

**Physiological ecology and future distributions
of two malaria vectors – *Anopheles arabiensis*
and *An. funestus***

by
Candice-Lee Lyons

*Dissertation presented for the degree of Doctor of Zoology in the Faculty
of Science at
Stellenbosch University*



Promotor: Prof. Steven L. Chown
Dr. John S. Terblanche
Prof. Maureen Coetzee

March 2013

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes one original paper published in a peer-reviewed journal and three unpublished publications. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

Date:March 2013.....

Abstract

Although malaria remains a major public health concern, especially in sub-Saharan Africa, little information exists on the physiological tolerances of malaria vectors. Here, I aimed to provide a comprehensive set of physiological tolerances for *Anopheles arabiensis* and *An. funestus*, by investigating thermal tolerance traits of adults, larvae and pupae; desiccation resistance of adults and development rate-temperature relationships for both vectors. Critical thermal limit (CT) and desiccation data showed significant effects of increasing adult age on reducing tolerance to temperature or dry conditions. Females of both species were more tolerant of high or low temperatures in CT experiments and were more desiccation tolerant than males in desiccation trials. *Anopheles funestus* was more desiccation tolerant than *An. arabiensis*, despite the common misconception that *An. arabiensis* is the more arid-adapted of the two species.

Comparisons between thermal tolerance traits of adult laboratory and wild strain progeny of both species indicated a high degree of similarity between critical thermal limits in wild and laboratory strains, suggesting that the use of laboratory populations of both mosquito strains can provide an accurate estimate of wild population responses to thermal change. Lethal temperature estimates for both vectors indicated a higher tolerance to high temperature in *An. arabiensis* larvae and pupae when compared with *An. funestus*, and a greater tolerance of high or low temperatures in adult females when compared with adult males.

Species differences between the vectors were further highlighted in development rate-temperature experiments. Under fluctuating and constant temperatures, *An. arabiensis* developed significantly faster than *An. funestus* and had higher survival to the adult stage. Under fluctuating temperatures, *An. arabiensis* developed faster or no different to constant temperatures, while survival under fluctuating temperatures was also comparable to constant temperature estimates. This faster development rate of this species is likely a consequence of the puddle-breeding nature of *An. arabiensis* and the need to develop to adulthood before

evaporation of breeding sites. *Anopheles funestus* on the other hand, showed reduced survival and development under fluctuating temperatures when compared with constant temperatures, probably as a result of the more thermally stable breeding sites usually used by this species.

Distribution data of these species, combined with developmental parameters in a process-based distribution model, suggests that both species will show range changes in response to climate change. Areas where these species were previously only present on a seasonal basis might become more suitable for vector population establishment and persistence, while areas on the northern margins of current distributions will become less favourable, leading to an overall southerly shift in habitat suitability for both species.

Increases in temperature and changes in rainfall patterns as predicted to occur with climate change are likely to impact the distribution of both malaria vectors. Combining the physiological tolerance data collected in this thesis in a future, planned mechanistic distribution model, will provide an accurate indication of potential range shifts of these vectors and hence, provide an indication of areas that may be at increased risk of malaria.

Opsomming

Alhoewel malaria 'n groot publieke gesondheidskwelling bly, veral in sub-Sahara Afrika, bestaan min inligting rakende die fisiologiese toleransies van malaria vektore. Hier het ek gepoog om 'n omvattende reeks van fisiologiese toleransies te voorsien vir *Anopheles arabiensis* en *An. funestus*, deur termiese verdraagsaamheidseienskappe, uitdrogingsweerstand en ontwikkelingstempo-temperatuur verhoudings vir beide vektore te ondersoek. Kritiese termiese limiet (CT) en uitdroging data het beduidende uitwerkings getoon van toenemende ouderdom op die vermindering van verdraagsaamheid teenoor temperatuur of droë toestande. Wyfies van beide spesies was meer verdraagsaam vir hoë of lae temperature in CT eksperimente en was meer verdraagsaam teenoor uitdroging as mannetjies in die uitdrogingsproewe. *Anopheles funestus* was meer verdraagsaam teenoor uitdroging as *An. arabiensis*, ten spyte van die algemene wanopvatting dat *An. arabiensis* die meer ariede aangepaste van die twee spesies is.

Vergelykings tussen termiese verdraagsaamheidseienskappe van laboratorium-en wilde stamlyn nageslagte van beide spesies het 'n hoë mate van ooreenkoms tussen kritieke termiese limiete in wilde en laboratorium stamlyne aangedui, wat voorstel dat die gebruik van laboratorium bevolkings van beide muskiet stamlyne 'n akkurate skatting kan gee van wilde bevolkingsreaksies tot termiese verandering. Fatale temperatuur beramings vir beide vektore het 'n hoër toleransie getoon by hoë temperature in *An. arabiensis* larwes en papies wanneer dit vergelyk word met *An. funestus*, en 'n groter verdraagsaamheid van hoë of lae temperature in wyfies, wanneer vergelyk word met mannetjies.

Spesies verskille tussen die vektore is verder uitgelig in die ontwikkelingstempo-temperatuur eksperimente. Onder wisselende en konstante temperature ontwikkel *An. arabiensis* aansienlik vinniger as *An. funestus* en het hoër oorlewing tot die volwasse stadium getoon. Onder wisselende temperature ontwikkel *An. arabiensis* vinniger of met geen verskil van konstante temperature nie, terwyl oorlewing onder wisselende temperature ook

vergelykbaar was met konstante temperatuur beramings. Die vinniger tempo van hierdie spesie is waarskynlik 'n gevolg van die poel-broeiende aard van *An. arabiensis* en die behoefte om tot volwassenheid te ontwikkel voor die verdamping van broeiplekke. *Anopheles funestus* aan die ander kant, het verminderde oorlewing en ontwikkeling onder wisselende temperature gewys wanneer dit vergelyk word met konstante temperature, waarskynlik as gevolg van die meer termies stabiele broeiplekke wat gewoonlik gebruik word deur hierdie spesie.

Verspreidingsdata van hierdie spesies, gekombineer met ontwikkelings-parameters in 'n proses-gebaseerde verspreidingsmodel, dui daarop dat beide spesies reeks veranderinge sal wys in reaksie tot klimaatsverandering. Gebiede waar hierdie spesies voorheen slegs teenwoordig was op 'n seisoenale basis, mag dalk meer geskik word vir vektor bevolkingsvestiging en volharding, terwyl areas op die noordelike grense van die huidige verspreidings minder gunstig sal word, wat sal lei tot algehele suidelike verskuiwing in die habitat geskiktheid vir beide spesies.

Toenames in temperatuur en veranderinge in reënvalpatrone, soos voorspel word om voor te kom met verandering van die klimaat, sal waarskynlik die verspreiding van malaria vektore beïnvloed. Deur die fisiologiese toleransie data, versamel in hierdie tesis, te kombineer met 'n toekoms, beplande meganistiese verspreidingsmodel, sal dit 'n akkurate aanduiding gee van die potensiele verspreidingsverskuiwings van hierdie vektore en dus 'n aanduiding gee van gebiede wat onder verhoogde risiko van malaria sal wees.

Acknowledgements

There are several people without which this work would not have been possible. Laboratory assistance and mosquito colony maintenance was provided by several staff and students at the Vector Control Reference Unit (VCRU) of the National Institute of Communicable Diseases in Johannesburg. Special thanks to Allison Gilbert, Ryan Norton, Zilindile Zulu, Belinda Spillings and Oliver Wood for assisting with mosquito feeding and maintenance.

The use of wild strains used in this study would not have been possible without the collecting efforts and assistance of Basil Brooke, Richard Hunt, Givemore Munhenga, Lizette Koekemoer, Michael Osaе and Belinda Spillings. Special thanks to Edwardo Nhamahango at the Maragra Sugar Estate in Mozambique for logistical support and assistance in the field. Many thanks to Givemore Munhenga and Belinda Spillings for assistance with PCR identifications of wild caught mosquitoes.

Several people commented on drafts of chapters, in particular, Samantha Stoffberg, Charlene Scheepers, Katelyn Faulkner, Elsje Kleynhans, Helene Basson, Chris Weldon and Jessica Allen. Sam, you constantly reminded me of the “light at the end of the tunnel” and without your help, support and discussion, the last few months would have been exceedingly difficult to get through, and I thank you for your many years of friendship. Charlene, your support and encouragement, and the fact that we were in the “same boat” so to speak, really made writing up just that little easier, so thank you for your time and energy in making me see the bigger picture. Kate, thanks for the many discussions and for working through tough problems with me. A very special thank you to Darren Kriticos for being an excellent teacher and for taking the time to discuss significant technical issues. Thanks to Peter Le Roux and Brigitte Braschler for always being willing to help and for several technical discussions. Many thanks to the admin staff at the Centre for Invasion Biology, in particular, Anel Garthwaite and Elrike Marais, for ensuring smooth running of all aspects of this project. And thanks for the technical assistance provided from Erika Nortje, even from a distance.

I would like to extend a heartfelt appreciation to Richard Hunt, Lizette Koekemoer, Basil Brooke and Maureen Coetzee for several discussions during my time at the VCRU and for being such spectacular hosts and making me feel truly welcome for the 18 months I spent in Johannesburg collecting data. To Maureen, it was an honour having you as a supervisor and I learnt a lot of what I know about malaria and mosquitoes from you.

Thanks also to John Terblanche for many hours of discussion, especially during the initial phases of this project and for several useful comments and suggestions throughout my PhD. Thank you to my family and friends for your support and encouragement over the three year period of my PhD. I know I was not always the nicest person to be around and often prioritized work over social activities, but I am grateful to have you all in my life and that you are all so understanding and accepting. To my parents, thank you for encouraging me to fulfill my dreams and to persevere to obtain my goals. To Jason, thank you for your support, encouragement and patience, especially during the difficult phases of writing up. And thank you for your help, wherever and in whatever way it was provided.

Last but definitely not least, I would very much like to thank Steven Chown for his endless support and assistance throughout my time at Stellenbosch University and the Centre for Invasion Biology. I was never short of assistance or equipment as a result of your supervisory role and I will remain forever appreciative of the time and effort spent on making this a wonderful experience in my scientific career. Thank you for your patience and your teaching and for giving me the motivation to keep going. Thank you for constantly making me question and think – key aspects which helped me achieve what I have. I thank you for instilling in me an even greater love for science brought on by your intellect and passion for what you do.

Table of Contents

Declaration	ii
Abstract	iii
Opsomming	v
Acknowledgements	vii
Chapter 1	
General Introduction	1
i. References	13
Chapter 2	
Thermal limits of wild and laboratory strains of two African malaria vector species, <i>Anopheles arabiensis</i> and <i>Anopheles funestus</i>	24
i. Introduction	25
ii. Methods	27
iii. Results	32
iv. Discussion	35
v. References	40
vi. Figure Legends	48
vii. Figures and Tables	50
viii. Supplementary Materials	64
Chapter 3	
Stable and fluctuating temperature effects on the development rate and survival of two malaria vectors, <i>Anopheles arabiensis</i> and <i>Anopheles funestus</i>	77
i. Introduction	78
ii. Methods	81
iii. Results	85

iv.	Discussion	86
v.	References	91
vi.	Figure Legends	100
vii.	Figures and Tables	102
viii.	Supplementary Materials	109

Chapter 4

Intrinsic and extrinsic factors interact to affect desiccation tolerance in adult mosquitoes: implications for malaria vector competence		120
i.	Introduction	121
ii.	Methods	123
iii.	Results	127
iv.	Discussion	131
v.	References	135
vi.	Figure Legends	144
vii.	Figures and Tables	145
viii.	Supplementary Materials	160

Chapter 5

Future distributions of <i>Anopheles arabiensis</i> and <i>Anopheles funestus</i> in Africa		161
i.	Introduction	162
ii.	Methods	164
iii.	Results	167
iv.	Discussion	169
v.	References	172
vi.	Figure Legends	180
vii.	Figures	181

viii.	Supplementary Materials	184
-------	-------------------------	-----

Chapter 6

	General Conclusion	192
--	--------------------	-----

i.	References	200
----	------------	-----

Chapter 1

General Introduction

“We do not inherit the Earth from our ancestors, we borrow it from our children”

Native American Proverb

Climate change and biological systems

Climate change and its impact on organisms is receiving much attention in the scientific literature (Root *et al.* 2003; Parmesan and Yohe 2003; Parmesan 2006). According to the Intergovernmental Panel on Climate Change (IPCC), temperatures are predicted to increase by between 1.1°C and 6°C within the next 100 years (Boko *et al.* 2007). Changes in mean annual, and daily, temperature variations are not only predicted, but are already occurring, as are changes in rainfall patterns (Hansen *et al.* 2012). Mean precipitation is expected to increase in the deep tropics and extratropics and decrease in the subtropics, with precipitation extremes increasing on a global scale (Hughes 2000; Kovats *et al.* 2001; Mc Michael *et al.* 2006; O’Gorman and Schneider 2009). Many mid- and low-latitude regions are expected to become drier with climate change, while average annual precipitation will increase in many other regions (Kovats *et al.* 2001; Helmuth *et al.* 2005; Mc Michael *et al.* 2006). For southern Africa, changes in temperature are likely to be in the region of 2°C and 4°C with rainfall predicted to decrease on the west coast and increase on the east coast (Eeley *et al.* 1999; Hulme *et al.* 2001; Knoesen *et al.* 2009).

