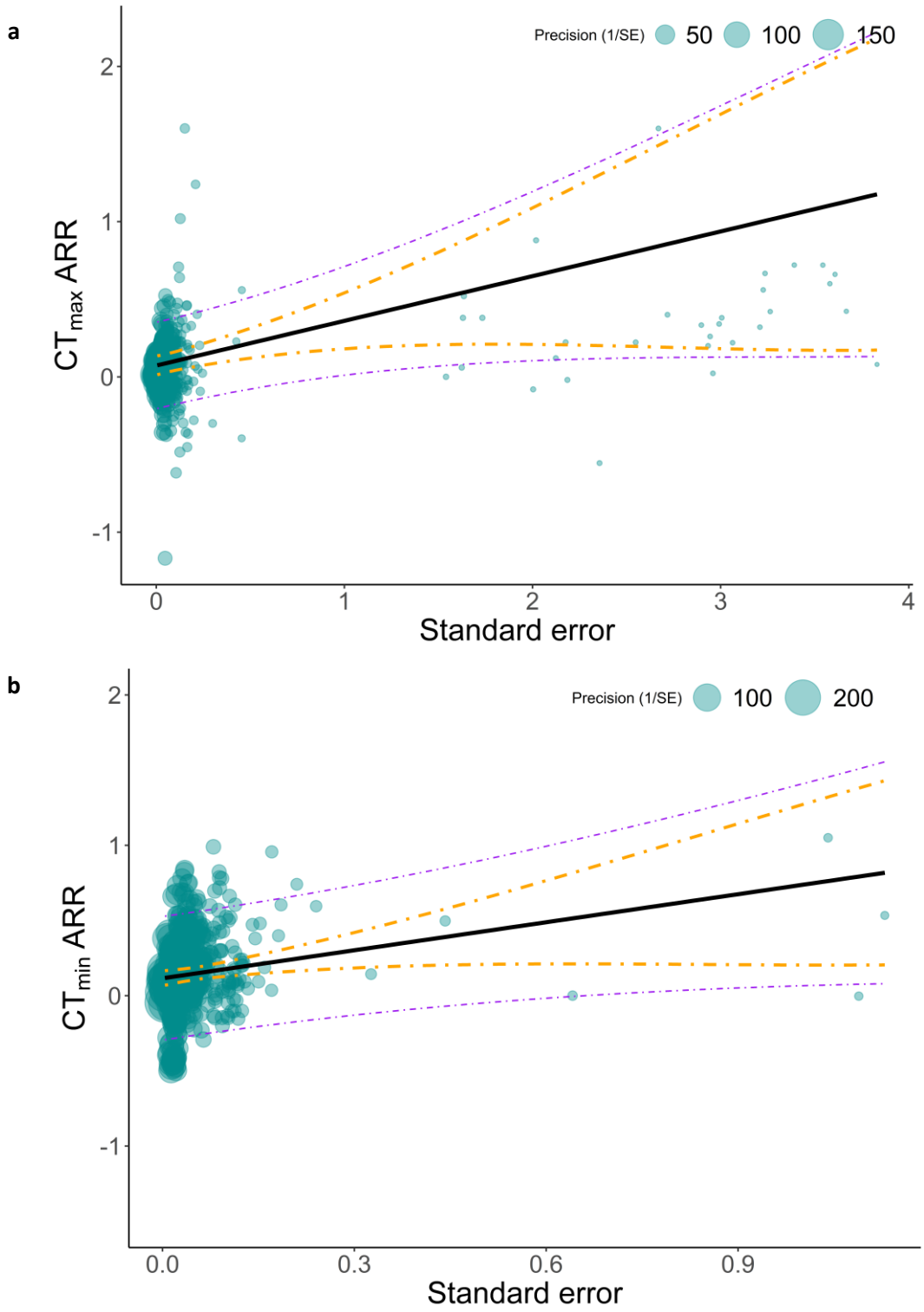
8.2 Appendix 2 - Chapter 2



Supplementary Figure 2.1 PRISMA exclusion procedure detailing how records were screened. First, duplicates were removed, then study titles were briefly screened to remove those clearly unsuitable e.g. non-insect species. Of the remaining articles, abstracts were assessed in detail and, finally, full methodology of selected studies was evaluated. Four articles were removed later in the analysis due studies measuring a very large number of insects at one time, meaning the sample size and therefore precision of the study was inflated. It was deemed that this many insects could not be assessed at once accurately.



Supplementary Figure 2.2 Phylogenetic tree used for (a) CT_{max} and (b) CT_{min} meta-analytic multi-level, random effects models. Phylogenetic trees were constructed using the Open Tree of Life and R packages 'rotl' and 'ape'.



Supplementary Figure 2.3 Relationship between (a) CT_{max} and (b) CT_{min} ARR (Acclimation Response Ratio) and standard error for Egger's regression test. A positive relationship shows positive publication bias. 95% confidence intervals are depicted by orange dotted lines, prediction intervals are purple dotted lines. The precision of the study (1/SE) is proportional to the size of each data point.

Supplementary Table 2.1 Heading descriptions for Supplementary Data from https://osf.io/cbhv4/

Heading	Description
rowid	ID of row
studyid	ID of study
popid	ID of insect population
ord	Order
fam	Family
gen	Genus
sp	Species
full_sp	Genus + species. Some species have been changed to synonyms or to higher names in their taxonomy so they are recognised in the synthetic tree of life.
habitat	Aquatic or terrestrial
source	Laboratory or field source. Lab if one or more generations is under lab conditions.
development	Holometabolous or hemimetabolous
acclim_stage	Life stage at which acclimation took place
ctm_stage	Life stage at which critical thermal limit assay took place
tsr	Whether acclimation was within a life stage or between stages to investigate the temperature-size rule.
sex	Male, female, mixed or both/unknown
lat	Latitude in degrees
stand_lat	Latitude in degrees away from equator
lat_bin	Latitude in 10 degree bins
mass	Wet mass of insect (mg) extracted from original papers
all_mass	Wet mass of insect (mg) extracted from original papers and other sources
acclim_temp	Acclimation temperature (°C)
cont_temp	Control temperature (°C)
acclim_dur	Duration of acclimation period in hours. Data were only included if acclimation was stated in hours. If recorded as the length of a life stage, the cell is left blank.
acclim_type	Constant or fluctuating temperature during acclimation
ctlimit	CT_{max} or CT_{min}
ramp_rate	Rate of temp ramp (°C/min)
groupID	Treatments within a group use pairwise comparisons to calculate ARR (e.g., 10-12°C, 12-15°C, 15-20°C).
treat	critical thermal limit of treatment group

treatSD	Standard deviation of treatment group
treatN	Sample size of treatment group
cont	critical thermal limit of control group
contSD	Standard deviation of control group
contN	Sample size of control group
simpctend	End point at which critical thermal limit is measured, simplified into groupings
data_type	The source of the data collected e.g. table, graph, directly from author
author	First author of paper
title	Paper title
journal	Journal name
year	Year of publication
webpage	Link to paper webpage
ARR	Calculated Acclimation Response Ratio
var	Calculated variance
precision	Calculated precision (1/Standard Error)
diff	Difference between two acclimation temperatures used for ARR calculation

Supplementary Table 2.2 Intercept multi-level meta-analytic, random effects models for upper (CT_{max}) and lower (CT_{min}) critical thermal limits, to test whether ARR (Acclimation Response Ratio) is significantly different from zero. Significant results (95% CIs do not span zero) are highlighted in bold. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. $R^2_{marg.}$: R^2 marginal, the variance explained only by moderators. $R^2_{cond.}$: R^2 conditional, the variance explained by moderators and random effects.

Limit	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
CT_{max}	803	**0.913**	**2.98**	**0.030**	**0.153**	-797.2	0.972	0.151	0.153	0.177	0.491	<0.001	0.495
CT_{min}	571	**0.147**	**7.17**	**0.106**	**0.188**	-292.4	0.991	0.361	<0.001	<0.001	0.630	<0.001	0.364

Supplementary Table 2.3 Univariate multi-level meta-analytic, random effects models for upper critical thermal limit (CT_{max}) ARR (Acclimation Response Ratio) for all moderators. Results for intercept models are displayed. Results are highlighted in bold where 95% CIs do not overlap between groups or where regressions are significant for continuous variables. (rob.) is where a robust model was used because the residuals were not homogeneous. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. $R^2_{marg.}$: R^2 marginal, the variance explained only by moderators. $R^2_{cond.}$: R^2 conditional, the variance explained by moderators and random effects.

Model	Comparison	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
~duration		521	<-0.001	-0.05	<-0.001	<0.001	-588.3	0.963	0.351	0.345	0.267	<0.001	<0.001	0.642
	Intercept	-	0.077	2.27	0.009	0.145	-	-	-	-	-	-	-	-
~ramp rate		803	0.020	0.45	-0.067	0.106	-794.8	0.973	0.162	0.479	0.160	0.171	0.001	0.508
	Intercept	-	0.085	2.44	0.015	0.155	-	-	-	-	-	-	-	-
~mass (rob.)		614	<0.001	0.94	-0.001	0.003	-689.3	0.976	0.032	0.443	0.313	0.188	0.047	0.567
	Intercept	-	0.142	2.74	0.024	0.153	-	-	-	-	-	-	-	-
~acclimation	**Early life**	803	**0.105**	**3.98**	**0.053**	**0.156**	-791.4	0.970	0.157	0.530	0.094	0.189	0.013	0.461
stage	**Adulthood**	-	**0.069**	**2.63**	**0.017**	**0.120**	-	-	-	-	-	-	-	-
	Whole life	-	0.076	2.63	0.019	0.132	-	-	-	-	-	-	-	-
	Several generations	-	0.080	1.85	-0.005	0.164	-	-	-	-	-	-	-	-
~source	Laboratory	803	0.105	2.87	0.032	0.177	-795.4	0.973	0.162	0.175	0.163	0.472	0.004	0.517
	Field	-	0.083	2.34	0.013	0.154	-	-	-	-	-	-	-	-
~habitat (rob.)	Aquatic	803	0.090	2.01	<0.001	0.180	-795.0	0.973	0.160	0.166	0.167	0.479	<0.001	0.507
	Terrestrial	-	0.093	2.74	0.025	0.161	-	-	-	-	-	-	-	-
~end point	Death	803	0.071	0.62	-0.160	0.302	-794.3	0.974	0.205	0.126	0.198	0.446	0.076	0.577
	Activity	-	**0.085**	**2.01**	**0.002**	**0.167**	-	-	-	-	-	-	-	-
	Clinging	-	**0.072**	**1.19**	**-0.047**	**0.191**	-	-	-	-	-	-	-	-
	Righting	-	**0.212**	**3.97**	**0.105**	**0.318**	-	-	-	-	-	-	-	-