Biological implications of temperature and precipitation changes include effects on organism abundance, distribution and evolution (Huey and Tewksbury 2009). For example, strong correlations exist between temperature and species distribution over a range of spatial and temporal scales (Helmuth *et al.* 2005). Generally, increased temperatures should benefit temperate species by giving them increased flexibility in their habitat ranges, but may constrain tropical species by restricting their habitat use and availability (Kearney *et al.* 2009). Empirical evidence for the biological impacts of climate change exists in the changes associated with species traits and communities, and four types of change that may occur as a result of climate change associated warming have been identified. First, the density of species may change in certain regions and ranges may shift poleward or upwards in elevation, to

areas where these species can tolerate surrounding conditions (i.e. temperature) (Hughes 2000; Midgley *et al.* 2002; Root *et al.* 2003; Guo *et al.* 2009; Kearney *et al.* 2009). An example of these distribution shifts exists in the poleward range expansions observed in many northern hemisphere bird species and in several butterfly species (Parmesan *et al.* 1999; Parmesan and Yohe 2003; Parmesan 2006). However, changes in species distributions and biodiversity are often not easily observable. On a broader scale, changes in phenology of species may be the most easily observed change resulting from climate change. This is the second type of change associated with climate change. Examples include an earlier onset of spring flowering and breeding events, or a later onset of autumnal events such as bird migrations (Crick and Sparks 1999; Hughes 2000; Midgley *et al.* 2002; Walther *et al.* 2002; Root *et al.* 2003; Guo *et al.* 2009). Third, species morphologies may change. This may be true of the body sizes (Gardner *et al.* 2011; Bickford *et al.* 2011) of some species over an extended period of time (Root *et al.* 2003), or those species with short generation times might undergo a type of microevolution in response to climate change (Hughes 2000). Finally, gene frequencies of species may shift in response to extended periods of climate change, with greater occurrence of the genes in a population which increase survival of a species or individual under altered climatic scenarios (e.g. Umina *et al.* 2005). These above-mentioned predicted effects as a result of climate change may also alter the competitive abilities of species and the interactions between species, with possible implications for species abundance and geographic ranges (Hughes 2000).

Climate change and species ranges

Whether or not species show range changes as a result of climate change depends largely on their ability to tolerate, adapt to, or behaviourally manipulate, their surroundings. A species' ability to tolerate, or adapt to, a particular environment will depend largely on its physiology,

which determines where it has the potential to survive and reproduce. Physiological constraints of species, and therefore distributions, are often determined by environmental conditions. Some of the physiological variables considered most important in determining a species' range, include its ability to withstand desiccation, its ability to adjust to changing temperatures and its ability to use its surroundings to manipulate microclimates to enhance and prolong survival. Currently, nearly 90% of investigated species are showing range shifts in the directions predicted as a result of physiological tolerances (Root *et al.* 2003; Rosenweig *et al.* 2008). Using a meta-analysis of over a hundred studies, Root *et al.* (2003) showed that many plant and animal species are showing range shifts in the direction predicted based on physiological tolerances to temperature. Thomas and Lennon (1999) showed that several bird species have shown population range changes, based on breeding site localities, and shifts in these breeding sites to beyond the previous northern range limits. Many studies infer range shifts as a result of climate change based on for example, density or population size changes of species over several years (see Parmesan 2006 for review). Others investigate species range margins and base conclusions on long term changes observed at these range margins (e.g. Parmesan *et al.* 1999; Pounds *et al.* 2006).

Temperature, precipitation and rising CO₂ levels are known to affect metabolic and development rates in many animals and processes such as photosynthesis and respiration in many plants (Hughes 2000; Guo *et al.* 2009; Irlich *et al.* 2009). For this reason, physiological traits should be included in predictions of species range changes (Helmuth *et al.* 2005). If a single physiological factor (e.g. body temperature) is a causative factor limiting a species distribution, then it is important to deduce which aspect of such a variable is the limiting factor (e.g. minimum or maximum temperature, mean temperature) (Helmuth *et al.* 2005). The physiological performance of organisms can also be affected through genetic adaptation brought on by environmental change, or through phenotypic plasticity in response to certain

environmental conditions (Helmuth *et al.* 2005). Depending on the physiological response of the organism concerned, climate change may result in acclimatization by the species (if changes are small), certain genotypes may be favoured (if climate change exceeds the plasticity of some but not all individuals), or all individuals in a population may die or migrate (if conditions are sufficiently severe) (Helmuth *et al.* 2005).

Malaria and climate change

The effect of climate change on species, and in particular species distributions, has important implications for humans, both in terms of pest species and disease (Parmesan 2006). Changes in the distributions of vectors may affect human disease incidence and transmission. On average, 800 000 people die of malaria annually (WHO 2010). Most of these deaths occur in Africa, where disease reporting occurs on a smaller scale than in other parts of the world and infrastructure for dealing with the disease is largely lacking (Martens *et al.* 1997; Nchinda 1998; Hay *et al.* 2002; Pascual *et al.* 2006). Malaria is largely confined to the tropics, with sub-Saharan Africa being the most affected region (Kiszewski *et al.* 2004; Molyneux 2009). Transmitted by *Anopheles* mosquitoes, this disease is likely to have strong links with climate because mosquitoes are one of the insect groups with a strong reliance on temperature and moisture for development, survival and reproduction (e.g. Bayoh and Lindsay 2004; Gray and Bradley 2005). Because of this, *Anopheles* mosquitoes should be highly susceptible to changing climate. However, how malaria as a disease is likely to be impacted by climate change remains uncertain, largely due to the complex interactions between vectors, the malaria-causing *Plasmodium* parasite and their environment, with survival of the parasite also being largely temperature-dependent (Thompson *et al.* 1997; Sachs and Malaney 2002; Molyneux 2009; Paaijmans *et al.* 2009). Warming may lead to a prolonged transmission season which may in turn lead to heightened transmission levels, thereby increasing malaria

related morbidity and mortality (Tanser *et al.* 2003 but see Reiter *et al.* 2004). Several studies have suggested that malaria will show an increase at its current range margins (Martens *et al.* 1997; Tanser *et al.* 2003) whereas others predict that there will be no overall change to the prevalence of the disease as a result of warming temperatures and changing rainfall patterns (Small *et al.* 2003). Indeed, debates about the overall impact of climate change on malaria are currently heated (Martens *et al.* 1999; Rogers and Randolph 2000; Hay *et al.* 2002; Reiter *et al.* 2004; Pascual *et al.* 2006; Githeko 2009).

The most prominent *Plasmodium falciparum* vectors in sub-Saharan Africa are *Anopheles arabiensis*, *An. funestus* and *An. gambiae* (e.g. Collins and Besansky 1994; Chan *et al.* 1999; Donnelly *et al.* 2001; Molyneux 2009). The spread of the disease is largely confined to regions favourable for the development, survival and persistence of these *Anopheles* vectors. Anopheline mosquitoes have a close association between breeding site availability and precipitation and hence, their distribution may therefore be closely associated with precipitation events (Tanser *et al.* 2003). Excessive rainfall may eliminate breeding grounds (Paaijmans *et al.* 2007), whereas too little rain may lead to dry conditions not conducive to larval breeding (McMichael *et al.* 2006).

As is the case for all holometabolous insects, *Anopheles* mosquitoes are characterized by four life stages – eggs, larvae, pupae and adults (Gullan and Cranston 1994; Clements 2000), each affected, to different degrees, by environmental conditions. Eggs, larvae and pupae for instance, are largely or wholly confined to water and are therefore likely to be constrained by their habitats (Clements 2000). Furthermore, although larvae and pupae are to some extent mobile, eggs are not and are therefore, in most instances, less tolerant of desiccation (Clements 1963; 2000). Adults on the other hand, are mobile and are able to make use of available microclimates (e.g. indoor resting behaviour during the hottest or driest parts of the day or year) (Paaijmans and Thomas 2011). Because of the different

environmental requirements and mobility of the various life stages, the influence of climate change on each of these stages is likely to differ. Furthermore, the response of adult mosquitoes to climate change could differ between ages and sexes, as has been found in several other insect species (Krebs and Loeschcke 1996; Bowler and Terblanche 2008), and given that females have to be able to tolerate rapid changes in temperature associated with blood ingestion (Benoit *et al.* 2011).

In addition to influencing suitable habitats, and thus distributions, temperature also affects mosquito feeding intervals, longevity and population density as well as the reproductive potential and survival of *Plasmodium* parasites (Small *et al.* 2003; Paaijmans *et al.* 2009). Temperature fluctuations can have substantial impacts on the parasite incubation period and hence, malaria transmission rates (Paaijmans *et al.* 2009). The impact of climate change on malaria will require an understanding not only of the responses of the parasite to changes in temperature and precipitation (outside of the limits of this thesis), but also of the mosquito vectors themselves.

Models of malaria transmission and the effects of temperature

Many factors combine to influence malaria transmission dynamics, several of which are strongly reliant on temperature (Kiszewski *et al.* 2004). Amongst others, malaria is influenced by vector biology, parasite biology, control efforts influencing vector survival, infectivity of mosquitoes, and the propensity of vectors to bite humans (Kiszewski *et al.* 2004; Kiware *et al.* 2012). Traditional models of malaria transmission such as those of Ross and MacDonald (reviewed in Smith and McKenzie 2004) make several assumptions about vector biology which could severely influence the transmission potential they estimate. Some of these assumptions include that mosquitoes do not experience age-related mortality or any evidence of senescence behaviour (Smith and McKenzie 2004; Smith *et al.* 2012). However,

evidence suggests that many malaria vectors do indeed senesce and that mortality is not constant with age (Dawes *et al.* 2009). Furthermore, these models also assume that mosquito population size remains constant, although this is clearly not observed in natural settings, with fluctuations in adult populations being observed across seasons and localities (Hamad *et al.* 2002). Several aspects of mosquito biology are strongly influenced by environmental conditions. These include development rate and growth, as well as survival. Under “less favourable” conditions, the resultant emergent adult population is greatly reduced, resulting in non-homogenous mosquito populations, and fluctuations in population size, largely dependent on temperature and rainfall.

These types of models also assume that mosquito behaviour is constant and that females bite all humans equally (Mahande *et al.* 2007). Contrary to this assumption, mosquitoes do in fact show preference for some people when compared to others (Kelly 2001; Smith and McKenzie 2004), and this behaviour is also largely a factor of human proximity to breeding sites, with those people closer to breeding sites, being bitten more frequently than those some distance away (Smith and McKenzie 2004). Some vectors are also more anthropophilic and endophilic than others, preferring to feed on humans indoors, while others display preference for cattle (*Anopheles arabiensis* for example) (Mahande *et al.* 2007). These behavioural traits and plasticity of these traits, with preferences of vectors being largely dependent on the population under investigation, could significantly influence the estimates made of the basic reproductive number (R_0) or even the entomological inoculation rate (EIR) both dependent on the number of infective mosquitoes biting humans (Smith *et al.* 2005; Smith *et al.* 2007). The early models of malaria transmission assisted in the development of these parameters (and others), which are still used in epidemiological studies today (Smith and McKenzie 2004).