	Stimulus response	-	**0.050**	**1.15**	**-0.035**	**0.136**	-	-	-	-	-	-	-	-
	Motor response	-	**0.078**	**1.92**	**-0.002**	**0.158**	-	-	-	-	-	-	-	-
~latitude		701	-0.001	-1.20	-0.002	<0.001	-607.8	0.972	0.294	0.071	0.148	0.459	0.004	0.530
	Intercept	-	0.115	2.81	0.033	0.198	-	-	-	-	-	-	-	-
~latitude (field-		187	-0.001	-0.72	-0.005	0.003	12.4	0.944	0.380	0.032	<0.001	0.532	0.010	0.441
caught subset)	Intercept	-	0.106	1.38	-0.053	0.265	-	-	-	-	-	-	-	-
~sex	Female	803	0.057	1.55	-0.016	0.130	-794.0	0.972	0.158	0.143	0.191	0.480	0.022	0.517
	Male	-	0.093	2.32	0.014	0.171	-	-	-	-	-	-	-	-
	Both	-	0.087	2.20	0.010	0.164	-	-	-	-	-	-	-	-
	Unknown	-	0.108	3.24	0.043	0.173	-	-	-	-	-	-	-	-
~development	**Holometabolous**	803	**0.045**	**1.48**	**-0.015**	**0.105**	-799.2	0.970	0.166	0.080	0.201	0.523	0.058	0.492
	Hemimetabolous	-	**0.135**	**3.95**	**0.067**	**0.203**	-	-	-	-	-	-	-	-
~development	Holometabolous	591	0.034	1.28	-0.020	0.087	-777.4	0.960	0.006	0.097	0.093	0.763	0.025	0.224
(juvenile subset)	Hemimetabolous	-	0.092	2.54	0.019	0.165	-	-	-	-	-	-	-	-
Temperature-size	Between	803	0.091	2.66	0.024	0.158	793.9	0.972	0.159	0.157	0.168	0.488	<0.001	0.498
rule	Within	-	0.092	2.95	0.031	0.153	-	-	-	-	-	-	-	-

Supplementary Table 2.4 Univariate multi-level meta-analytic, random effects models for lower critical thermal limit (CT_{min}) ARR (Acclimation Response Ratio) for all moderators. Results for intercept models are displayed. Results are highlighted in bold where 95% CIs do not overlap between groups or where regressions are significant for continuous variables. (rob.) is where a robust model was used because the residuals were not homogeneous. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. $R^2_{marg.}$: R^2 marginal, the variance explained only by moderators. $R^2_{cond.}$: R^2 conditional, the variance explained by moderators and random effects.

Model	Comparison	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
~*duration* (rob.)		375	<0.001	0.64	<-0.001	<0.001	-291.8	0.989	0.232	0.016	<0.001	0.741	0.018	0.264
	Intercept	-	0.098	5.52	0.062	0.133	-	-	-	-	-	-	-	-
~*ramp rate* (rob.)		571	0.017	0.31	-0.091	0.125	-290.7	0.992	0.367	<0.001	<0.001	0.625	<0.001	0.370
	Intercept	-	0.142	5.41	0.089	0.194	-	-	-	-	-	-	-	-
~*mass*		464	<-0.001	-0.63	-0.001	<0.001	-205.8	0.992	0.992	0.399	<0.001	<0.001	0.594	0.002
	Intercept	-	0.151	5.72	0.098	0.204	-	-	-	-	-	-	-	-
~*acclimation stage*	**Early life**	571	**0.196**	**6.36**	**0.136**	**0.257**	-289.7	0.992	0.372	<0.001	<0.001	0.619	0.016	0.385
	Adulthood	-	**0.129**	**5.43**	**0.082**	**0.176**	-	-	-	-	-	-	-	-
	Whole life	-	0.128	3.27	0.051	0.204	-	-	-	-	-	-	-	-
	Several generations	-	0.164	2.47	0.034	0.295	-	-	-	-	-	-	-	-
~*source* (rob.)	**Laboratory**	571	**0.163**	**8.83**	**0.126**	**0.200**	-292.3	0.991	0.348	<0.001	<0.001	0.643	0.012	0.359
	Field	-	**0.112**	**6.21**	**0.076**	**0.147**	-	-	-	-	-	-	-	-
~*habitat*	Aquatic	571	0.038	0.43	-0.139	0.215	-291.9	0.991	0.358	<0.001	<0.001	0.633	0.010	0.368
	Terrestrial	-	0.153	7.31	0.111	0.195	-	-	-	-	-	-	-	-
~*end point* (rob.)	**Death**	571	**0.052**	**1.59**	**-0.013**	**0.117**	-283.4	0.992	0.376	<0.001	<0.001	0.616	0.023	0.393
	Activity	-	0.152	2.52	0.032	0.272	-	-	-	-	-	-	-	-
	Clinging	-	**0.150**	**4.65**	**0.085**	**0.214**	-	-	-	-	-	-	-	-
	Natural position	-	**0.038**	**2.14**	**0.003**	**0.073**	-	-	-	-	-	-	-	-

	Righting	-	**0.168**	**4.11**	**0.086**	**0.249**	-	-	-	-	-	-	-	-
	Stimulus response	-	**0.146**	**4.16**	**0.076**	**0.216**	-	-	-	-	-	-	-	-
	Motor response	-	**0.172**	**4.06**	**0.087**	**0.256**	-	-	-	-	-	-	-	-
~latitude		471	-0.001	-0.97	-0.002	0.001	-207.1	0.990	0.369	<0.001	<0.001	0.622	0.004	0.375
	Intercept	-	0.169	4.26	0.089	0.250	-	-	-	-	-	-	-	-
~latitude (field		144	-0.001	-1.64	-0.002	<0.001	-188.7	0.946	<0.001	0.048	<0.001	0.898	0.019	0.069
subset; rob.)	Intercept	-	0.127	4.02	0.062	0.191	-	-	-	-	-	-	-	-
~sex	Female	571	0.186	5.80	0.123	0.249	-289.5	0.991	0.359	<0.001	<0.001	0.632	0.024	0.377
	Male	-	0.158	4.26	0.085	0.231	-	-	-	-	-	-	-	-
	Both	-	0.164	5.19	0.102	0.226	-	-	-	-	-	-	-	-
	Unknown	-	0.096	2.75	0.028	0.165	-	-	-	-	-	-	-	-
~development	Holometabolous	571	0.138	5.83	0.090	0.186	-291.1	0.992	0.3633	<0.001	<0.001	0.629	0.004	0.368
	Hemimetabolous	-	0.174	4.22	0.091	0.257	-	-	-	-	-	-	-	-
~development	Holometabolous	473	0.126	4.51	0.070	0.183	-263.4	0.991	0.429	<0.001	<0.001	0.562	0.013	0.440
(juvenile subset)														
	Hemimetabolous	-	0.189	3.60	0.083	0.296	-	-	-	-	-	-	-	-
Temperature-size	Between	571	0.114	2.88	0.036	0.191	-291.1	0.992	0.395	<0.001	<0.001	0.597	0.007	0.402
rule	Within	-	0.152	6.87	0.108	0.195	-	-	-	-	-	-	-	-

Supplementary Table 2.5 Multivariate multi-level meta-analytic, random effects models for upper critical thermal limit (CT_{max}) ARR (Acclimation Response Ratio). Top four best models are shown, calculated using the 'dredge' from the MuMin package. Estimate: difference in ARR compared to the reference group or the coefficient for regressions. Reference groups are indicated in subscript. Results are highlighted in bold where 95% CIs do not overlap between groups or where regressions are significant for continuous variables. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. R^2 marg.: R^2 marginal, the variance explained only by moderators. R^2 cond.: R^2 conditional, the variance explained by moderators and random effects.

Rank	Model	Comparison	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
1	***~development*** ***hemimetabolous***	**Holometabolous**	803	**-0.090**	**-2.12**	**-0.175**	**-0.006**	-799.2	0.970	0.165	0.077	0.204	0.524	0.059	0.491
2	***~development*** ***hemimetabolous*** ***+***	**Holometabolous**	803	**-0.073**	**-2.02**	**-0.145**	**-0.001**	-792.5	0.969	0.165	0.037	0.214	0.551	0.064	0.467
	acclimation stage	**Early life**	-	**0.034**	**2.29**	**0.005**	**0.064**	-	-	-	-	-	-	-	-
	adulthood	Whole life	-	0.007	0.43	-0.024	0.038	-	-	-	-	-	-	-	-
		Several generations	-	0.009	0.24	-0.062	0.080	-	-	-	-	-	-	-	-
3	***~development*** ***hemimetabolous*** *+ source* *field-caught*	**Holometabolous**	803	**-0.095**	**-2.12**	**-0.185**	**-0.006**	-797.5	0.971	0.176	0.087	0.197	0.510	0.061	0.507
		Laboratory		0.023	0.82	-0.033	0.080								
4	***~development*** ***hemimetabolous*** ***+***	**Holometabolous**	803	**-0.078**	**-2.04**	**-0.154**	**-0.002**	-790.4	0.969	0.178	0.044	0.209	0.538	0.065	0.481
	acclimation stage	**Early life**	-	**0.033**	**2.21**	**0.004**	**0.063**	-	-	-	-	-	-	-	-
	adulthood *+ source* *field-caught*	Whole life	-	0.005	0.32	-0.027	0.037	-	-	-	-	-	-	-	-
		Several generations	-	0.007	0.18	-0.065	0.078	-	-	-	-	-	-	-	-
		Laboratory	-	0.019	0.69	-0.036	0.074	-	-	-	-	-	-	-	-

Supplementary Table 2.6 Multivariate multi-level meta-analytic, random effects models for lower critical thermal limit (CT_{min}) ARR (Acclimation Response Ratio). Top four best models are shown, calculated using the 'dredge' from the MuMin package. Estimate: difference in ARR compared to the reference group or the coefficient for regressions. Reference groups are indicated in subscript. Results are highlighted in bold where 95% CIs do not overlap between groups or where regressions are significant for continuous variables. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. R^2 marg.: R^2 marginal, the variance explained only by moderators. R^2 cond.: R^2 conditional, the variance explained by moderators and random effects.

Rank	Model	Comparison	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
1	***~source*** $_{field\text{-}caught}$ **(rob.)**	**Laboratory**	571	0.052	**2.24**	**0.006**	**0.098**	-292.3	0.991	0.348	<0.001	<0.001	0.643	0.012	0.359
2	*Intercept model*	-	571	**0.147**	**7.17**	**0.106**	**0.188**	-292.4	0.991	0.361	<0.001	<0.001	0.630	<0.001	0.364
3	***~sex*** $_{female}$ *+*	Male	571	-0.024	-0.68	-0.048	<0.001	-289.1	0.992	0.375	<0.001	<0.001	0.616	0.055	0.413
	acclimation stage	Mixed	-	-0.024	-0.61	-0.098	0.050	-	-	-	-	-	-	-	-
	$_{adulthood}$ (rob.)	**Unknown**	**-**	**0.124**	**-2.54**	**-0.211**	**-0.036**	-	-	-	-	-	-	-	-
		Early life	-	0.084	2.52	0.027	0.141	-	-	-	-	-	-	-	-
		Whole life	-	-0.014	-0.33	-0.195	0.168	-	-	-	-	-	-	-	-
		Several generations	-	0.022	0.32	-0.144	0.188	-	-	-	-	-	-	-	-
4	*~habitat*$_{aquatic}$	Terrestrial	571	0.115	1.27	-0.067	0.297	-291.9	0.991	0.358	<0.001	<0.001	0.633	0.010	0.368

Supplementary Table 2.7 Conditional average best moderators from multivariate multi-level meta-analytic, random effects models for upper thermal limit (CT_{max}) ARR (Acclimation Response Ratio), ranked by AICc. Estimate: difference in ARR compared to the reference group or the coefficient for regressions. Significant results (95% CIs do not overlap between groups or significant regressions for continuous variables) are highlighted in bold. Reference groups are indicated in subscript. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval.

Moderator	estimate	SE	z	CI.lb	CI.ub
Development: holometabolous $_{\text{hemimetabolous}}$	**-0.080**	0.030	**2.65**	**-0.139**	**-0.020**
Acclimation stage: Early life $_{\text{adulthood}}$	**0.036**	0.015	**2.46**	**0.007**	**0.065**
Acclimation stage: Several generations $_{\text{adulthood}}$	0.008	0.036	0.22	-0.063	0.079
Acclimation stage: Whole life $_{\text{adulthood}}$	0.007	0.016	0.46	-0.024	0.038
Source: Laboratory $_{\text{field-caught}}$	0.019	0.026	0.72	-0.032	0.070
Ramp rate	0.013	0.041	0.31	-0.068	0.093

Supplementary Table 2.8 Full average best moderators from multivariate multi-level meta-analytic, random effects models for upper thermal limit (CT_{max}) ARR (Acclimation Response Ratio), ranked by AICc. Estimate: difference in ARR compared to the reference group or the coefficient for regressions. Significant results (95% CIs do not overlap between groups or significant regressions for continuous variables) are highlighted in bold. Reference groups are indicated in subscript. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval.

Moderator	estimate	SE	z	CI.lb	CI.ub
Development: holometabolous $_{\text{hemimetabolous}}$	**-0.080**	0.030	**2.65**	**-0.139**	**-0.021**
Acclimation stage: Early life $_{\text{adulthood}}$	0.015	0.020	0.74	-0.025	0.055
Acclimation stage: Several generations $_{\text{adulthood}}$	0.003	0.024	0.14	-0.043	0.049
Acclimation stage: Whole life $_{\text{adulthood}}$	0.003	0.011	0.28	-0.018	0.024
Source: Laboratory $_{\text{field-caught}}$	0.005	0.016	0.33	-0.026	0.037
Ramp rate	0.002	0.015	0.10	-0.027	0.030

Supplementary Table 2.9 Conditional average best moderators from multivariate multi-level meta-analytic, random effects models for lower thermal limit (CT_{min}) ARR (Acclimation Response Ratio), ranked by AICc. Estimate: difference in ARR compared to the reference group or the coefficient for regressions. Significant results (95% CIs do not overlap between groups or significant regressions for continuous variables) are highlighted in bold. Reference groups are indicated in subscript. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval.

Moderator	estimate	SE	z	CI.lb	CI.ub
Source: Laboratory $_{\text{field-caught}}$	0.049	0.036	1.38	-0.021	0.119
Acclimation stage: Early life $_{\text{adulthood}}$	**0.079**	0.034	**2.34**	**0.0128**	**0.144**
Acclimation stage: Several generations $_{\text{adulthood}}$	0.030	0.067	0.45	-0.102	0.162
Acclimation stage: Whole life $_{\text{adulthood}}$	-0.005	0.040	0.11	-0.083	0.074
Sex: Male $_{\text{female}}$	-0.027	0.035	0.77	-0.096	0.042
Sex: Mixed $_{\text{female}}$	-0.025	0.039	0.64	-0.100	0.051
Sex: Unknown $_{\text{female}}$	**-0.121**	0.047	**2.56**	**-0.213**	**-0.028**
Habitat: Terrestrial $_{\text{aquatic}}$	0.114	0.093	1.23	-0.068	0.2961
Development: Holometabolous $_{\text{hemimetabolous}}$	-0.050	0.048	1.06	-0.143	0.043
Ramp rate	0.021	0.069	0.30	-0.114	0.155

Supplementary Table 2.10 Full average best moderators from multivariate multi-level meta-analytic, random effects models for lower thermal limit (CT_{min}) ARR (Acclimation Response Ratio), ranked by AICc. Estimate: difference in ARR compared to the reference group or the coefficient for regressions. Significant results (95% CIs do not overlap between groups or significant regressions for continuous variables) are highlighted in bold. Reference groups are indicated in subscript. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval.

Moderator	**estimate**	**SE**	**z**	**CI.lb**	**CI.ub**
Source: Laboratory $_{\text{field-caught}}$	0.023	0.035	0.67	0.091	-0.045
Acclimation stage: Early life $_{\text{adulthood}}$	0.042	0.046	0.90	0.132	-0.049
Acclimation stage: Several generations $_{\text{adulthood}}$	0.016	0.051	0.31	0.116	-0.084
Acclimation stage: Whole life $_{\text{adulthood}}$	-0.002	0.029	0.08	0.055	-0.060
Sex: Male $_{\text{female}}$	-0.009	0.024	0.39	0.039	-0.057
Sex: Mixed $_{\text{female}}$	-0.009	0.026	0.33	0.042	-0.059
Sex: Unknown $_{\text{female}}$	-0.042	0.064	0.66	0.083	-0.167
Habitat: Terrestrial $_{\text{aquatic}}$	0.046	0.081	0.57	0.205	-0.113
Development: Holometabolous $_{\text{hemimetabolous}}$	-0.014	0.034	0.42	0.053	-0.082
Ramp rate	0.001	0.013	0.05	0.026	-0.024

8.2.1 Sensitivity analyses

Supplementary Table 2.11 Leave-one-out sensitivity analysis for multi-level meta-analytic, random effects models of upper thermal limit (CT_{max}) ARR (Acclimation Response Ratio). Individual studies, species or families were removed iteratively to check for outliers. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval.

Moderator	estimate	z	SE	CI.lb	CI.ub
Study	0.091	2.96	0.031	0.030	0.153
Species	0.091	2.97	0.031	0.030	0.153
Family	0.091	2.96	0.031	0.030	0.153

Supplementary Table 2.12 Leave-one-out sensitivity analysis for multi-level meta-analytic, random effects models of lower thermal limit (CT_{min}) ARR (Acclimation Response Ratio). Individual studies, species or families were removed iteratively to check for outliers. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval.

Moderator	estimate	z	SE	CI.lb	CI.ub
Study	0.147	7.08	0.021	0.105	0.189
Species	0.147	7.11	0.021	0.105	0.189
Family	0.147	7.04	0.021	0.105	0.189

Supplementary Table 2.13 Intercept multi-level meta-analytic, random effects models for upper and lower thermal limits, CT_{max} and CT_{min} respectively, without fluctuating data. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. $R^2_{marg.}$: R^2 marginal, the variance explained only by moderators. $R^2_{cond.}$: R^2 conditional, the variance explained by moderators and random effects. Significant results (95% CIs do not span 0) are highlighted in bold.

Limit	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
CT_{max}	738	**0.085**	**2.84**	**0.025**	**0.146**	-713.6	0.971	0.030	0.175	0.198	0.567	<0.001	0.416
CT_{min}	556	**0.154**	**7.18**	**0.111**	**0.197**	-276.6	0.992	0.363	<0.001	<0.001	0.629	<0.001	0.366

Supplementary Table 2.14 Intercept multi-level meta-analytic, random effects models for upper and lower thermal limit, CT_{max} and CT_{min} respectively, without Drosophilidae data. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. $R^2_{marg.}$: R^2 marginal, the variance explained only by moderators. $R^2_{cond.}$: R^2 conditional, the variance explained by moderators and random effects. Significant results (95% CIs do not span 0) are highlighted in bold.

Limit	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
CT_{max}	421	**0.094**	**2.77**	**0.026**	**0.162**	-267.4	0.978	0.427	0.132	0.205	0.314	<0.001	0.679
CT_{min}	377	**0.121**	**4.73**	**0.070**	**0.173**	-339.6	0.981	<0.001	0.088	0.178	0.715	<0.001	0.27

Supplementary Table 2.15 Intercept multi-level meta-analytic, random effects models for upper and lower thermal limit, CT_{max} and CT_{min} respectively, with Drosophilidae data only. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. $R^2_{marg.}$: R^2 marginal, the variance explained only by moderators. $R^2_{cond.}$: R^2 conditional, the variance explained by moderators and random effects. Significant results (95% CIs do not span 0) are highlighted in bold.