In addition to vector biology and specifically behaviour, malaria incidence and prevalence is also largely determined by the parasite's relationship with temperature. Various models exist to explain the relationship between temperature and parasite development (e.g. Paaijmans *et al.* 2009; Mordecai *et al.* 2012) and MacDonald was one of the first practitioners to realize the importance of parasite development in malaria epidemiology (see Smith and McKenzie 2004). At excessively high temperatures, parasite development and growth is retarded, leading to an overall reduction in parasite numbers within an infective female mosquito (Dawes *et al.* 2009). Recent work has argued that the optimal temperature for malaria transmission and hence, parasite development, is much lower than previously thought (Mordecai *et al.* 2012). Furthermore, under conditions of fluctuating temperatures, parasite development is influenced differently to what it is under conditions of constant temperature (Paaijmans *et al.* 2009). At fluctuations around the lower mean of 21°C, parasite development is increased, lowering the extrinsic incubation period (EIP), and hence, leading to increased malaria potential in regions that may previously have been thought of as unsuitable for transmission (Paaijmans *et al.* 2009). In addition, temperatures that fluctuate above a mean of 21°C, are thought to increase the EIP, with the overall result that malaria incidence or potential for transmission is reduced (Paaijmans *et al.* 2009). Traditional models of malaria transmission fail to incorporate daily temperature fluctuations although these fluctuations are becoming more important in light of recent climate change research (Hansen *et al.* 2012).

As a disease whose is influenced by several factors, malaria remains a concern for large parts of Africa and Asia. Although it is difficult to obtain all the necessary information required to model disease transmission and although some of these “classic models” such as the Ross and MacDonald models do not perform well in malaria-endemic regions (Smith and McKenzie 2004), understanding the vectors' biology should provide a good starting point on

which to base models, reducing the assumptions through experimentation and field observation where possible.

Species distribution models

Determining the impact of climate change on the distribution ranges of *Anopheles* species and consequently, on the extent of malaria as a disease, requires the use of some form of distribution model. Species distribution models are of two broad categories. The first is the conventional “top-down” modelling approach, inferring species responses to climate change (for example) based on current known distributions and the environmental conditions associated with these realized niches (Phillips *et al.* 2006; Peterson *et al.* 2007). The approaches generally used to construct such models, also known as species distribution models, are enjoying much current attention (e.g. Elith and Leathwick 2009; Araújo and Peterson 2012). The second approach is a “bottom-up” mechanistic model which uses *a priori* information on the species to infer its fundamental niche, in the absence of any known distribution data, and based solely on where, for example, its physiological tolerances would enable it to survive (Kearney 2006; Kearney and Porter 2009).

Mechanistic models require the inclusion of species-specific physiological data – much of which is lacking for African malaria vectors. Several studies have investigated physiological tolerances of *An. gambiae* and only a handful have investigated physiological tolerances in *An. arabiensis* (Bayoh and Lindsay 2003, 2004; Kirby and Lindsay 2004; Gray and Bradley 2005). None have investigated the thermal tolerance, development rate-temperature relationships or the desiccation tolerance of *An. funestus* despite the importance of this species in transmitting malaria, especially in sub-Saharan Africa. A third type of modelling approach is a partial mechanistic model, using a combination of mechanistic and correlative techniques. One such approach is that of the CLIMEX modelling software developed by Sutherst and Maywald (1985). Although this approach does not incorporate an

extensive amount of physiology, it allows the user to choose species-defined parameters, based on known biology, including developmental parameters and thresholds, degree-days to complete one generation and the ability of a species to diapause under unfavourable environmental conditions of temperature or light (Sutherst *et al.* 2007). This approach provides a good starting point for many investigators interested in species range changes as a result of climate change or the invasive potential of some species (e.g. Robinson and Hoffmann 2001; Lozier and Mills 2011).

Aims and outline of thesis

The main aim of this thesis is to examine the physiological tolerances of two of Africa's most prominent malaria vectors, *Anopheles arabiensis* and *An. funestus*, with the goal of providing data required for mechanistic distribution modelling of these vectors. Using only a portion of this data, an initial indication of potential projected range changes of these species is made.

Chapter two investigates the thermal physiology of *Anopheles arabiensis* and *An. funestus*. The lethal temperature limits to survival of fourth instar larvae and the pupal stages of each species are determined. Additionally, the influence of sex and age of adults in determining these species' lethal temperature limits are investigated. The extent of phenotypic plasticity (Hoffmann *et al.* 2003; Chown and Terblanche 2007) of each of these vectors is determined in critical thermal limits experiments of adults of different ages, following different acclimation treatments. Furthermore, given the loss of tolerance often associated with laboratory colonies in other species (e.g. Harshman and Hoffmann 2000; Terblanche and Chown 2007) and in *Anopheles* mosquitoes (Huho *et al.* 2007), the differences in critical thermal minima and maxima between early generation wild progeny and laboratory strains of both species is determined.

Chapter three investigates the development rate-temperature relationships of both vectors, under a range of constant and fluctuating temperatures. Given the importance of climate change in influencing thermal variability on daily and seasonal cycles (Hansen *et al.* 2012), understanding the impact of fluctuating temperatures on such an important aspect to mosquito population dynamics may provide greater insight into the effects of climate change on mosquito populations. Responses of various insect species to fluctuating and constant temperatures differs (Hagstrum and Leach 1972; Brittain and Campbell 1991) and to obtain as accurate as possible physiological data required for distribution models, an understanding of the roles of these fluctuating temperatures is crucial.

Chapter four investigates the desiccation tolerance of these vectors across different ages, sexes, and temperature and humidity combinations. Given the seasonality of malaria in some areas, and hence, the absence of the disease during the dry season (Hay *et al.* 1998; Huestis *et al.* 2011), desiccation resistance (enabling overwintering) of *An. arabiensis* and *An. funestus* is one likely mechanism by which this resurgence during the rains may be accomplished. Several studies have pointed to the possibility of overwintering phenotypes of malaria vectors (Lehmann *et al.* 2010; Huestis *et al.* 2011) but only a few have investigated the desiccation resistance of *An. gambiae s.l.* (which includes *An. arabiensis*) malaria vectors (e.g. Gray and Bradley 2005; Rocca *et al.* 2009).

In Chapter five, a preliminary distribution map of *An. arabiensis* and *An. funestus* is provided, using the development rate-temperature data collected in chapter three. Given the narrow thermal niches for development from egg to adult of each species (chapter three) and the wider thermal ranges of adults (chapter two), distribution of these species may be limited by breeding site availability, and hence, distribution models based on this information may indicate suitable potential niches for both vectors. For the third African malaria vector, *An.*

gambiae s.s. this kind of information has already shown close matches to the distribution of this species across Africa (Lindsay and Bayoh 2004).

References

- Araújo, M.B. and Peterson, A.T. 2012. Uses and misuses of bioclimatic envelope modelling. *Ecology* **93**: 1527-1539.
- Bayoh, M.N. and Lindsay, S.W. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Bulletin of Entomological Research* **93**: 375-381.
- Bayoh, M.N. and Lindsay, S.W. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Medical and Veterinary Entomology* **18**: 174-179.
- Benoit, J.B., Lopez-Martinez, G., Patrick, K.R., Phillips, Z.P., Krause, T.B. and Denlinger, D.L. 2011. Drinking a hot blood meal elicits a protective heat shock response in mosquitoes. *Proceedings of the National Academy of Sciences of the U.S.A.* **108**: 8026-8029.
- Bickford, D.P., Sheridan, J.A. and Howard, S.D. 2011. Climate change responses: forgetting frogs, ferns and flies? *Trends in Ecology and Evolution* **26**: 553-554.
- Boko, M., Niang, I., Nyong, A., Vogel, C., Githeko, A., Medany, M., Osman-Elasha, B., Tabo, R. and Yanda, P. 2007. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Edited by Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. Cambridge: Cambridge University Press, UK: 433-467.

- Bowler, K. and Terblanche, J.S. 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biological Reviews* **83**: 339-355.
- Brittain, J.E. and Campbell, I.C. 1991. The effect of temperature on egg development in the Australian mayfly genus *Coloburiscoides* (Ephemeroptera: Coloburiscidae) and its relationship to distribution and life history. *Journal of Biogeography* **18**: 231-235.
- Chan, N.Y., Ebi, K.L., Smith, F., Wilson, T.F. and Smith, A.E. 1999. An integrated assessment framework for climate change and infectious diseases. *Environmental Health Perspectives* **107**: 329-337.
- Chown, S.L. and Terblanche, J.S. 2007. Physiological diversity in insects: Ecological and evolutionary contexts. *Advances in Insect Physiology* **33**: 50-152.
- Clements, A.N. 1963. *The Physiology of Mosquitoes*. The MacMillan Company, New York, USA.
- Clements, A.N. 2000. *The Biology of Mosquitoes: Development, Nutrition and Reproduction*. CABI Publishing, Oxfordshire, UK.
- Collins, F.H. and Besansky, N.J. 1994. Vector biology and the control of malaria in Africa. *Science* **264**: 1874-1875.
- Crick, H.Q.P. and Sparks, T.H. 1999. Climate change related to egg-laying trends. *Nature* **399**: 423-424.
- Dawes, E.J., Churcher, T.S., Zhuang, S., Sinden, R.E. and Basáñez, M-G. 2009. *Anopheles* mortality is both age- and *Plasmodium*-density dependent: implications for malaria transmission. *Malaria Journal* **8**: 228.
- Donnelly, M.J., Licht, M.C. and Lehmann, T. 2001. Evidence for recent population expansion in the evolutionary history of the malaria vectors *Anopheles arabiensis* and *Anopheles gambiae*. *Molecular Biology and Evolution* **18**: 1353-1364.

- Eeley, H.A.C., Lawes, M.J. and Piper, S.E. 1999. The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *Journal of Biogeography* **26**: 595-617.
- Elith, J. and Leathwick, J.R. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution and Systematics* **40**: 677-697.
- Gardner, J.L. Peters, A., Kearney, M.R., Joseph, L. and Heinsohn, R. 2011. Declining body size: a third universal response to warming? *Trends in Ecology and Evolution* **26**: 285-291.
- Githeko, A.K. 2009. Malaria and climate change. Commonwealth Health Ministers Update: 40-43.
- Gray, E.M. and Bradley, T.J. 2005. Physiology of desiccation resistance in *Anopheles gambiae* and *Anopheles arabiensis*. *American Journal of Tropical Medicine and Hygiene* **73**: 553-559.
- Gullan, P.J. and Cranston, P.S. 1994. *The Insects: An Outline of Entomology*. First Edition. Chapman and Hall, London, UK.
- Guo, K., Hao, S-G., Sun, O.J. and Kang, L. 2009. Differential responses to warming and increased precipitation among three contrasting grasshopper species. *Global Change Biology* **15**: 2539-2548.
- Hagstrum, D.W. and Leach, C.E. 1972. Role of constant and fluctuating temperatures in determining development time and fecundity of three species of stored-products Coleoptera. *Annals of the Entomological Society of America* **66**: 407-410.
- Hamad, A.A., Nugud, A.E.H.D., Arnot, D.E., Giha, H.A., Abdel-Muhsin, A.A., Satti, G.M.H., Theander, T.G., Creasey, A.M., Babiker, H.A. and Elnaiem, D.A. 2002. A

- marked seasonality of malaria transmission in two rural sites in eastern Sudan. *Acta Tropica* **83**: 71-82.
- Hansen, J., Sato, M. and Ruedy, R. 2012. Perception of climate change. Proceedings of the National Academy of Sciences of the U.S.A.: Early Edition: pnas.org/cgi/doi/10.1073/pnas.1205276109.
- Harshman, L.G. and Hoffmann, A.A. 2000. Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends in Ecology and Evolution* **15**: 32-36.
- Hay, S.I., Snow, R.W. and Rogers, D.J. 1998. Predicting malaria seasons in Kenya using multitemporal meteorological satellite sensor data. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**: 12-20.
- Hay, S.I., Rogers, D.J., Randolph, S.E., Stern, D.I., Cox, J., Shanks, G.D. and Snow, R.W. 2002. Hot topic or hot air? Climate change and malaria resurgence in East African highlands. *Trends in Parasitology* **18**: 530-534.
- Helmuth, B., Kingsolver, J.G. and Carrington, E. 2005. Biophysics, physiological ecology and climate change: does mechanism matter? *Annual Review of Physiology* **67**: 177-201.
- Hoffmann, A.A., Sørensen, J.G. and Loeschcke, V. 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* **28**: 175-216.
- Huestis, D.L., Yaro, A.S., Traoré, A.I., Adamou, A., Kassogué, Y., Diallo, M., Timbine, S., Dao, A. and Lehmann, T. 2011. Variation in metabolic rate of *Anopheles gambiae* and *A. arabiensis* in a Sahelian village. *Journal of Experimental Biology* **214**: 2345-2353.
- Huey, R.B. and Tewksbury, J.J. 2009. Can behaviour douse the fire of climate warming? *Proceedings of the National Academy of Sciences of the U.S.A.* **106**: 3647-3648.

- Hughes, L. 2000. Biological consequences of global warming: is the signal already apparent? *Trends in Ecology and Evolution* **15**: 56-61.
- Huho, B.J., Ng'habi, K.R., Killeen, G.F., Nkwengulila, G., Knols, B.G.J. and Ferguson, H.M. 2007. Nature beats nurture: a case study of the physiological fitness of free-living and laboratory-reared male *Anopheles gambiae s.l.* *Journal of Experimental Biology* **210**: 2939-2947.
- Hulme, M., Doherty, R., Ngara, T., New, M. and Lister, D. 2001. African climate change: 1900-2100. *Climate Research* **17**: 145-168.
- Irlich, U.M., Terblanche, J.S., Blackburn, T.M. and Chown, S.L. 2009. Insect-rate temperature relationships: environmental variation and the metabolic theory of ecology. *American Naturalist*. **174**: 819-835.
- Kearney, M. 2006. Habitat, environment and niche: what are we modelling? *OIKOS* **115**: 186-191.
- Kearney, M. and Porter, W. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters* **12**: 334-350.
- Kearney, M., Porter, W.P., Williams, C., Ritchie, S. and Hoffmann, A.A. 2009. Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: the dengue mosquito *Aedes aegypti* in Australia. *Functional Ecology* **23**: 528-538.
- Kelly, D.W. 2001. Why are some people bitten more than others? *Trends in Parasitology* **17**: 578-581.
- Kirby, M.J. and Lindsay, S.W. 2004. Responses of adult mosquitoes of two sibling species, *Anopheles arabiensis* and *An. gambiae s.s.* (Diptera: Culicidae), to high temperatures. *Bulletin of Entomological Research* **94**: 441-448.

- Kiszewski, A., Mellinger, A., Spielman, A., Malaney, P., Sachs, S.E. and Sachs, J. 2004. A global index representing the stability of malaria transmission. *American Journal of Tropical Medicine and Hygiene* **70**: 486-498.
- Kiware, S.S., Chitnis, N., Moore, S.J., Devine, G.J., Majambere, S. and Killeen, G.F. 2012. Simplified models of vector control impact upon malaria transmission by zoophagic mosquitoes. *PLoS ONE* **7**: e37661.
- Knoesen, D., Schulze, R., Pringle, C., Summerton, M., Dickens, C. and Kunz, R. 2009. Water for the future: impacts of climate change on water resources in the Orange-Senqu River basin. Report to NeWater, a project funded under the Sixth Research Framework of the European Union. Institute of Natural Resources, Pietermaritzburg, South Africa.
- Kovats, R.S., Campbell-Lendrum, D.H., McMichael, A.J., Woodward, A. and Cox, St. H. 2001. Early effects of climate change: do they include changes in vector-borne disease? *Philosophical Transactions of the Royal Society of London B* **356**: 1057-1068.
- Krebs, R.A. and Loeschcke, V. 1996. Acclimation and selection for increased resistance to thermal stress in *Drosophila buzzatii*. *Genetics* **142**: 471-479.
- Lehmann, T., Dao, A., Yaro, A.S., Adamou, A., Kassogue, Y., Diallo, M., Sékou, T. and Coscaron-Arias, C. 2010. Aestivation of the African malaria mosquito, *Anopheles gambiae* in the Sahel. *American Journal of Tropical Medicine and Hygiene* **83**: 601-606.
- Lindsay, S.W. and Bayoh, M.N. 2004. Mapping members of the *Anopheles gambiae* complex using climate data. *Physiological Entomology* **29**: 204-209.
- Lozier, J.D. and Mills, N.J. 2011. Predicting the potential invasive range of light brown apple moth (*Epiphyas postvittana*) using biologically informed and correlative species distribution models. *Biological Invasions* **13**: 2409-2421.

- Mahande, A., Mosha, F. Mahande, J. and Kweka, E. 2007. Feeding and resting behaviour of malaria vector, *Anopheles arabiensis* with reference to zoophylaxis. *Malaria Journal* **6**: 100.
- Martens, W.J.M., Jetten, T.H. and Focks, D.A. 1997. Sensitivity of malaria, schistosomiasis and dengue to global warming. *Climatic Change* **35**: 145-156.
- Martens, P., Kovats, R.S., Nijhof, S., de Vries, P., Livemore, M.T.J., Bradley, D.J., Cox, J. and McMichael, A.J. 1999. Climate change and future populations at risk of malaria. *Global Environmental Change* **9**: S89-S107.
- McMichael, A.J., Woodruff, R.E. and Hales, S. 2006. Climate change and human health: present and future risks. *Lancet* **367**: 859-569.
- Midgley, G.F., Hannah, L., Millar, D., Rutherford, M.C. and Powrie, L.W. 2002. Assessing the vulnerability of species richness to anthropogenic climate change in a biodiversity hotspot. *Global Ecology and Biogeography* **11**: 445-451.
- Molyneux, D.H. 2009. Climate change and vector-borne diseases (VBDs): Redrawing the epidemiology map. *Public Health Journal* **20**: 8-18.
- Mordecai, E.A., Paaijmans, K.P., Johnson, L.R., Balzer, C., Ben-Horin, T., Moor, E., McNally, A., Pawar, S., Ryan, S.J., Smith, T.C. and Lafferty, K.D. 2012. Optimal temperature for malaria transmission is lower than previously predicted. *Ecology Letters*: doi: 10.1111/ele.12015.
- Nchinda, T.C. 1998. Malaria: A re-emerging disease in Africa. *Emerging Infectious Diseases* **4**: 398-404.
- O’Gorman, P.A. and Schneider, T. 2009. The physical basis for increases in precipitation extremes in simulations of 21st-century climate change. *Proceedings of the National Academy of Sciences* **106**: 14773-14777.

- Paaijmans, K.P., Wandago, M.O., Githeko, A.K. and Takken W. 2007. Unexpected high losses of *Anopheles gambiae* larvae due to rainfall. *PLoS ONE* **2**: e1146. doi:10.1371/journal.pone.0001146.
- Paaijmans, K.P., Read, A.F. and Thomas, M.B. 2009. Understanding the link between malaria risk and climate. *Proceedings of the National Academy of Sciences of the U.S.A.* **106**: 13844-13849.
- Paaijmans, K.P. and Thomas, M.B. 2011. The influence of mosquito resting behaviour and associated microclimate for malaria risk. *Malaria Journal* **10**: 183.
- Parnesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D. and Descimon, H. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**: 579–583.
- Parnesan, C. and Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37-42.
- Parnesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* **37**: 637-669.
- Pascual, M., Ahumada, J.A., Chaves, L.F., Rodó, X. and Bouma, M. 2006. Malaria resurgence in the East African highlands: Temperature trends revisited. *Proceedings of the National Academy of Sciences of the U.S.A.* **103**: 5829-5834.
- Peterson, A.T., Papeş, M. and Eaton, M. 2007. Transferability and model evaluation in ecological niche modelling: a comparison of GARP and Maxent. *Ecography* **30**: 550-560.
- Phillips, S.J., Anderson, R.P. and Shapire, R.E. 2006. Maximum entropy modelling of species geographic distributions. *Ecological Modelling* **190**: 231-259.
- Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.R.N., La Marca, E., Masters, K.L., Merino-Vitero, A., Puschendorf, R., Ron, S.R.,

- Sanchez-Azofeifa, A., Still, C.J. and Young, B.E. 2006. Widespread amphibian extinctions from epidemic driven by global warming. *Nature* **439**: 161-167.
- Reiter, P., Thomas, C.J., Atkinson, P.M., Hay, S.I., Randolph, S.E. Rogers, D.J., Shanks, G.D., Snow, R.W. and Spielman, A. 2004. Global warming and malaria: a call for accuracy. *Lancet Infectious Diseases* **4**: 323-324.
- Robinson, M.T. and Hoffmann, A.A. 2001. The pest status and distribution of three cryptic blue oat mite species (*Penthaleus* spp.) and redlegged earth mite (*Halotydeus destructor*) in southeastern Australia. *Experimental and Applied Acarology* **25**: 699-716.
- Rocca, K.A.C., Gray, E.M., Constantini, C. and Besansky, N.J. 2009. 2La chromosomal inversion enhances thermal tolerance of *Anopheles gambiae* larvae. *Malaria Journal* **8**: 147.
- Rogers, D.J. and Randolph, S.E. 2000. The global spread of malaria in a future, warmer world. *Science* **289**: 1763-1766.
- Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenweig, C. and Pounds, J.A. 2003. Fingerprints of global warming on wild animals and plants. *Nature* **421**: 57-60.
- Rosenweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q., Casassa, G., Menzel, A., Root, T.L., Estrella, N., Seguin, B., Tryjanowski, P., Liu, C., Rawlins, S. and Imeson, A. 2008. Attributing physical and biological impacts to anthropogenic climate change. *Nature* **453**: 353-358.
- Sachs, J. and Malaney, P. 2002. The economic and social burden of malaria. *Nature* **415**: 680-685.
- Small, J., Goetz, S.J. and Hay, S.I. 2003. Climatic suitability for malaria transmission in Africa, 1911-1995. *Proceedings of the National Academy of Sciences of the U.S.A.* **100**: 15341-15345.

- Smith, D.L. and McKenzie, F.E. 2004. Statics and dynamics of malaria infection in *Anopheles* mosquitoes. *Malaria Journal* **3**: 13.
- Smith, D.L., Dushoff, J., Snow, R.W. and Hay, S.I. 2005. The entomological inoculation rate and *Plasmodium falciparum* infection in African children. *Nature* **438**: 492-495.
- Smith, D.L., McKenzie, F.E., Snow, R.W. and Hay, S.I. 2007. Revisiting the basic reproductive number for malaria and its implications for malaria control. *PLoS Biology* **5**: e42.
- Smith, D.L., Battle, K.E., Hay, S.I., Barker, C.M., Scott, T.W. and McKenzie, F.E. 2012. Ross, MacDonald and a theory for the dynamics and control of mosquito-transmitted pathogens. *PLoS Pathogens* **8**: e1002588.
- Sutherst, R.W. and Maywald, G.F. 1985. A computerized system for matching climates in ecology. *Agriculture, Ecosystems and Environment* **13**: 281-299.
- Sutherst, R.W., Maywald, G.F. and Bourne, A.S. 2007. Including species interactions in risk assessments for global change. *Global Change Biology* **13**: 1843-1859.
- Tanser, F.C., Sharp, B. and le Sueur, D. 2003. Potential effects of climate change on malaria transmission in Africa. *Lancet* **362**: 1792-1798.
- Terblanche, J.S. and Chown, S.L. 2007. Factory flies are not equal to wild flies. *Science* **317**: 1678.
- Thomas, C.D. and Lennon, J.L. 1999. Birds extend their ranges northwards. *Nature* **399**: 213.
- Thompson, M.C., Connor, S.J., Milligan, P. and Flasse, S.P. 1997. Mapping malaria risk in Africa: What can satellite data contribute? *Parasitology Today* **13**: 313-318.
- Umina, P.A., Weeks, A.R., Kearney, M.R., McKechnie, S.W. and Hoffmann, A.A. 2005. A rapid shift in classic clinal pattern in *Drosophila* reflecting climate change. *Science* **308**: 691-693.