Limit	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
CT_{max}	382	0.036	0.45	-0.140	0.212	-533.2	0.971	0.014	0.325	0.111	0.521	<0.001	0.463
CT_{min}	194	**0.225**	**3.20**	**0.066**	**0.383**	-14.3	0.997	0.477	<0.001	<0.001	0.520	<0.001	0.479

Supplementary Table 2.16 Univariate multi-level meta-analytic, random effects models for upper critical thermal limit (CT_{max}) ARR (Acclimation Response Ratio), with Drosophilidae data only. Significant results (95% CIs do not overlap between groups or significant regressions for continuous variables) are highlighted in bold. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. $R^2_{marg.}$: R^2 marginal, the variance explained only by moderators. $R^2_{cond.}$: R^2 conditional, the variance explained by moderators and random effects.

Model	Comparison	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
~duration		149	**<-0.001**	**-2.03**	**<-0.001**	**<-0.001**	-413.5	0.779	<-0.001	0.115	0.115	0.549	0.053	0.333
	Intercept	-	0.044	4.97	0.023	0.066	-	-	-	-	-	-	-	-
~ramp rate		382	0.002	0.02	-0.245	0.249	-528.7	0.959	0.018	0.104	0.104	0.732	<0.001	0.237
	Intercept	-	0.042	1.97	-0.006	0.091	-	-	-	-	-	-	-	-
~mass		351	-0.003	-0.19	-0.038	0.032	-461.3	0.964	0.016	0.122	0.122	0.703	<0.001	0.270
	Intercept	-	0.047	1.49	-0.025	0.119	-	-	-	-	-	-	-	-
~acclimation stage	Early life	382	0.052	2.24	0.006	0.098	-528.5	0.959	0.009	0.112	0.112	0.726	0.011	0.251
	Adulthood	-	0.025	1.32	-0.012	0.062	-	-	-	-	-	-	-	-
	Whole life	-	0.051	2.92	0.017	0.085	-	-	-	-	-	-	-	-
~endpoint	Activity	382	0.032	0.032	-0.012	0.075	-527.5	0.959	0.013	0.110	0.110	0.726	0.008	0.249
	Stimulus response	-	0.056	0.056	0.009	0.103	-	-	-	-	-	-	-	-
	Motor response	-	0.047	0.047	-0.05	0.140	-	-	-	-	-	-	-	-
~latitude		380	<0.001	-0.21	-0.001	0.001	-524.1	0.959	0.029	0.102	0.102	0.727	<0.001	0.242
	Intercept	-	0.046	1.79	-0.013	0.106	-	-	-	-	-	-	-	-
~sex	**Female**	382	**0.028**	**1.59**	**-0.007**	**0.063**	-533.3	0.959	0.008	0.111	0.111	0.728	0.017	0.253
	Male	-	**0.060**	**3.29**	**0.024**	**0.096**	-	-	-	-	-	-	-	-

Supplementary Table 2.17 Univariate multi-level meta-analytic, random effects models for lower critical thermal limit (CT_{min}) ARR (Acclimation Response Ratio), with Drosophilidae data only. Significant results (95% CIs do not overlap between groups or significant regressions for continuous variables) are highlighted in bold. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. $R^2_{marg.}$: R^2 marginal, the variance explained only by moderators. $R^2_{cond.}$: R^2 conditional, the variance explained by moderators and random effects.

Model	Comparison	k	estimate	t	CI.lb	CI.ub	AICc	I^2_{total}	I^2_{study}	$I^2_{phylogeny}$	$I^2_{species}$	I^2_{row}	$R^2_{marg.}$	$R^2_{cond.}$
~duration		41	**-0.001**	**-5.70**	**-0.002**	**-0.001**	-10.9	0. 997	0.088	<0.001	<0.001	0.091	0.469	0.516
	Intercept	-	0.171	3.61	-0.431	0.773	-	-	-	-	-	-	-	-
~ramp rate		194	0.011	0.04	-0.500	0.523	-13.0	0. 997	0.494	<0.001	<0.001	0.502	<0.001	0.496
	Intercept	-	0.222	2.24	-0.007	0.450	-	-	-	-	-	-	-	-
~mass		184	-0.005	-0.23	-0.056	0.045	-3.6	0. 997	0.473	<0.001	<0.001	0.524	<0.001	0.475
	Intercept	-	0.233	3.08	0.058	0.409	-	-	-	-	-	-	-	-
~acclimation stage	Early life	194	0.356	3.78	0.170	0.543	-20.3	0.997	0.595	<0.001	<0.001	0.402	0.077	0.628
	Adulthood	-	0.241	2.65	0.061	0.421	-	-	-	-	-	-	-	-
	Whole life	-	0.121	1.32	-0.060	0.302	-	-	-	-	-	-	-	-
	Activity	194	0.149	1.07	-0.208	0.506	-13.9	0.997	0.529	<0.001	<0.001	0.468	0.107	0.581
	Clinging	-	0.084	0.36	-0.507	0.675	-	-	-	-	-	-	-	-
	Righting	-	0.333	1.80	-0.144	0.809	-	-	-	-	-	-	-	-
	Stimulus response	-	0.343	2.48	-0.012	0.699	-	-	-	-	-	-	-	-
	Motor response	-	0.072	0.30	-0.547	0.691	-	-	-	-	-	-	-	-
~latitude		191	-0.001	-0.36	-0.003	0.002	-10.4	0.997	0.502	<0.001	<0.001	0.495	0.001	0.503
	Intercept	-	0.237	2.41	-0.004	0.475	-	-	-	-	-	-	-	-
~sex	Female	194	0.229	2.77	0.066	0.392	-11.9	0.997	0.500	<0.001	<0.001	0.497	0.009	0.506
	Male	-	0.197	2.21	0.021	0.372	-	-	-	-	-	-	-	-
	Mixed	-	0.293	1.35	-0.222	0.809	-	-	-	-	-	-	-	-

8.2.2 Publication bias

Supplementary Table 2.18 Egger's regression test (two-sided) for intercept multi-level, random effects meta-analytic model for upper critical thermal limit (CT_{max}) ARR (Acclimation Response Ratio). SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. Significant results are highlighted in bold.

Moderator	k	estimate	t	SE	CI.lb	CI.ub
Intercept	803	0.073	2.37	0.031	0.011	0.134
√variance	-	**0.288**	2.17	0.133	**0.028**	**0.548**

Supplementary Table 2.19 Egger's regression test (two-sided) was significant for intercept multi-level, random effects meta-analytic model for upper critical thermal limit (CT_{max}) ARR (Acclimation Response Ratio) so SE^2 was run as a moderator to find predicted estimate without publication bias. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. Significant results are highlighted in bold.

Moderator	k	estimate	t	SE	CI.lb	CI.ub
Intercept	803	0.091	2.97	0.031	0.030	0.152
SE^2	-	**0.043**	0.67	0.06v5	**-0.084**	**0.170**

Supplementary Table 2.20 Egger's regression test (two-sided) for the best multi-level, random effects meta-analytic model (as selected using the Mumin package) for upper critical thermal limit (CT_{max}) ARR (Acclimation Response Ratio). SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. Significant results are highlighted in bold.

Moderator	k	estimate	t	SE	CI.lb	CI.ub
Intercept	803	0.116	3.43	0.034	0.048	0.183
√variance	-	**0.288**	2.17	0.133	**0.028**	**0.548**
Development: Homogeneous	-	**-0.088**	-2.14	0.041	**-0.169**	**-0.006**

Supplementary Table 2.21 Egger's regression test (two-sided) for intercept multi-level, random effects meta-analytic model for lower critical thermal limit (CT_{min}) ARR (Acclimation Response Ratio). SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. Significant results are highlighted in bold.

Moderator	k	estimate	t	SE	CI.lb	CI.ub
Intercept	571	0.116	4.67	0.025	0.066	0.165
√variance	-	**0.621**	2.21	0.282	**0.068**	**1.174**

Supplementary Table 2.22 Egger's regression test (two-sided) was significant for intercept multi-level, random effects meta-analytic model for lower critical thermal limit (CT_{min}) ARR (Acclimation Response Ratio) so SE^2 was run as a moderator to find predicted estimate without publication bias. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. Significant results are highlighted in bold.

Moderator	k	estimate	t	SE	CI.lb	CI.ub
Intercept	571	0.144	6.94	0.021	0.102	0.185
SE^2	-	**0.635**	1.28	0.497	**-0.341**	**1.611**

Supplementary Table 2.23 Egger's regression test (two-sided) for the best multi-level, random effects meta-analytic model (as selected using the Mumin package) for lower critical thermal limit (CT_{min}) ARR (Acclimation Response Ratio). SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. Significant results are highlighted in bold.

Moderator	k	estimate	t	SE	CI.lb	CI.ub
Intercept	571	0.068	1.92	0.035	-0.003	0.139
√variance	-	**0.695**	2.46	0.283	**0.140**	**1.249**
Source: laboratory	-	0.064	1.85	0.035	-0.004	0.133

Supplementary Table 2.24 Univariate multi-level meta-analytic, random effects models for upper critical thermal limit (CT_{max}) ARR (Acclimation Response Ratio), investigating year as a moderator. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. Significant results are highlighted in bold.

Moderator	k	estimate	t	SE	CI.lb	CI.ub
Intercept	803	1.73	0.42	4.157	-6.59	10.046
Year	-	-0.001	-0.39	0.002	-0.005	0.003

Supplementary Table 2.25 Univariate multi-level meta-analytic, random effects models for lower critical thermal limit (CT_{min}) ARR (Acclimation Response Ratio), investigating year as a moderator. SE: standard error. CI.lb: lower bound of the 95% confidence interval; CI.ub: upper bound of the 95% confidence interval. Significant results are highlighted in bold.

Moderator	k	estimate	t	SE	CI.lb	CI.ub
Intercept	571	4.103	0.60	6.809	-9.574	17.780
SE^2	-	-0.002	-0.58	0.003	-0.009	0.005

8.3 Appendix 3 - Chapter 3

Supplementary Table 3.1 Mean ± SE temperature and relative humidity of acclimation treatments for each species recorded by iButton data logger. Origin is the location where flies were originally collected for colony establishment by IAEA.

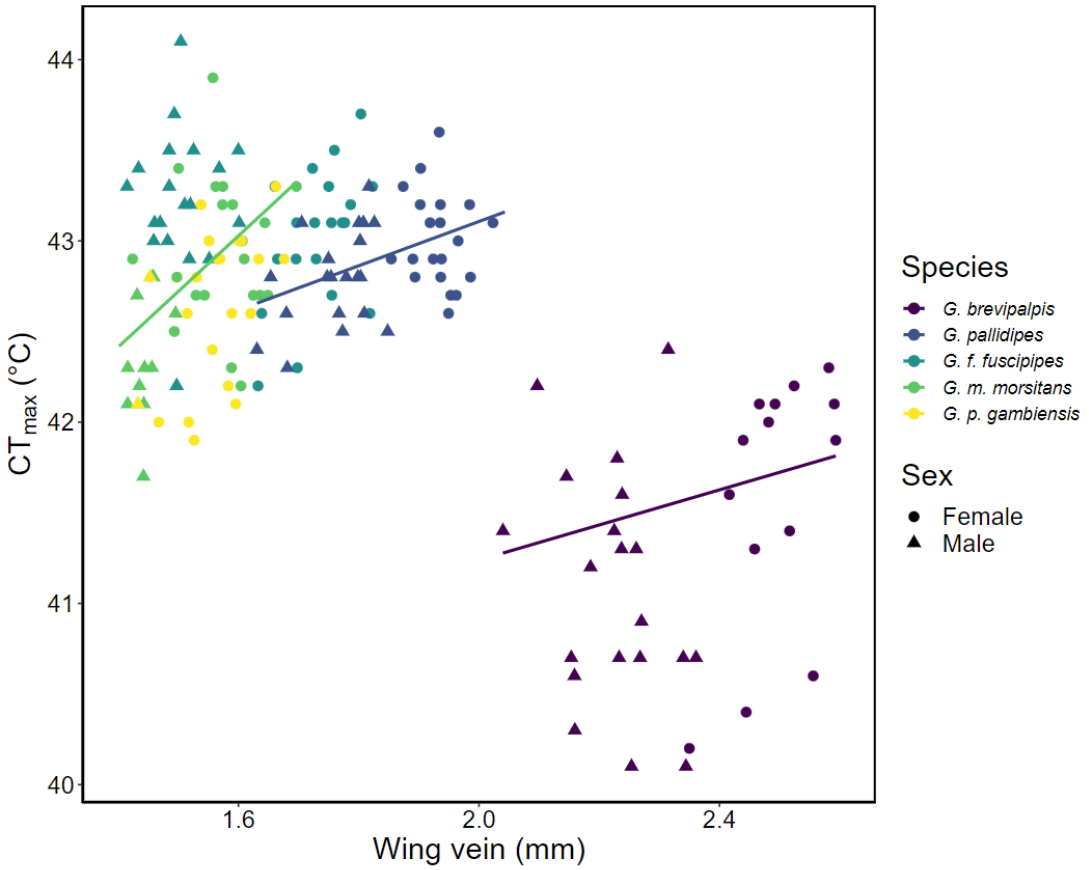
Species	Sub-genera	Origin	Treatment	Temperature ± SD	Humidity ± SD
G. brevipalpis	Fusca	Kibwezi Forest	25	24.9 ± 0.3	78.8 ± 0.6
		Kenya	30	30.6 ± 1.1	81.6 ± 4.8
G. f. fuscipes	Palpalis	Central African	25	24.9 ± 0.3	78.5 ± 0.7
		Republic	30	30.8 ± 0.8	78.6 ± 3.7
G. p. gambiensis	Palpalis	Burkina Faso,	25	24.9 ± 0.3	78.8 ± 0.6
		Pout Senegal	30	30.5 ± 0.6	79.9 ± 5.9
G. m. morsitans	Morsitans	Zimbabwe	25	24.9 ± 0.3	78.8 ± 0.6
			30	30.4 ± 1.0	81.6 ± 5.0
G. pallidipes	Morsitans	Tororo Uganda	25	25.2 ± 0.1	76.3 ± 1.4
			30	30.7 ± 0.1	78.0 ± 2.36

Supplementary Table 3.2 Critical Thermal Maximum (CT_{max}) mean ± Standard Error (SE) of tsetse flies (*Glossina* spp.) acclimated to either 25°C (basal) or 30°C for five consecutive days. CT_{max} was measured by ramping assay beginning at 25°C and ramping at 0.1°C/min.

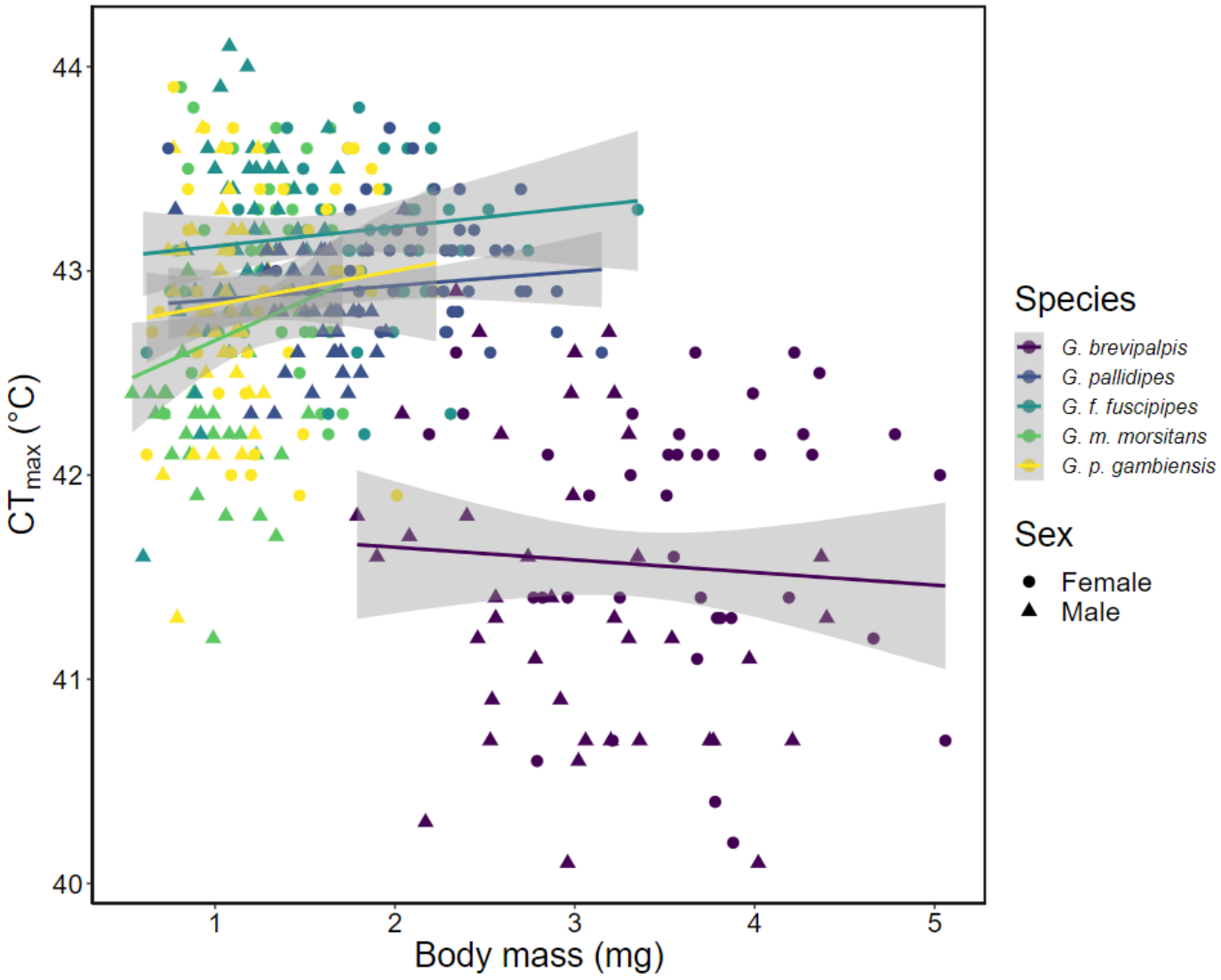
Species	Adult acclimation temperature	CT_{max} (°C)	SE	n
G. brevipalpis	25	41.3	0.11	40
	30	41.8	0.10	40
G. pallidipes	25	42.9	0.04	40
	30	42.9	0.05	40
G. f. fuscipes	25	43.1	0.64	39
	30	43.2	0.74	39
G. m. morsitans	25	42.6	0.08	38
	30	42.8	0.09	40
G. p. gambiensis	25	42.6	0.07	40
	30	43.2	0.06	40

Supplementary Table 3.3 Single-species linear models investigating the effect of acclimation and sex on Critical Thermal Maximum (CT_{max}) in tsetse (*Glossina* spp.). Flies were either acclimated at 25 or 30 for five days preceding the assay. Mean differences between groups and slopes are given ± SE.