Walther, G-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J-M., Hoegh-Guldberg, O. and Bairlein, F. 2002. Ecological responses to recent climate change. *Nature* **416**: 389-395.

World Health Organization: World Malaria Report 2010. Geneva, Switzerland.
[<http://www.who.int/malaria>].

Chapter 2

Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*



Published as: Lyons, C.L., Coetzee, M., Terblanche, J.S. and Chown, S.L. 2012. Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*.

Malaria Journal **11**: 226.

Introduction

Malaria affects large parts of Africa and Asia and is responsible for nearly 800,000 deaths annually. Despite interventions resulting in a reduction in global malaria mortality in the last 10 years (WHO 2010), much concern still exists that in regions where malaria is either endemic, seasonal or has been present in the recent past, climate change might affect its presence and/or prevalence. Forecasts of the effects of climate change on the disease are controversial. Some sources indicate a possible spread of malaria at its current distribution margins (Martens *et al.* 1997), whilst others suggest that climate change will decrease the disease burden in many parts of its current range (Small *et al.* 2003). In southern Africa, malaria already presents a significant health risk (Da Silva *et al.* 2004), and how climate change will influence malaria incidence in this region (Rogers and Randolph 2000; Tanser *et al.* 2003; van Lieshout 2004), depends on several factors which remain poorly understood. These include the form of the change in climate (New *et al.* 2006), the environmental responses of the vectors (Kearney *et al.* 2009; Kearney and Porter 2009), parasite-host interactions (Paaijmans *et al.* 2009; Pascual *et al.* 2009), and how interventions might interact with these changes (Rogers 2006; Rogers and Randolph 2006).

In southern Africa, *Plasmodium falciparum*, the causative agent of cerebral malaria and the most common of the malarias in Africa, is transmitted by three primary vector species – *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus* (Gillies and Coetzee 1987; Collins and Besansky 1994). Current climate change forecasts for the parts of the region where malaria is endemic suggest an increase in both temperature and rainfall, both of which could increase the numbers of mosquitoes and hence the number of cases of the disease (Boko *et al.* 2007). However, many factors remain to be clarified, including how the vectors will respond to such changed climatic conditions. Information on the response of

vectors in southern Africa to a variety of conditions is necessary to forecast any change in malaria burden due to changing climates.

Understanding the likely future abundance and distribution of free-living organisms (including malaria vectors) usually involves some form of species distribution modelling, either using environmental niche modelling or a more mechanistic approach (Kearney and Porter 2009). Both approaches have been used to estimate the impacts of climate change on mosquito vectors (Martin and Lefebvre 1995; Hay *et al.* 2002; Lindsay and Bayoh 2004), and it has been suggested that a combination of the two can provide the most insight because both the fundamental and realized niches can be estimated (or a sound assessment made of all the factors influencing abundance and distribution) (Kearney and Porter 2009). For mechanistic models, typically a range of basic physiological information is required, such as thermal tolerance limits, desiccation resistance and development rate (Crozier 2004; Kearney *et al.* 2009).

Because many insect species show phenotypic plasticity (Chown and Terblanche 2007), because the sexes often differ in their thermal response (Krebs and Loeschke 1996), and because tolerances may change with age, and age is an important feature of structured population models (Kareiva *et al.* 1990; Bowler and Terblanche 2008), these aspects should ideally be investigated too. A further complication is the fact that for many vectors, populations long-established in the laboratory are used for assessments, but laboratory adaptation might affect the outcome of the assays (Harshman and Hoffmann 2000; Sgró and Partridge 2000; Huho *et al.* 2007). In consequence, these factors must be considered when providing information that can be used for mechanistic niche modelling.

For *Anopheles* mosquitoes, information on physiological tolerances required for such species distribution modelling is largely lacking. Of the three primary southern African malaria vectors, *An. gambiae* has been the most widely studied from this perspective,

followed by *An. arabiensis*, but information on the physiological responses of *An. funestus* is largely absent. Furthermore, the immature forms of *An. arabiensis* and *An. funestus* have rarely been considered (Kirby and Lindsay 2009).

Here, comprehensive assessments of the thermal tolerances of these species, their phenotypic responses to short-term changes in the thermal environment, and an estimate of the extent of laboratory adaptation of these thermal tolerance traits are provided. Additionally, information on the upper and lower lethal temperature limits of the larval and pupal stages of both species is provided. Finally, how climate change might affect vector populations, and hence malaria transmission is briefly considered.

Methods

Laboratory strains

Two long-established laboratory colonies held at the Vector Control Reference Unit in Johannesburg were used for all investigations of thermal tolerance. *Anopheles arabiensis* was taken from the KGB colony, originally established in 1975 from Kanyemba in the Zambezi Valley, Zimbabwe (R.H. Hunt, pers. comm.) and *An. funestus* from the FUMOZ colony established in 2000 from southern Mozambique (Hunt *et al.* 2005). These colonies are maintained at a population size of at least several thousand each, under an insectary temperature of 25°C (\pm 1°C) and 80% relative humidity (verified using repeated measures with a Masons Hygrometer, Brannan, UK) with 12:12 light/dark cycle and 45 min dusk/dawn simulation. Larvae are fed a mixture of ground-up dog biscuits and yeast extracts and females are offered a blood meal three times weekly and allowed to lay eggs two to three times weekly. All adults are provided with a 10% sugar water solution *ad libitum*.

Wild populations

Anopheles arabiensis females were collected from Malahapanga in the Kruger National Park, South Africa (22° 53.23 S, 31° 02.22 E) in October 2010. Wild *An. funestus* females were collected from villages surrounding the Maragra Sugar Estate in southern Mozambique (25° 27.41 S, 32° 46.59 E) in April 2011. Adult anophelines were collected using active-search techniques from inside huts or houses or from indoor animal dwellings using a flashlight and 30 cm glass aspirator. Females were transported back to the laboratory within three days for egg-laying in polystyrene cups with rough surfaces, at a density of 20 females per 250 ml and were provided with a ball of cotton wool moistened with 10% sugar water solution. Egg batches from these females were kept separate until positive species identifications of the wild adults were made using standard PCR methods (Scott *et al.* 1993; Koekemoer *et al.* 2002). The progeny of at least 80 individual females was used to establish a laboratory colony of the wild strains, with the fifth to seventh generations being used in experiments on *An. arabiensis*, and the first generation used in experiments on *An. funestus*. Different generations were used as a result of the inherent difficulties associated with establishing *An. funestus* colonies compared with *An. arabiensis* colonies (R.H. Hunt, pers. comm.). These colonies were kept under the same conditions as the laboratory strains.

Critical thermal limits (CTL)

Three age groups for each of the laboratory strains were used. *An. arabiensis* adults were 10-, 15- and 20-day olds, while *An. funestus* adults were 10-, 20- and 30-day olds. These ages were chosen because of the different lengths of the gonotrophic cycle and different adult longevities of the two species (Hunt *et al.* 2005; Munhenga *et al.* 2011). Only two adult age comparisons for the wild *An. arabiensis* strain (10- and 15-day olds) and wild *An. funestus*

strain (10- and 20-day olds) were possible due to low colony numbers and the requirement to make assessments before 10 generations in the laboratory.

Between 20 and 40 individual males and females from all age groups were exposed to each of three acclimation treatments prior to CT determinations. Adult mosquitoes were acclimated for a period of five to seven days at 20°C, 25°C or 30°C and a RH > 80% at either insectary conditions (25°C) or using PTC-1 Peltier portable temperature control cabinets (Sable Systems, Las Vegas, Nevada, USA, 20 ± 1°C and 30 ± 1°C). Humidity in the insectary was checked using a Masons Thermohygrometer (Brannan, UK). At 20°C and 30°C, humidity was maintained through the use of distilled water (checked using a Hygrochron i-button, DS 1923-F5, Maxim/Dallas Semiconductor, Sunnyvale, CA, USA). Each acclimation treatment was maintained on a 12L:12D cycle for the five or seven day period. Most insect species show acclimation responses in less than seven days (Weldon *et al.* 2011). Following these acclimation treatments, ten individuals (comprising five individuals of each sex) per individual trial were placed into a double-jacketed insulated chamber connected to a programmable water bath (Grant LTC-12 Series, Grant Instruments, Ltd., Cambridge, UK). For each age group and acclimation treatment of each species, a total of four replicate trials were completed. CT_{min} experiments started at 20°C while CT_{max} started at 25°C, decreasing or increasing at a rate of 0.25°C/min, respectively after an equilibration period of 10 min. While it has been shown that rate of temperature change can significantly alter the upper thermal tolerances of various insect species, the current rate was chosen as one comparable with many other studies (Chown *et al.* 2009). The CT_{min} was regarded as the point where individuals displayed reduced motor function (i.e. onset of spasms) and could not cling to the tip of a paint brush, while CT_{max} was regarded as the point where individuals displayed reduced motor function following a period of rapid flight (Lutterschmidt and Hutchison 1997). At no point were individuals removed from the trial chambers for

assessments of motor function (i.e. individuals were continuously subjected to the thermal assay).

Lethal temperature limits

Lethal temperature (LT) determinations of larvae and pupae, most appropriate for less mobile stages (Chown and Nicolson 2004), for both species were carried out on six groups of ten individuals each, per life stage (n=60 per exposure temperature). The plunging technique was used instead of a ramping protocol (Chown and Nicolson 2004; Mitchell and Hoffmann 2010). Each replicate (i.e. group of ten individuals) was exposed for a period of two hours to temperatures ranging from -12°C to 8°C for LLT (lower lethal temperature) and from 34°C to 44°C for ULT (upper lethal temperature) in 2°C increments to ensure that 0% and 100% survival of test individuals was recorded. A water temperature of $24 \pm 0.5^{\circ}\text{C}$ was used as a control and survival at this temperature was 100%. Temperatures were maintained through the use of programmable water baths (Grant LTD-20 and GR150 R4 Series, Grant Instruments, Ltd., Cambridge, UK). Following the two-hour exposure, experimental groups were returned to water at 24°C ($\pm 1^{\circ}\text{C}$) and survival was scored every 24 hours until either eclosion to adulthood or complete mortality occurred. Percentage survival was then scored as the percentage of the ten individuals that eclosed. Larvae were fed daily on the same larval food as the colony strains.

Adult lethal temperature experiments were performed on five groups of ten individual males and females each (n=50 individuals per sex, per temperature), acclimated at only one temperature (25°C , RH 80%). The three age groups (*An. arabiensis*: 10-, 15- and 20-days; *An. funestus* 10-, 20- and 30-days), were used in the upper lethal temperature and the LLT experiments, with the exception of the LLT determinations for *An. arabiensis* adults where only two age groups (10- and 15-day olds) were used due to unexpected mortality in the

colony. Each replicate (i.e. group of ten individuals) was exposed to a given temperature in the range -6°C to 16°C for LLT determinations and 24°C to 38°C for ULT determinations, for a period of four hours to ensure that 0% and 100% survival temperature was measured for both LLT and ULT. This four-hour temperature exposure was chosen as an estimate of the length of time of the hottest period in the day, to which mosquitoes would be exposed, based on generalized daily temperature profile data which show that for many regions, including those of tropical Africa, high daytime temperatures are maintained for approximately four hours (Kingsolver 1979; Bonan 2002). Experiments were conducted in a SANYO incubator (MIR-154, SANYO Electric Co. Ltd., Osaka, Japan). A temperature of $25 \pm 1^{\circ}\text{C}$ was chosen as a control and survival at this temperature was close to 100%. Adults were immediately removed from the exposure temperature following the four-hour period, given sugar water and left to recover at 25°C ($\pm 1^{\circ}\text{C}$) and relative humidity of 80%. Survival was scored as the percentage of the ten adults still living, 24 hours after the experiment concluded.