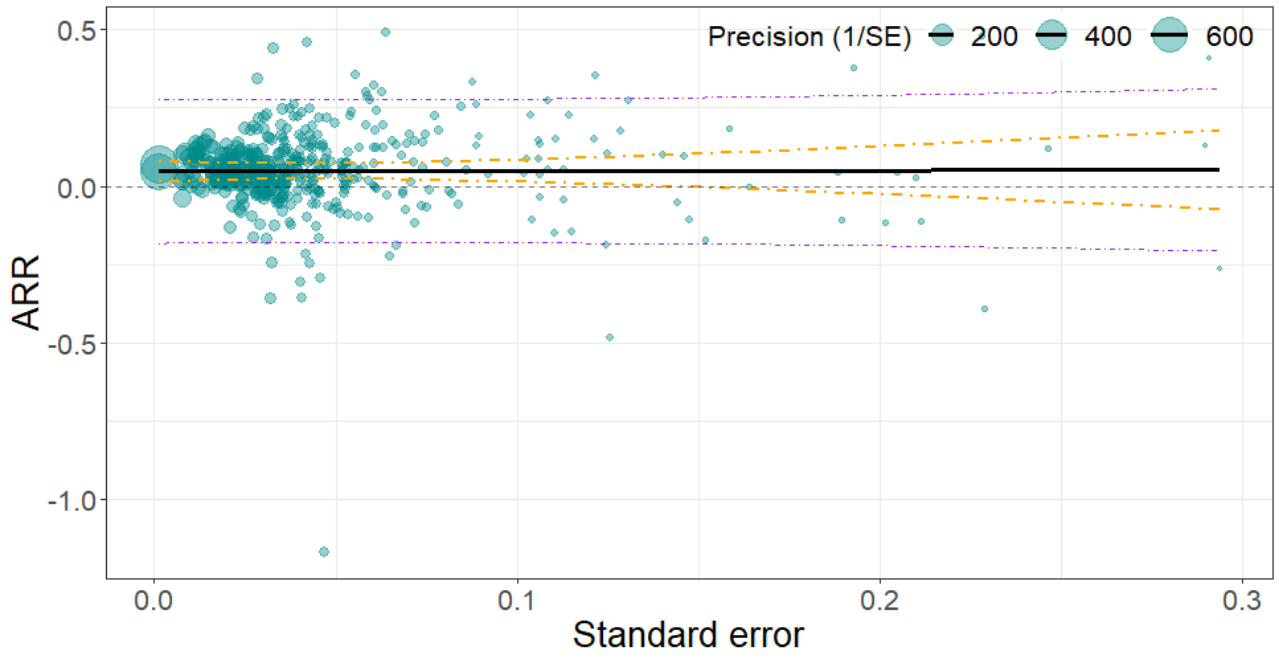
Model	Variable	Mean difference ± SE	t-statistic	p-value	F-statistic	DF	p-value	R^2
		Parameters			Model			
G. brevipalpis	**Intercept (25°C)**	**41.47 ± 0.12**	**332.0**	**<0.0001**	**8.95**	**2, 76**	**<0.001**	**0.19**
	Treatment (30°C)	**0.52 ± 0.14**	**3.61**	**<0.001**				
	Sex (female - male)	**-0.33 ± 0.14**	**-2.26**	**0.03**				
G. pallidipes	**Intercept (25°C)**	**43.03 ± 0.05**	**827.9**	**<0.0001**	**9.76**	**2, 77**	**<0.001**	**0.20**
	Treatment (30°C)	0.04 ± 0.06	0.63	0.53				
	Sex (female - male)	**-0.26 ± 0.06**	**-4.37**	**<0.0001**				
G. f. fuscipes	**Intercept (25°C)**	**43.02 ± 0.10**	**451.7**	**<0.0001**	1.77	2, 74	0.16	0.07
	Treatment (30°C)	**0.30 ± 0.14**	**2.21**	**0.03**				
	Sex (female - male)	0.22 ± 0.14	1.59	0.12				
	Treatment x sex	-0.38 ± 0.19	-1.98	0.05				
G. m. morsitans	**Intercept (25°C)**	**42.96 ± 0.08**	**516.3**	**<0.0001**	**25.44**	**2, 74**	**<0.0001**	**0.41**
	Treatment (30°C)	0.17 ± 0.10	1.79	0.08				
	Sex (female - male)	**-0.68 ± 0.10**	**-6.97**	**<0.0001**				
G. p. gambiensis	**Intercept (25°C)**	**42.62 ± 0.08**	**505.9**	**<0.0001**	**19.61**	**2, 77**	**<0.0001**	**0.34**
	Treatment (30°C)	**0.60 ± 0.10**	**6.17**	**<0.0001**				
	Sex (female - male)	-0.11 ± 0.10	-1.08	0.28				



Supplementary Figure 3.1 Relationship between basal CT_{max} and wing vein size (mm). *Glossina* spp. are distinguished by different colours. Lines represent significant linear regressions within species groups. The linear regression for *G. brevipalpis* was near to significant (p = 0.08) so has been included. Circles resemble female flies and triangles resemble males. Species are given in mean size order from largest to smallest by dry mass.



Supplementary Figure 3.2 Relationship between CT_{max} and body mass (mg). *Glossina* spp. are distinguished by different colours. Lines represent linear regressions within species groups. Circles resemble female flies and triangles resemble males. CT_{max} is represented for individuals acclimated at both 25°C and 30°C. N ~ 40 per treatment/sex/species.



Supplementary Figure 3.3 Relationship between Critical Thermal Maximum ARR (Acclimation Response Ratio) and standard error for Egger's regression test. A positive relationship shows positive publication bias. 95% confidence intervals are depicted by orange dotted lines, prediction intervals are purple dotted lines. The precision of the study (1/SE) is proportional to the size of each data point.

8.4 Appendix 4 - Chapter 4

Supplementary Figure 4.1 Cage to house five tsetse flies (*Glossina pallidipes*), in a ratio of 3 females to 2 males. 150 ml cylindrical containers (70 mm x 73 mm) had 2.5 mm mesh fabric to allow L3 larvae to drop through for collection.

Supplementary Table 4.1 Actual mean and SD (Standard Deviation) temperature and relative humidity (RH) of each heatwave treatment during two hours in the water bath for three consecutive days (six hours total) recorded by iButton. The sampling rate was every 30 seconds. Note that the 40°C heatwave for trial 1 was removed from the experiment due to not reaching within ±0.5°C of the required temperature.

Trial	Treatment	Temperature	SD	Humidity (RH)	SD
1	25	24.8	0.1	83.7	11.3
1	36	35.6	0.5	78.3	8.9
1	38	37.5	0.8	85.1	5.4
1	40	38.6	1.9	73.5	2.1
2	25	24.9	0.1	84.8	4.3
2	36	35.7	1.0	82.1	7.0
2	38	37.6	0.8	74.7	10.0
2	40	39.9	1.2	87.4	3.9

Supplementary Table 4.2 Generalised linear model with binomial distribution investigating the effect of heatwave temperature on proportion survival in adult tsetse (*G. pallidipes*) 24 hours post-heatwave. Comparisons are made with the 25°C control group. Significant values are highlighted in bold. n = number of cages per treatment. Each cage contained approximately 10 flies. Values are back transformed.

Heatwave temperature (°C)	% mortality	Odds ratio	SE	n	z-value	p-value
Intercept (25)	**1.8**	**-3.72**	**0.54**	**14**	**-6.85**	**<0.0001**
36	6.0	1.03	0.64	14	1.61	0.11
38	**7.6**	**1.25**	**0.62**	**13**	**2.01**	**0.04**
40	**100**	**8.53**	**1.53**	**5**	**5.57**	**<0.0001**

Supplementary Table 4.3 Multivariate Cox proportional-hazards random effects model examining survival risk of tsetse (*G. pallidipes*) after heatwave (n = 366 flies) over a period of six weeks. Hazard ratios are given with 95% confidence intervals. Hazard ratios >1 suggest higher risk of death, and <1 suggest lower risk of death compared to the reference group. Significant results are in bold font. SD = standard deviation.

Variable	n	Hazard ratio (95% CIs)	z-value	p-value
Fixed effects				
Temperature (25)	117	Reference		
36	124	1.68 (0.90 – 3.13)	1.64	0.10
38	125	**2.32 (1.27 – 4.23)**	**2.75**	**0.006**
Exposed sex (Female)	212	Reference		
Male	154	**0.52 (0.32 – 0.85)**	**-2.64**	**0.008**
Random effects	**SD**	**Variance**		
Cage code	0.82	0.67		

Supplementary Table 4.4 Multivariate Cox proportional-hazards random effects model examining probability of first larviposition in tsetse (*G. pallidipes*) per cage after heatwave (n = 143 cages). Cages generally consisted of three females and two males. Two models were run depending on whether females or males were exposed to heatwave. Comparisons are made with the 25°C control group. Hazard ratios of greater than 1 suggest fewer days until first larviposition, and below 1, more days until first larviposition compared to the reference group. Hazard ratios are given with 95% confidence intervals. Significant results are in bold font. Cage code was included as a random effect in the model.

Model	Variable	n	Hazard ratio	z-value	p-value
Female	**Fixed effects**				
	Temperature (25)	23			
	36	25	0.71 (0.32 - 1.58)	-0.85	0.40
	38	23	**0.16 (0.07 - 0.39)**	**-4.13**	**<0.0001**
	Random effects	**SD**	**Variance**		
	Cage code	0.76	0.58		
Male	**Fixed effects**				
	Temperature (25)	24			
	36	25	0.72 (0.40 - 1.28)	-1.13	0.26
	38	28	0.63 (0.36 - 1.11)	-1.60	0.11
	Random effects	**SD**	**Variance**		
	Cage code	0.02	<0.001		

Supplementary Table 4.5 Hurdle model results investigating how female heatwave affect TFLs (Thermal Fertility Limits) and TSF (Thermal Sensitivity of Fertility) in tsetse. The model is in two parts, the first part of the model analysed the non-zero data only, using a Poisson with log link. The second part of the model analysed the likelihood of infertility (0) versus fertility (1) at each time point with logit link. Comparisons are made with the 25°C control group. Results are back transformed from log scale. Cage code was used as a random factor, allowing for different slopes between cages over time. Values are back transformed.

Variable	Odds ratios (95% confidence intervals	z-value	p-value
Count part (TSF)			
Temperature (25)	**0.13 (0.06 - 0.28)**	**-5.08**	**<0.001**
36	1.02 (0.30 - 3.50)	0.04	0.97
38	2.61 (0.66 - 10.24)	1.37	0.17
Time	1.01 (0.97 - 1.05)	0.56	0.57
Temperature (36) x Time	0.98 (0.92 - 1.04)	-0.67	0.50
Temperature (38) x Time	0.95 (0.89 - 1.02)	-1.30	0.19
Zero part (TFL)			
Temperature (25)	2.09 (1.34 - 3.25)	3.27	0.001
36	1.34 (0.72 - 2.50)	0.91	0.36
38	**3.31 (1.62 - 6.76)**	**3.29**	**<0.001**
Temperature (36) x Time	0.99 (0.96 - 1.01)	-1.26	0.21
Temperature (38) x Time	0.99 (0.96 - 1.02)	-0.46	0.65
	0.97 (0.94 - 1.00)	**-1.79**	**0.07**
Random effects	**SD**	**Corr.**	
Intercept	0.26		
zi intercept	0.010	-0.80	

Supplementary Table 4.6 Hurdle model results investigating how male heatwave affect TFLs (Thermal Fertility Limits) and TSF (Thermal Sensitivity of Fertility) in tsetse. The model is in two parts, the first part of the model analysed the non-zero data only, using a Poisson with log link. The second part of the model analysed the likelihood of infertility (0) versus fertility (1) at each time point with logit link. Comparisons are made with the 25°C control group. Results are back transformed from log scale. Cage code was used as a random factor, allowing for different slopes between cages over time. Values are back transformed.

Variable	Odds ratios (95% confidence intervals	z-value	p-value
Count part (TFL)			
Temperature (25)	0.22 (0.15 - 0.33)	-7.40	<0.0001
36	1.05 (0.68 - 1.60)	0.21	0.83
38	1.62 (1.11 - 2.37)	2.49	0.01
Time	0.99 (0.97 - 1.00)	-1.43	0.15
Zero part (TSF)			
Temperature (25)	1.48 (1.10 - 1.98)	2.61	<0.01
36	1.17 (0.87 - 1.57)	1.03	0.30
38	1.34 (1.00 - 1.79)	1.98	0.05
Time	0.99 (0.97 - 1.00)	-2.11	0.04
Random effects	**SD**	**Corr.**	
Intercept	0.20		
zi intercept	0.01	0.27	

Supplementary Table 4.7 General linear model results investigating the effect of female heatwave on overall population size per cage. Population size at each time point was calculated by adding the number of living individuals to the number of births. Mean differences between groups are given ± Standard Error. Comparisons are made with the 25°C control group.

Variable	Mean difference ± SE	z-value
Temperature (25)	4.969 ± 0.222	22.36
36	-0.377 ± 0.308	-1.22
38	-0.575 ± 0.314	-1.83
Time	-0.008 ± 0.003	-2.89
Temperature (36) x Time	-0.007 ± 0.004	-1.89
Temperature (38) x Time	-0.010 ± 0.004	-2.53
Random effects	**Variance**	**SD**
Cage code (intercept)	1.04	1.02
Residual	0.51	0.72

Supplementary Table 4.8 General linear model results investigating the effect of male heatwave on overall population size per cage. Population size at each time point was calculated by adding the number of living individuals to the number of births. Mean differences between groups are given ± Standard Error. Comparisons are made with the 25°C control group.

Variable	Mean difference ± SE	z-value
Temperature (25)	5.236 ± 0.130	40.42
36	-0.060 ± 0.181	-0.33
38	-0.055 ± 0.177	-0.31
Time	-0.002 ± 0.003	-0.52
Temperature (36) x Time	-0.010 ± 0.005	-2.10
Temperature (38) x Time	-0.007 ± 0.004	-1.57
Random effects	**Variance**	**SD**
Cage code (intercept)	0.27	0.52
Residual	0.73	0.85

Supplementary Table 4.9 Multivariate general linear random effects model examining offspring CT_{max} of tsetse (*G. pallidipes*) after parental heatwave (n = 240 flies). SE = standard error, SD = standard deviation. Significant results are in bold font.

Variable	n	Estimate ± SE	t-value	p-value
Fixed effects				
Temperature (25)	80	**42.61 ± 0.12**	**341.3**	**<0.0001**
36	78	**0.21 ± 0.08**	**2.53**	**0.01**
38	82	0.16 ± 0.08	1.91	0.06
Pupal mass	240	**0.75 ± 0.16**	**4.61**	**<0.001**
Random effects	**SD**	**Variance**		
Cage code	0.29	0.08		

Supplementary Table 4.10 Abortion data for each treatment group. Abortions were counted as larvae in the first or second stage.

Treatment (°C)	Sex treated	Abortion number
25	Female	7
36	Female	6
38	Female	6
25	Male	5
36	Male	2
38	Male	12

8.5 Appendix 5 - Chapter 5

Supplementary Table 5.1 Mean mass (mg) of tsetse (*G. m. morsitans*) pupae from different maternal age groups (weeks). Mean differences between groups are given ± standard deviation (SD). ANOVA was used to compare differences between groups with full statistics (adjusted R^2; F statistic; degrees of freedom) displayed below the comparison statistics.

Mother age (weeks)	Mass (mg) ± SD	Mean difference ± SD	t-value	p-value
Reference	25.35 ± 0.21			
4 - 5	**26.80 ± 0.19**	**1.45 ± 0.28**	**5.19**	**<0.001**
6 - 7	**27.07 ± 0.18**	**1.72 ± 0.27**	**6.27**	**<0.001**
8 - 9	**26.34 ± 0.22**	**0.99 ± 0.30**	**3.30**	**0.001**

Adj. R^2 = 0.085; F = 14.6; df = 3, 436

Supplementary Table 5.2 Pupal development time (mean ± standard deviation (SD)) of female and male tsetse (*G. m. morsitans*) under two constant temperature regimes: 25°C and 31°C.

Temperature (°C)	Sex	N	Development time ± SD (days)
25	Female	109	30.61 ± 2.78
	Male	109	32.12 ± 2.81
31	Female	113	19.64 ± 0.55
	Male	97	21.40 ± 0.59

Supplementary Table 5.3 Generalised Least Squares model examining the effect of constant 25°C or 31°C on pupal development time (days) in tsetse (*G. m. morsitans*). Variances were assumed to be unequal across temperature treatments. All pupae that emerged were considered in the analysis. Significant results are in bold font. Comparisons are made with the reference group and results are back-transformed from the log scale.

Variable (reference group)	Estimate (95% CIs)	t-value	p-value
Intercept	**31.66 (30.20 - 33.19)**	**144.0**	**<0.0001**
Pupal temperature (25)	**0.64 (0.63 - 0.66)**	**-47.4**	**<0.0001**
Sex (female)	**1.05 (1.02 - 1.08)**	**3.9**	**0.0001**
Pupal mass	1.00 (1.00 - 1.00)	-1.7	0.09
Temp x sex	**1.04 (1.01 - 1.06)**	**2.7**	**0.006**

Supplementary Table 5.4 Post-hoc analysis examining the interaction between sex and pupal temperature on development time. The Tukey method was used for P value adjustment. Results have not been back transformed from the log scale.

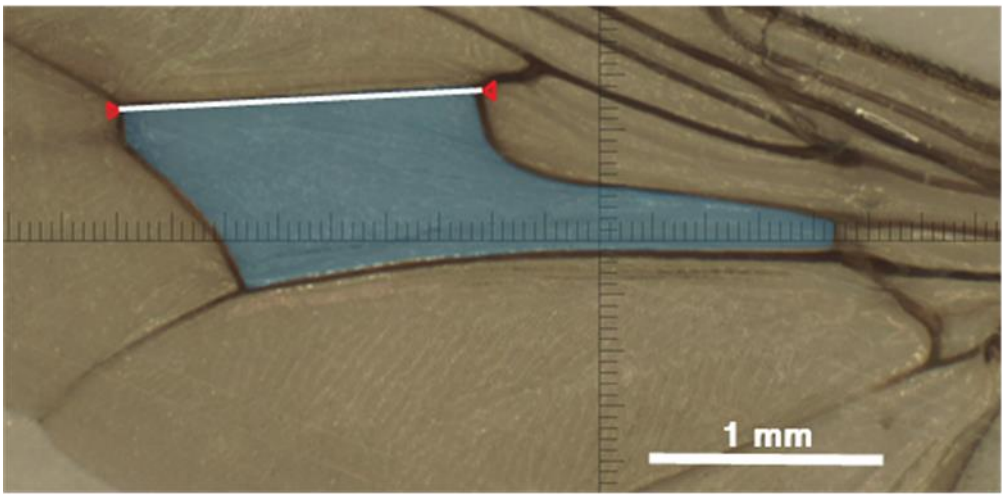
Comparison	Estimate	SE	df	t-value	p-value
25/female - 31/female	**0.440**	**0.009**	**252**	**47.4**	**<0.0001**
25/female - 25/male	**-0.049**	**0.013**	**216**	**-3.9**	**0.0008**
25/female - 31/male	**0.354**	**0.009**	**259**	**37.9**	**<0.0001**
31/female - 25/male	**-0.488**	**0.009**	**252**	**-52.6**	**<0.0001**
31/female - 31/male	**-0.085**	**0.004**	**208**	**-22.2**	**<0.0001**
25/male - 31/male	**0.403**	**0.009**	**259**	**43.1**	**<0.0001**

Supplementary Table 5.5 Linear models examining the effect of pupal temperature (constant 25°C or 31°C), sex and maternal age on adult mass (mg) and wing length (mm) in tsetse (*G. m. morsitans*). Mean differences between groups are given ± standard deviation (SD). Full statistics (adjusted R^2; F statistic; degrees of freedom) are displayed for each model below the comparison statistics.

		Wing vein (mm)			Adult mass (mg)		
Variable	**Group**	**Estimate ± SD**	**t-value**	**p-value**	**Estimate ± SD**	**t-value**	**p-value**
Intercept		**1.61 ± 0.007**	**224.0**	**<0.0001**	**22.09 ± 0.27**	**81.4**	**<0.0001**
Pupal temp. (25)	31	**-0.021 ± 0.006**	**-3.53**	**0.0004**	**-1.19 ± 0.22**	**-5.39**	**<0.0001**
Sex (female)	Male	**-0.16 ± 0.006**	**-27.3**	**<0.0001**	**-0.79 ± 0.22**	**-3.59**	**0.0003**
Maternal age	4 - 5	0.013 ± 0.008	1.54	0.12	**1.43 ± 0.31**	**4.57**	**<0.0001**
(weeks)	6 - 7	0.015 ± 0.008	1.80	0.07	**1.71 ± 0.31**	**5.57**	**<0.0001**
	8 - 9	-0.0019 ± 0.009	-0.21	0.83	**0.81 ± 0.33**	**2.43**	**0.015**
		Adj. R^2 = 0.66; F = 151.8; df = 379, 5			Adj. R^2 = 0.14; F = 14.87; df = 422, 5		

Supplementary Table 5.6 Mean mass ± standard deviation (SD) (mg) loss of tsetse (*G. m. morsitans*) during pupation and adulthood. Pupae were kept at either constant 25°C or 31°C for development and transferred to either a matched or unmatched temperature upon emergence for adulthood, given as pupal / adult temperature.