Data analysis

Normality and homogeneity of variances were examined using Shapiro-Wilk's tests and Levene's tests, respectively (Statistica v. 11, StatSoft, Tulsa, Oklahoma, USA). Some deviations from normality were observed, but the model assumptions were generally met (supplementary materials, Table S1) and the sample sizes sufficiently large to allow for the use of parametric general linear models (GLM) (Quinn and Keough 2002), as implemented in R (v. 2.13.1) (R Foundation for Statistical Computing, Vienna, Austria). The first model examined the effects of age, acclimation, sex and strain on the variables CT_{min} and CT_{max} for each species. Because significant effects of strain or an interaction with strain were found for CT_{min}/max, models were then run separately for each strain incorporating age, sex and acclimation as predictor variables. As an estimate of effect size, the mean percent deviation in

CTmin/max from the grand mean per group was calculated by subtracting from each factor mean, the grand mean, and dividing this by the grand mean, multiplied by 100 to obtain a percentage (Table S2). The sign of this % deviation from the mean provides an indication of whether or not each factor had on average a lower (negative) or higher (positive) CTmin/max than the grand mean.

The mean (\pm S.E.) lethal temperature at which 50% of the sample population died (LT₅₀) for each species and life stage, in relation to age and sex (for adults) was determined through the use of logistic regression with binomial distributions (logit link) in R (v.2.13.1). Using Hochberg's GT-2 method as described in (Sokal and Rohlf 1995), lower and upper 95% confidence limits for each group were calculated using the means and standard errors obtained from logistic regression analyses. Mean LT₅₀ (\pm 95% C.I.) for each group were plotted. Overlapping confidence intervals indicate no significant difference between groups.

Results

Critical thermal limits of Anopheles funestus

No significant differences in CTmax were found between the wild and laboratory strains of *An. funestus* mosquitoes, and the interactions involving strain were generally not significant, except in a single case (Table 1, Figure 1). Only acclimation affected CTmax values significantly in both strains (Table 1), although the effect size was typically $\leq 2^{\circ}\text{C}$, with the significant three-way interaction between strain, age and acclimation not being clearly interpretable (Figure 1). However, it is clear that the overall acclimation response is less in the laboratory than wild strain in both males and females, explaining the significant two-way interaction between strain and acclimation. Clearly, some difference in the effects of acclimation, sex and age exists among strains and therefore the models were run separately for each strain (Table 2). Acclimation and age have much greater effects on CTmax in the

wild than in the laboratory strains, with some differences in the interactions too. However, the total variation in CT_{max} was *c.* 3°C (Figure 1, Table S2). Generally, higher acclimation treatments resulted in higher CT_{max}, and younger adults and females tended to have higher CT_{max} values (Figures 1 and 2). CT_{min} values differed between the *An. funestus* strains, which also showed significant differences in response to acclimation (Table 1). The strongest acclimation response was found in the colony strain and specifically in 10-day old males and females, whereas by comparison differences among other ages and among genders and acclimation treatments at other ages were much reduced (Figure 1). Maximum effect size of *c.* 4°C was found following different acclimation treatments in 10-day old colony females (Figure 1, Table S2). When the models were run independently for the two strains it became clear that in each strain, sex, age and acclimation temperature had significant effects, but in somewhat different ways among strains, with the effects tending to be most pronounced in the laboratory strains (Table 3). Across the full set of treatments, the maximum difference in CT_{min} was *c.* 6°C (Figure 1).

Critical thermal limits of Anopheles arabiensis

In *An. arabiensis*, with the exception of two-way interactions between sex and age, and sex and acclimation treatment, as well as several three-way interactions between strain, sex, age and acclimation treatment, no other effects on CT_{max} were significant, and especially not the main effects in the model (Table 4). It does appear that females have higher CT_{max} values than males (Figure 2), but these effects were not readily distinguished in the full model. When the models were implemented separately for each strain, the sex effect was significant (Table 5, Figure 2), as was the effect of acclimation for the wild strain, largely reflecting the large effect of the 30°C acclimation treatment on 15-day old males (Figure 3). By contrast,

age and various interactions did not have significant effects on the laboratory strain. The overall range of CT_{max} values was *c.* 3°C (Figure 3).

CT_{min} responded strongly to acclimation treatments, and age, sex and strain were also all significant in *An. arabiensis* (Table 4). The wild strain tended to have lower CT_{min} values than the laboratory strain, while 10-day old females in the laboratory colony showed the strongest response to acclimation (Table 6, Figure 3), just as was the case in *An. funestus*. In the wild strain, females tended to have a lower CT_{min} than males (Figure 2), and acclimation had a strong, generally linear effect on CT_{min} (Table 6, Figure 3). However, in the laboratory strain, although all of the main effects and interactions were significant (Table 6), the responses were non-linear among acclimation treatments, and the variation among age groups at a given acclimation $\leq 1.5^\circ\text{C}$ (Figure 3). Overall, among strains, ages, sexes and acclimation treatments the variation in CT_{min} was *c.* 5°C (Figure 3, Table S2).

Lethal temperature limits

Lower lethal temperature (LLT) in *An. funestus* was approximately -1°C to -2°C for all stages and age groups examined, with the exception of the larvae (mean \pm 95% C.I., $1.94^\circ\text{C} \pm 0.62^\circ\text{C}$), and 30-day old adult males (mean \pm 95% C.I., $0.68^\circ\text{C} \pm 0.83^\circ\text{C}$), which were less tolerant of low temperature (Figure 4). In *An. arabiensis*, the situation was similar, with larvae likewise showing the least tolerance of low temperatures (mean \pm 95% C.I., $1.59^\circ\text{C} \pm 0.71^\circ\text{C}$), and adult males being the least resistant of all groups (10-day old males mean \pm 95% C.I., $3.66^\circ\text{C} \pm 0.98^\circ\text{C}$; 15-day old males mean \pm 95% C.I., $3.48^\circ\text{C} \pm 0.83^\circ\text{C}$). Lower lethal limits in the adults were generally 8-11°C less than the CT_{min}. The full range of LLT values spanned *c.* 6°C (Figure 4).

Upper lethal temperatures (ULT) across the full range of stages, ages and species varied by *c.* 11°C. In both species, larvae and pupae had the highest ULT, with *An. arabiensis*

having more tolerant immature stages than *An. funestus* (Figure 5). Females of both species tended to have higher ULT than males, with the most heat sensitive group being the males of *An. arabiensis*. The lethal temperature estimates were typically 8-10°C lower than the CT_{max} estimates, indicating a much reduced scope for long-term tolerance of high temperature in the adults.

Discussion

Laboratory colonies are used for a wide range of investigations of insect responses to changing environmental conditions. These include investigations of the responses of mosquitoes to various thermal conditions (e.g. Bayoh and Lindsay 2003), and to pathogens and insecticides (Blanford *et al.* 2009). However, as has now been demonstrated in a range of arthropod taxa, laboratory adaptation and acclimation can take place rapidly, affecting some traits, but not others and affecting sexes differentially (Harshman and Hoffmann 2000; Sgró and Partridge 2000; Huho *et al.* 2007). In consequence, extrapolations to the field situation, such as is required for mechanistic niche modelling or assessments of the outcomes of control interventions, may be compromised, making estimations of the extent of differences among laboratory and field strains essential.

The current results indicate that differences in mean CT_{min} or CT_{max} among the wild and laboratory strains of *An. arabiensis* and *An. funestus* typically did not exceed 2°C. In most instances differences between strains were approximately 1°C. The 2°C difference among wild and laboratory strains was observed for CT_{min} in the longest-lived colony (35 years) of *An. arabiensis* and might indicate a loss of thermal tolerance after extensive exposure to constant laboratory colony conditions. Differences in the acclimation responses between the wild and laboratory strains were also evident. However, the range of acclimation responses over all treatments was similar for both strains except in younger females of the laboratory colonies. Thus, results suggest that although caution is required when

extrapolating laboratory thermal tolerance data to the field, as is recommended for other aspects of malaria biology (e.g. Aguilar *et al.* 2005), at least for the species examined here, using thermal tolerance data from laboratory colonies may provide a reasonable approximation of expected responses in the field (but see also Huho *et al.* 2007) although further investigations between field and laboratory strains should be undertaken (e.g. see Griffiths *et al.* 2005).

Other biologically significant sources of variation in thermal tolerance limits, especially in the context of understanding and forecasting responses to environmental change, are those associated with age, sex and short-term responses to change (phenotypic plasticity) (Hoffmann *et al.* 2003; Chown and Terblanche 2007; Bowler and Terblanche 2008). Several recent studies have shown that upper lethal limits or limits to activity in insects and other ectotherms are typically much less variable, both among populations and species, and over time (through plasticity or responses to selection) than are lower limits (Chown 2001; Griffiths *et al.* 2005; Sunday *et al.* 2011). The same pattern was found here for the adults and in addition the extent of variation amongst the age groups in CTmax and ULT tended to be fairly narrow. Thus, whilst increasing temperatures may benefit the species in cooler areas (contributing perhaps to rising malaria incidence as is the case in East Africa (e.g. Alonso *et al.* 2011), where they are close to their thermal limits rising temperatures may act to suppress populations. Indeed, constrained upper thermal limits may be the mechanistic basis, together with the thermal sensitivity of immature development (see Bayoh and Lindsay 2003; unpublished data), for the forecast range declines of *An. gambiae* and *An. arabiensis* in northern and west Africa and increases in south-eastern Africa (Peterson 2009). In this respect, males might be more sensitive than females given the 1-2°C difference in CTmax, which may well be associated with the blood-feeding habits of females (Benoit *et al.* 2011).

Transmission of malaria is dependent on the effects of ambient temperature on the *Plasmodium* parasite, and on the effect of ambient temperature on the vector species. Lower limits to *Plasmodium* development are *c.* 16°C. However, although parasite development rate increases with increasing temperatures, temperatures above *c.* 30°C are detrimental to parasite development and could therefore, have consequences for transmission (Paaijmans *et al.* 2009). Transmission of malaria is also dependent on the ability of adults to withstand high temperatures (Craig *et al.* 1999) and the greater sensitivity of older mosquitoes to high temperatures, as found here in most cases, may cause female death before the parasite migrates to the salivary glands and can be transmitted. The complexity of this interaction between the sensitivity to temperature of malaria parasites and their vectors has been noted previously (Paaijmans *et al.* 2009). In consequence, rising temperatures may not only reduce mosquito population densities, but also the extent to which the malaria parasite is transmitted. The dynamics of this interaction are likely to be complicated by the habits of the vector (indoor or outdoor species), temperature variability, and the nature of the host-parasite interaction (Pascual *et al.* 2009; Alonso *et al.* 2011). Nonetheless, the finding that temperature sensitivity, at least for critical limits, increases with age is in keeping with other studies of thermal responses in insects (Bowler and Terblanche 2008). For mosquitoes, mortality is highly age- and infection-dependent (Dawes *et al.* 2009). Even in the absence of *Plasmodium* ookinetes, mortality of females increases with an increase in age, suggesting the potential for female anophelines to senesce (Dawes *et al.* 2009). As a confounding factor to malaria transmission, the presence of large numbers of parasite ookinetes in the mosquito midgut greatly increases the mortality experienced within a population and reduces overall mosquito longevity (Dawes *et al.* 2009) adding to the potential for reduced overall mosquito populations, and hence, the potential for reduced malaria transmission, with increasing environmental temperatures as a consequence of climate change.