Variable	**Group**	**n**	**Pupal mass loss ± SD (mg)**	**Pupal mass loss rate (mg/day)**	**Adult mass loss ± SD (mg)**	**Adult mass loss rate (mg/day)**
Temperature	25 / 25	109	3.78 ± 4.72	0.12	8.8 ± 1.92	1.065
	25 / 31	109			11.0 ± 2.76	2.187
	31 / 25	103	4.75 ± 1.36	0.23	8.9 ± 2.63	1.059
	31 / 31	107			10.4 ± 2.93	2.087
Sex	Female	222	4.00 ± 1.35	0.16	10.1 ± 2.79	1.519
	Male	206	4.53 ± 1.36	0.17	9.5 ± 2.66	1.423



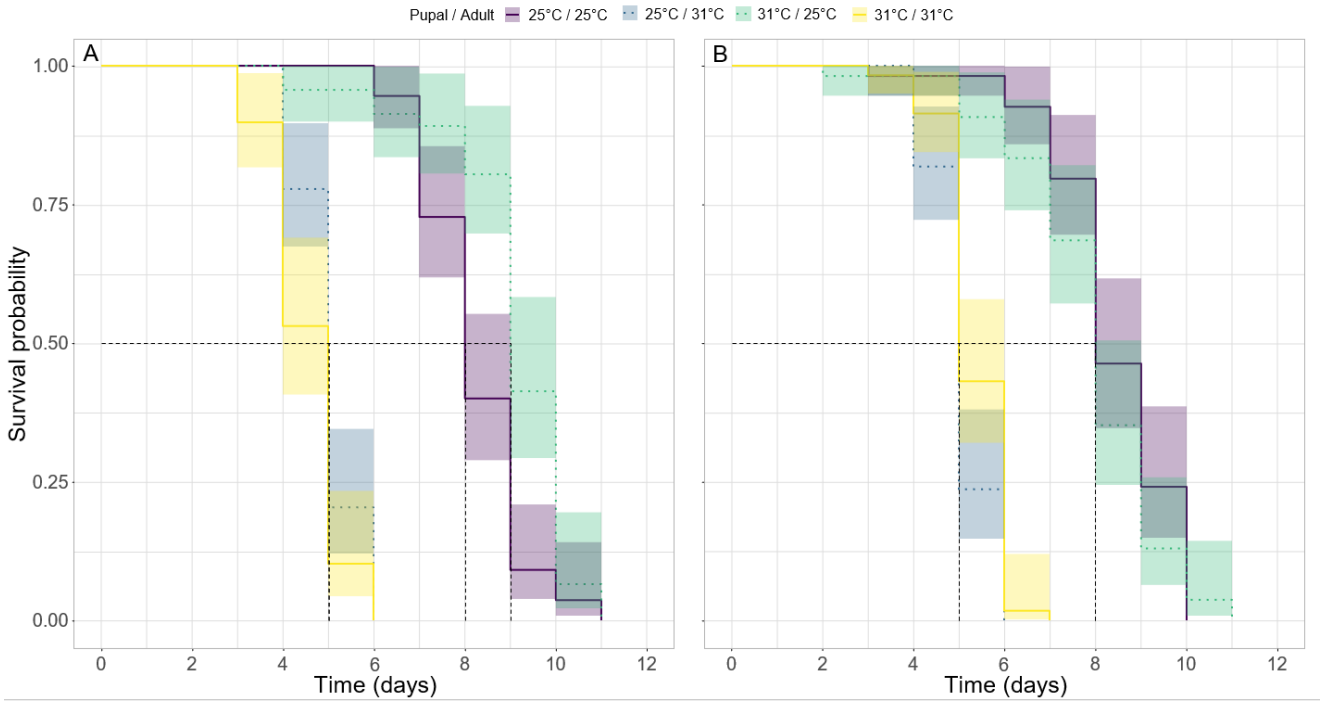
Supplementary Figure 5.1 Ventral view of tsetse (*G. m. morsitans*) wing with hatchet cell highlighted in blue. The length of the upper vein of the hatchet cell, marked between two red arrows, was taken as a proxy for size.

Supplementary Table 5.7 Multivariate General Linear Models examining the effect of four temperature regimes and sex on pupal and adult mass loss in unfed tsetse (*G. m. morsitans*). Pupae were kept at either constant 25°C or 31°C for development and transferred to either a matched or unmatched temperature upon emergence for adulthood, given as pupal / adult temperature. Mean differences between groups are given ± standard deviation (SD). Full statistics (adjusted R^2; F statistic; degrees of freedom) are displayed for each model below the comparison statistics.

			Pupal			Adult		
Variable	**Group**	**N**	**Mean difference ± SD**	**t-value**	**p-value**	**Mean difference ± SD**	**t-value**	**p-value**
Temperature	25 / 25	109	*Reference*			*Reference*		
	25 / 31	109				**2.15 ± 0.35**	**6.17**	**<0.001**
	31 / 25	103				0.04 ± 0.35	0.12	0.91
	31 / 31	107	**0.25 ± 0.029**	**8.56**	**<0.001**	**1.52 ± 0.35**	**4.35**	**<0.001**
Sex	Female	222	*Reference*			*Reference*		
	Male	206	**0.15 ± 0.029**	**5.01**	**<0.001**	**-0.61 ± 0.25**	**-2.47**	**0.014**
			Adj. R^2 = 0.18; F = 47.6; df = 425, 2			Adj. R^2 = 0.12; F = 15.7; df = 423, 4		

Supplementary Table 5.8 Post-hoc analysis examining the effect of four temperature regimes on total mass loss over pupation and adulthood in unfed tsetse (*G. m. morsitans*). Temperature is given as pupal / adult. Pairwise comparison mean differences between groups are given with standard error (SE). The Tukey method was used for P value adjustment. Significant differences are in bold font.

Comparison	Estimate	SE	df	t-value	p-value
25 / 25 - 25 / 31	**-2.01**	**0.35**	**424**	**-5.72**	**<0.0001**
25 / 25 - 31 / 25	**-0.94**	**0.36**	**424**	**-2.65**	**0.04**
25 / 25 - 31 / 31	**-2.46**	**0.35**	**424**	**-6.99**	**<0.0001**
25 / 31 - 31 / 25	**1.06**	**0.36**	**424**	**2.99**	**0.02**
25 / 31 - 31 / 31	-0.46	0.35	424	-1.30	0.57
31 / 25 - 31 / 31	**-1.52**	**0.36**	**424**	**-4.26**	**0.0002**



Supplementary Figure 5.2 Probability of survival of A.) male and B.) female adult tsetse (*G. m. morsitans*) over time (days) in absence of blood meals under four temperature regimes throughout pupation and adulthood. Temperature was maintained at constant 25°C or 31°C and are given as pupal / adult temperature. Matched pupal and adult temperature is depicted by solid lines and unmatched temperatures are depicted by dot-dash lines. 95% confidence intervals are shaded and black dotted lines mark where 50% of the population have survived.

Supplementary Table 5.9 Post-hoc comparison for interaction between treatment temperature and sex for Multivariate Cox Proportional-Hazards Model examining survival risk of tsetse (*G. m. morsitans*) when deprived of blood meals under four temperature regimes, given as pupal / adult. Pairwise comparison mean differences between groups are given with standard error (SE). The Tukey method was used for P value adjustment. Significant differences are in bold font.

Comparison	Estimate	SE	z ratio	p-value
25 / 25 female - 25 / 31 female	**-3.83**	**0.29**	**-13.3**	**<0.0001**
25 / 25 female - 31 / 25 female	-0.26	0.20	-1.4	0.88
25 / 25 female - 31 / 31 female	**-3.39**	**0.27**	**-12.4**	**<0.0001**
25 / 25 female - 25 / 25 male	-0.21	0.19	-1.1	0.96
25 / 25 female - 25 / 31 male	**-3.87**	**0.29**	**-13.5**	**<0.0001**
25 / 25 female - 31 / 25 male	0.58	0.20	2.8	0.08
25 / 25 female - 31 / 31 male	**-4.14**	**0.29**	**-14.2**	**<0.0001**
25 / 31 female - 31 / 25 female	**3.56**	**0.28**	**12.6**	**<0.0001**
25 / 31 female - 31 / 31 female	0.44	0.19	2.3	0.30
25 / 31 female - 25 / 25 male	**3.62**	**0.28**	**12.8**	**<0.0001**
25 / 31 female - 25 / 31 male	-0.04	0.19	-0.2	1
25 / 31 female - 31 / 25 male	**4.40**	**0.30**	**14.6**	**<0.0001**
25 / 31 female - 31 / 31 male	-0.31	0.20	-1.6	0.77
31 / 25 female - 31 / 31 female	**-3.13**	**0.27**	**-11.6**	**<0.0001**
31 / 25 female - 25 / 25 male	0.05	0.19	0.3	1
31 / 25 female - 25 / 31 male	**-3.60**	**0.28**	**-12.7**	**<0.0001**
31 / 25 female - 31 / 25 male	**0.84**	**0.20**	**4.1**	**0.001**
31 / 25 female - 31 / 31 male	**-3.87**	**0.29**	**-13.4**	**<0.0001**
31 / 31 female - 25 / 25 male	**3.18**	**0.27**	**11.9**	**<0.0001**
31 / 31 female - 25 / 31 male	-0.48	0.19	-2.5	0.20
31 / 31 female - 31 / 25 male	**3.97**	**0.29**	**13.8**	**<0.0001**
31 / 31 female - 31 / 31 male	**-0.75**	**0.20**	**-3.8**	**0.004**
25 / 25 male - 25 / 31 male	**-3.66**	**0.28**	**-12.9**	**<0.0001**
25 / 25 male - 31 / 25 male	**0.79**	**0.20**	**3.9**	**0.003**
25 / 25 male - 31 / 31 male	**-3.93**	**0.29**	**-13.7**	**<0.0001**
25 / 31 male - 31 / 25 male	**4.45**	**0.30**	**14.7**	**<0.0001**
25 / 31 male - 31 / 31 male	-0.27	0.20	-1.4	0.88
31 / 25 male - 31 / 31 male	**-4.71**	**0.31**	**-15.5**	**<0.0001**