Variation found among the lethal and critical thermal limits for the two anopheline species is typical of that found in a range of other taxa (Hoffmann *et al.* 2003; Terblanche *et al.* 2011). Activity tends to cease well before the lower lethal limit in adults, whilst the upper lethal limits tend to be somewhat lower than the short-term tolerances represented by CT_{max}. The latter may in part be explained by the differences between the two techniques used to measure these variables and the rate at which temperature was changed during the ramping method used for CT_{max} estimation. Slower rates often, though not always, result in lower CT_{max} values (Chown *et al.* 2009; Allen *et al.* 2012). Nonetheless, these thermal traits might also be under different genetic control (Hoffmann 2010). Irrespective, it is clear that the most pronounced differences in ULT were found among the stages, with the immatures having ULTs 2-10°C higher than those of the adults. Such among-stage variation is common in other insects and usually reflects their exposure to different conditions (Bowler and Terblanche 2008). For *An. arabiensis* and *An. funestus*, as with many other species where the adults are more mobile than the immatures, greater tolerance to high temperatures can be expected in the immature stages. Behavioural regulation is more straightforward for a highly mobile individual living in air than for a much less active individual living in a thermally conductive medium such as water (see also Huey 1991). In particular, the adults of both species are highly anthropophilic, with *An. arabiensis* displaying more exophilic behaviour than *An. funestus* (Gillies and Coetzee 1987; Coetzee *et al.* 2000). This behaviour of the adults, combined with their mobility, means that they are able to escape unfavourable temperatures and make use of indoor-resting behaviour during the hottest or coldest parts of the day (Paaijmans and Thomas 2011). However, behavioural avoidance of temperature extremes is likely limited for larvae of *An. funestus* and is probably largely absent for larval *An. arabiensis*, owing to their breeding habits (see Table 7).

In absolute terms, the larvae of both *An. arabiensis* (ULT₅₀ c. 41°C) and *An. funestus* (ULT₅₀ c. 38°C) were able to survive higher temperatures (to eclosion) than are those of *An. gambiae* s.s. (ULT₅₀ c. 32°C) (Bayoh and Lindsay 2004), although generally the lethal limits were within the range found for anophelines (Muirhead-Thomson 1938; Love and Whelchel 1957; Benedict *et al.* 1991; Raghavendra *et al.* 2010). *Anopheles arabiensis* breeds in shallow, temporary pools or puddles, while *An. funestus* prefers to breed in semi-permanent to permanent water bodies (Gillies and Coetzee 1987) (Table 7). The smaller water bodies are likely to show much greater thermal variation than the latter simply on the grounds of volume alone, and are also likely to offer less opportunity for microhabitat selection. Thus, the high upper thermal tolerances of *An. arabiensis* in the immature stages are not unexpected. Nonetheless, how the lethal limits determined here relate to thermal limits to development over the entire immature stage, given that the latter are typically narrower than the former (Hoffmann 2010) needs to be explored, especially in determining the environmental limits to distribution both at the upper and lower temperature extremes. Interactions between changing climates and lower development limits may account for forecasts of expansion of *An. arabiensis* into cooler areas as climates warm (Peterson 2009), given that such interactions can reasonably account for current coarse-scale distributions of *An. gambiae* s.s. (Bayoh and Lindsay 2003). Furthermore, interactions between climate and upper development limits may change the seasonality of occurrence or lead to range limitation, depending on interactions with rainfall (see e.g. Paaijmans *et al.* 2007). Investigations of the relationship between lethal and development limits for both *An. arabiensis* and *An. funestus* are currently underway (unpublished data), and should provide insights into changing distribution patterns and the extent to which they match those forecast on the basis of environmental niche modelling alone (see Kearney *et al.* 2009).

This study has shown that with the necessary caution, laboratory colonies provide an initial basis for investigating physiological tolerances of *An. arabiensis* and *An. funestus* to both high and low temperatures. In addition, it suggests that limited variation in upper thermal limits may well account for forecasts of declining distributions in already warm areas as temperatures rise, whilst sensitivity of development may be more significant a limiting factor in cool areas, given low, lower lethal limits. Finally, this study has demonstrated substantial physiological differences in tolerance between two of the main malaria vectors in southern Africa, which will have to be taken into account when forecasting responses to environmental change of all kinds, including the ways in which water bodies are manipulated to account for expected changes in rainfall regimes.

References

- Aguilar, R., Dong, Y., Warr, E. and Dimopoulos, G. 2005. *Anopheles* infection responses; laboratory models versus field malaria transmission systems. *Acta Tropica* **95**: 285-291.
- Allen, J.L., Clusella-Trullas, S. and 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). *Journal of Insect Physiology* **58**: 669-678.
- Alonso, D., Bouma, M.J. and Pascual, M. 2011. Epidemic malaria and warmer temperatures in recent decades in an East African highland. *Proceedings of the Royal Society B* **278**: 1661-1669.
- Bayoh, M.N. and Lindsay, S.W. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Bulletin of Entomological Research* **93**: 375-381.
- Bayoh, M.N. and Lindsay, S.W. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector *Anopheles gambiae* in the laboratory. *Medical and Veterinary Entomology* **18**: 174-179.

- Benedict, M.Q., Cockburn, A.F. and Seawright, J.A. 1991. Heat-shock mortality and induced thermotolerance in larvae of the mosquito *Anopheles albimanus*. *Journal of the American Mosquito Control Association* **7**: 547-550.
- Benoit, J.B., Lopez-Martinez, G., Patrick, K.R., Phillips, Z.P., Krause, T.B. and Denlinger, D.L. 2011. Drinking a hot blood meal elicits a protective heat shock response in mosquitoes. *Proceedings of the National Academy of Sciences of the U.S.A.* **108**: 8026-8029.
- Blanford, S., Read, A.F. and Thomas, M.B. 2009. Thermal behaviour of *Anopheles stephensi* in response to infection with malaria and fungal entomopathogens. *Malaria Journal* **8**: 72.
- Boko, M., Niang, I., Nyong, A., Vogel, C., Githeko, A., Medany, M., Osman-Elasha, B., Tabo, R. and Yanda, P. 2007. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Edited by Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. Cambridge: Cambridge University Press, UK: 433-467.
- Bonan, G.B. 2002. *Ecological Climatology: Concepts and Applications*. Cambridge: Cambridge University Press, UK.
- Bowler, K. and Terblanche, J.S. 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biological Reviews* **83**: 339-355.
- Chown, S.L. 2001. Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* **47**: 649-660.
- Chown, S.L. and Nicolson, S.W. 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford: Oxford University Press, UK.

- Chown, S.L. and Terblanche, J.S. 2007. Physiological diversity in insects: Ecological and evolutionary contexts. *Advances in Insect Physiology* **33**: 50-152.
- Chown, S.L., Jumbam, K.R., Sørensen, J.G. and Terblanche, J.S. 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology* **23**: 133-140.
- Coetzee, M., Craig, M. and le Sueur, D. 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today* **16**: 74-77.
- Collins, F.H. and Besansky, N.J. 1994. Vector biology and the control of malaria in Africa. *Science* **264**: 1874-1875.
- Craig, M.H., Snow, R.W. and le Sueur, D. 1999. A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitology Today* **15**: 105-111.
- Crozier, L. 2004. Warmer winters drive butterfly range expansion by increasing survivorship. *Ecology* **85**: 231-241.
- Da Silva, J., Garanganga, B., Teveredzi, V., Marx, S.M., Mason, S.J. and Connor, S.J. 2004. Improving epidemic malaria planning, preparedness and response in Southern Africa. *Malaria Journal* **3**: 37.
- Dawes, E.J., Churcher, T.S., Zhuang, S., Sinden, R.E. and Basáñez, M-G. 2009. *Anopheles* mortality is both age- and *Plasmodium*-density dependent: implications for malaria transmission. *Malaria Journal* **8**: 228.
- Gillies, M.T. and Coetzee, M. 1987. *A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region)*. Publications of the South African Institute of Medical Research, Johannesburg, South Africa.
- Griffiths, J.A., Schiffer, M. and Hoffmann, A.A. 2005. Clinal variation and laboratory adaptation in the rainforest species *Drosophila birchii* for stress resistance, wing size, wing shape and development time. *Journal of Evolutionary Biology* **18**: 213-222.

- Harshman, L.G. and Hoffmann, A.A. 2000. Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends in Ecology and Evolution* **15**: 32-36.
- Hay, S.I., Rogers, D.J., Randolph, S.E., Stern, D.I., Cox, J., Shanks, G.D. and Snow, R.W. 2002. Hot topic or hot air? Climate change and malaria resurgence in East African highlands. *Trends in Parasitology* **18**: 530-534.
- Hoffmann, A.A., Sørensen, J.G. and Loeschcke, V. 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* **28**: 175-216.
- Hoffmann, A.A. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. *Journal of Experimental Biology* **213**: 870-880.
- Huey, R.B. 1991. Physiological consequences of habitat selection. *American Naturalist* **137**: S91-S115.
- Huho, B.J., Ng'habi, K.R., Killeen, G.F., Nkwengulila, G., Knols, B.G.J. and Ferguson, H.M. 2007. Nature beats nurture: a case study of the physiological fitness of free-living and laboratory-reared male *Anopheles gambiae s.l.*. *Journal of Experimental Biology* **210**: 2939-2947.
- Hunt, R.H., Brooke, B.D., Pillay, C., Koekemoer, L.L. and Coetzee, M. 2005. Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector *Anopheles funestus*. *Medical and Veterinary Entomology* **19**: 271-275.
- Kareiva, P., Mullen, A. and Southwood, R. 1990. Population dynamics in spatially complex environments: theory and data [and discussion]. *Philosophical Transactions of the Royal Society B* **330**: 175-190.
- Kearney, M., Porter, W.P., Williams, C., Ritchie, S. and Hoffmann, A.A. 2009. Integrating biophysical models and evolutionary theory to predict climatic impacts on species'

- ranges: the dengue mosquito *Aedes aegypti* in Australia. *Functional Ecology* **23**: 528-538.
- Kearney, M. and Porter, W. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters* **12**: 334-350.
- Kingsolver, J.G. 1979. Thermal and hydric aspects of environmental heterogeneity in the pitcher plant mosquito. *Ecological Monographs* **49**: 357-376.
- Kirby, M.J. and Lindsay, S.W. 2009. Effect of temperature and inter-specific competition on the development and survival of *Anopheles gambiae sensu stricto* and *An. arabiensis* larvae. *Acta Tropica* **109**: 118-123.
- Koekemoer, L.L., Kamau, L., Hunt, R.H. and Coetzee, M. 2002. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *American Journal of Tropical Medicine and Hygiene* **6**: 804-811.
- Krebs, R.A. and Loeschcke, V. 1996. Acclimation and selection for increased resistance to thermal stress in *Drosophila buzzatii*. *Genetics* **142**: 471-479.
- Lindsay, S.W. and Bayoh, M.N. 2004. Mapping members of the *Anopheles gambiae* complex using climate data. *Physiological Entomology* **29**: 204-209.
- Love, G.J. and Whelchel, J.G. 1957. Lethal effects of high temperatures on the immature stages of *Anopheles quadrimaculatus*. *Ecology* **38**: 570-576.
- Lutterschmidt, W.I. and Hutchison, V.H. 1997. The critical thermal maximum: data to support the onset of spasms as the definitive endpoint. *Canadian Journal of Zoology* **75**: 1553-1560.
- Martens, W.J.M., Jetten, T.H. and Focks, D.A. 1997. Sensitivity of malaria, schistosomiasis and dengue to global warming. *Climatic Change* **35**: 145-156.
- Martin, P.H. and Lefebvre, M.G. 1995. Malaria and climate: sensitivity of malaria potential transmission to climate. *Ambio* **24**: 200-207.

- Mitchell, K.A. and Hoffmann, A.A. 2010. Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. *Functional Ecology* **24**: 694-700.
- Muirhead-Thomson, R.C. 1938. The reactions of mosquitoes to temperature and humidity. *Bulletin of Entomological Research* **29**: 125-140.
- Munhenga, G., Brooke, B.D., Chirwa, T.F., Hunt, R.H., Coetzee, M., Govender, D. and Koekemoer, L.L. 2011. Evaluating the potential of the sterile insect technique for malaria control: relative fitness and mating compatibility between laboratory colonized and a wild population of *Anopheles arabiensis* from the Kruger National Park, South Africa. *Parasites and Vectors* **4**: 208.
- New, M., Hewitson, B., Stephenson, D.B., Tsiga, A., Kruger, A., Manhique, A., Gomez, B., Coelho, C.A.S., Masisi, D.N., Kululanga, E., Mbambalala, E., Adesina, F., Saleh, H., Kanyanga, J., Adosi, J., Bulane, L., Fortunata, L., Mdoka, M.L. and Lajoie, R. 2006. Evidence of trends in daily climate extremes over southern and west Africa. *Journal of Geophysical Research* **111**: D14102. doi:10.1029/2005JD006289.
- Paaijmans, K.P., Wandago, M.O., Githeko, A.K. and Takken W. 2007. Unexpected high losses of *Anopheles gambiae* larvae due to rainfall. *PLoS ONE* **2**: e1146. doi:10.1371/journal.pone.0001146.
- Paaijmans, K.P., Read, A.F. and Thomas, M.B. 2009. Understanding the link between malaria risk and climate. *Proceedings of the National Academy of Sciences of the U.S.A.* **106**: 13844-13849.
- Paaijmans, K.P., Imbahale, S.S., Thomas, M.B. and Takken, W. 2010. Relevant microclimate for determining the development rate of malaria mosquitoes and possible implications of climate change. *Malaria Journal* **9**: 196.

- Paaijmans, K.P. and Thomas, M.B. 2011. The influence of mosquito resting behaviour and associated microclimate for malaria risk. *Malaria Journal* **10**: 183.
- Pascual, M., Dobson, A.P. and Bouma, M.J. 2009. Underestimating malaria risk under variable temperatures. *Proceedings of the National Academy of Sciences USA* **106**: 13645-13646.
- Peterson, A.T. 2009. Shifting suitability for malaria vectors across Africa with warming climates. *BMC Infectious Diseases* **9**: 1-6.
- Quinn, G.P. and Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press, UK.
- Raghavendra, K., Barik, T.K., and Adak, T. 2010. Development of larval thermotolerance and its impact on adult susceptibility to malathion insecticide and *Plasmodium vivax* infection in *Anopheles stephensi*. *Parasitology Research* **107**: 1291-1297.
- Rogers, D.J. 2006. Models for vectors and vector-borne diseases. *Advances in Parasitology* **62**: 1-35.
- Rogers, D.J. and Randolph, S.E. 2000. The global spread of malaria in a future, warmer world. *Science* **289**: 1763-1766.
- Rogers, D.J. and Randolph, S.E. 2006. Climate change and vector-borne disease. *Advances in Parasitology* **62**: 345-381.
- Scott, J.A., Brogdon, W.G. and Collins, F.H. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* **49**: 520-529.
- Sgró, C.M. and Partridge, L. 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *American Naturalist* **156**: 341-353.

- Small, J., Goetz, S.J. and Hay, S.I. 2003. Climatic suitability for malaria transmission in Africa, 1911-1995. *Proceedings of the National Academy of Sciences of the U.S.A.* **100**: 15341-15345.
- Sokal, R.R. and Rohlf, F.J. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Company, New York, USA.
- Sunday, J.M., Bates, A.E. and Dulvy, N.K. 2011. Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B* **278**: 1823-1830.
- Tanser, F.C., Sharp, B. and le Sueur, D. 2003. Potential effect of climate change on malaria transmission in Africa. *Lancet* **362**: 1792-1798.
- Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C. and Chown, S.L. 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. *Journal of Experimental Biology* **214**: 3713-3725.
- van Lieshout, M., Kovats, R.S., Livermore, M.T.J. and Martens, P. 2004. Climate change and malaria: analysis of the SRES climate and socio-economic scenarios. *Global Environmental Change* **14**: 87-99.
- Weldon, C.W., Terblanche, J.S. and Chown, S.L. 2011. Time-course for attainment and reversal of acclimation to constant temperature in two *Ceratitis* species. *Journal of Thermal Biology* **36**: 479-485.
- World Health Organization: World Malaria Report 2010. Geneva, Switzerland. [<http://www.who.int/malaria>].

Figure Legends

Figure 1. The effects of age, sex and acclimation temperature on CTmax (a, b) and CTmin (c, d) in laboratory (a, c) and wild (b, d) strains of adult *Anopheles funestus*.

Figure 2. Sex differences in CTmin (left) and CTmax (right) between the wild and laboratory strains of adult *Anopheles funestus* (top) and *An. arabiensis* (bottom).

Figure 3. The effects of age, sex and acclimation temperature on CTmax (a, b) and CTmin (c, d) in laboratory (a, c) and wild (b, d) strains of adult *Anopheles arabiensis*.

Figure 4. Lower lethal temperatures for 50% of the sample population ($LLT_{50} \pm 95\%$ confidence intervals for *Anopheles funestus* (left of the dashed line) and *An. arabiensis* (right of the dashed line) larval, pupal and adult stages. LLT_{50} data for adults include the influence of sex and age for both species. Differences in lower case letters indicate significant differences between groups, within and amongst species, while numbers below each line indicate sample size. Adults were exposed to temperature treatments for a period of four hours and larvae and pupae, for a period of two hours.

Figure 5. Upper lethal temperature for 50% of the sample population ($ULT_{50} \pm 95\%$ confidence intervals for *Anopheles funestus* (left of the dashed line) and *An. arabiensis* (right of the dashed line) larval, pupal and adult stages. ULT_{50} data for adults include the influence of sex and age for both species. Differences in lower case letters indicate significant differences between groups, within and amongst species, while numbers below each line

indicate sample size. Adults were exposed to temperature treatments for a period of four hours and larvae and pupae, for a period of two hours.

Figures and Tables

Fig. 1

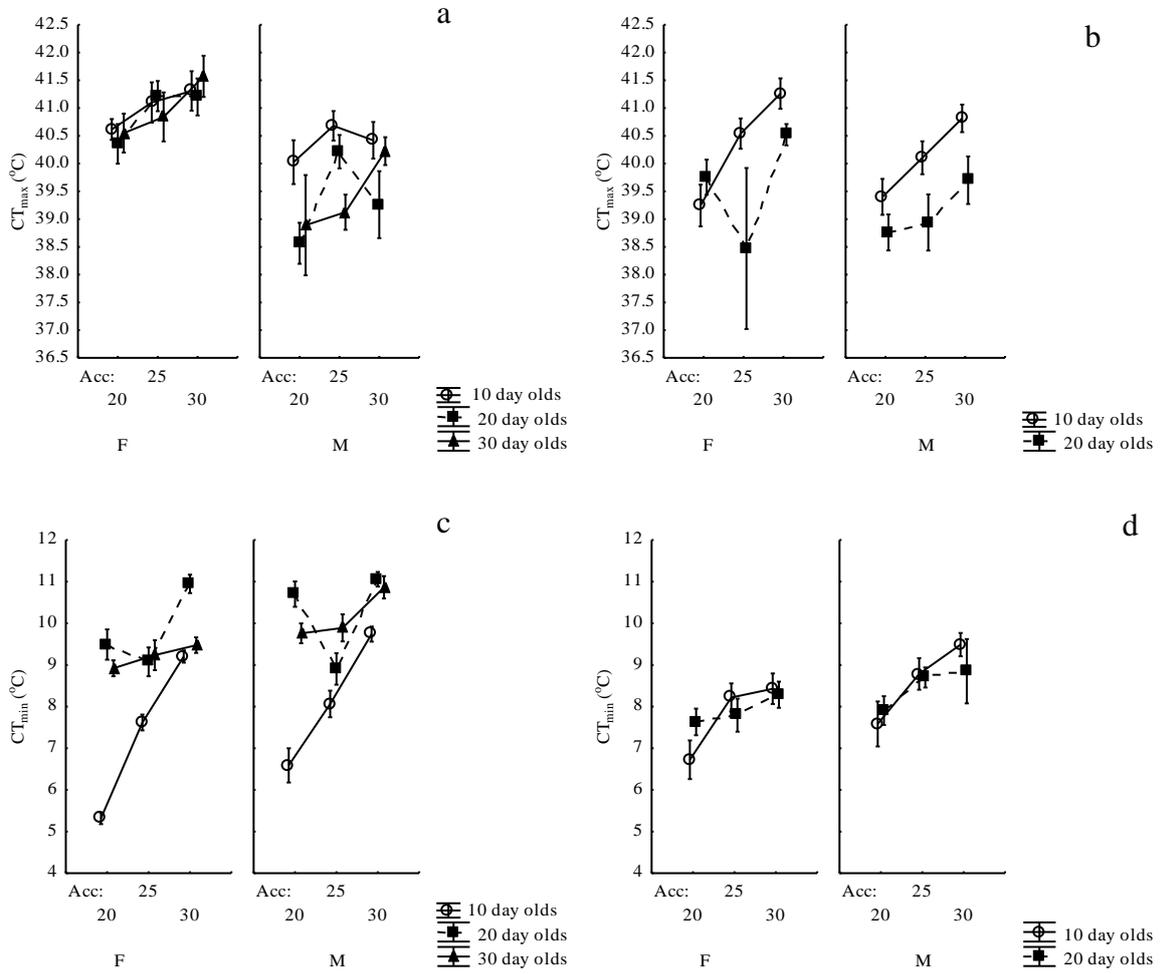


Fig. 2

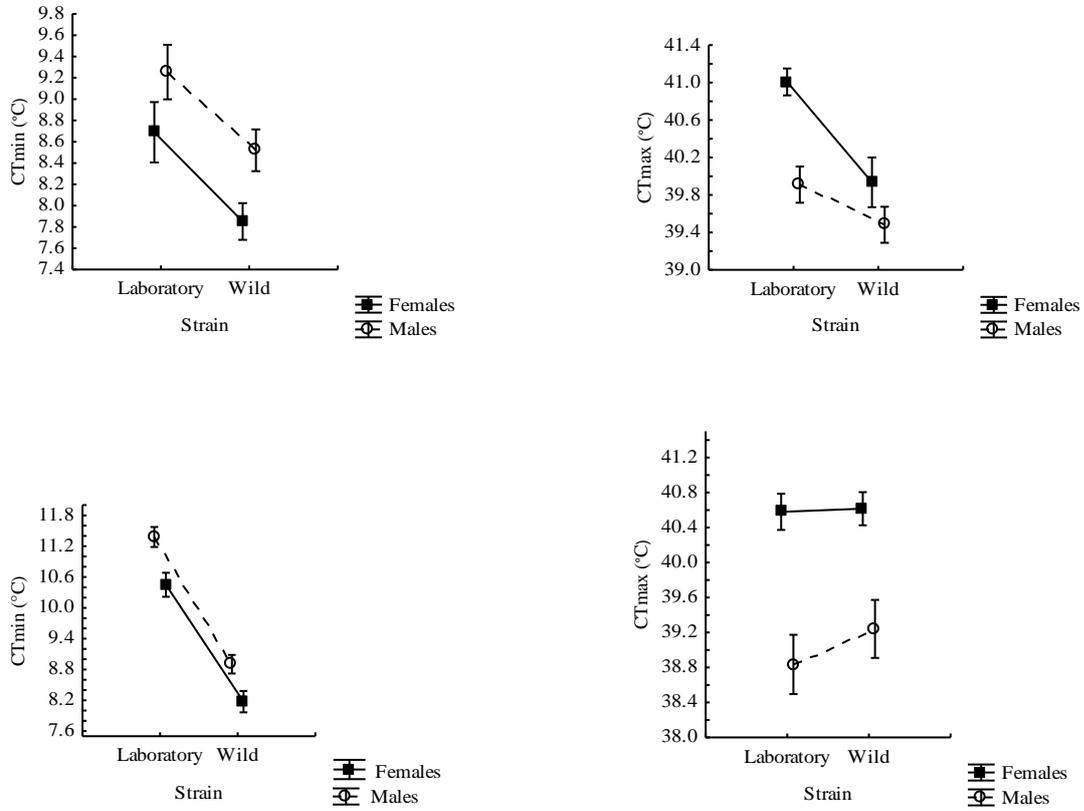


Fig. 3

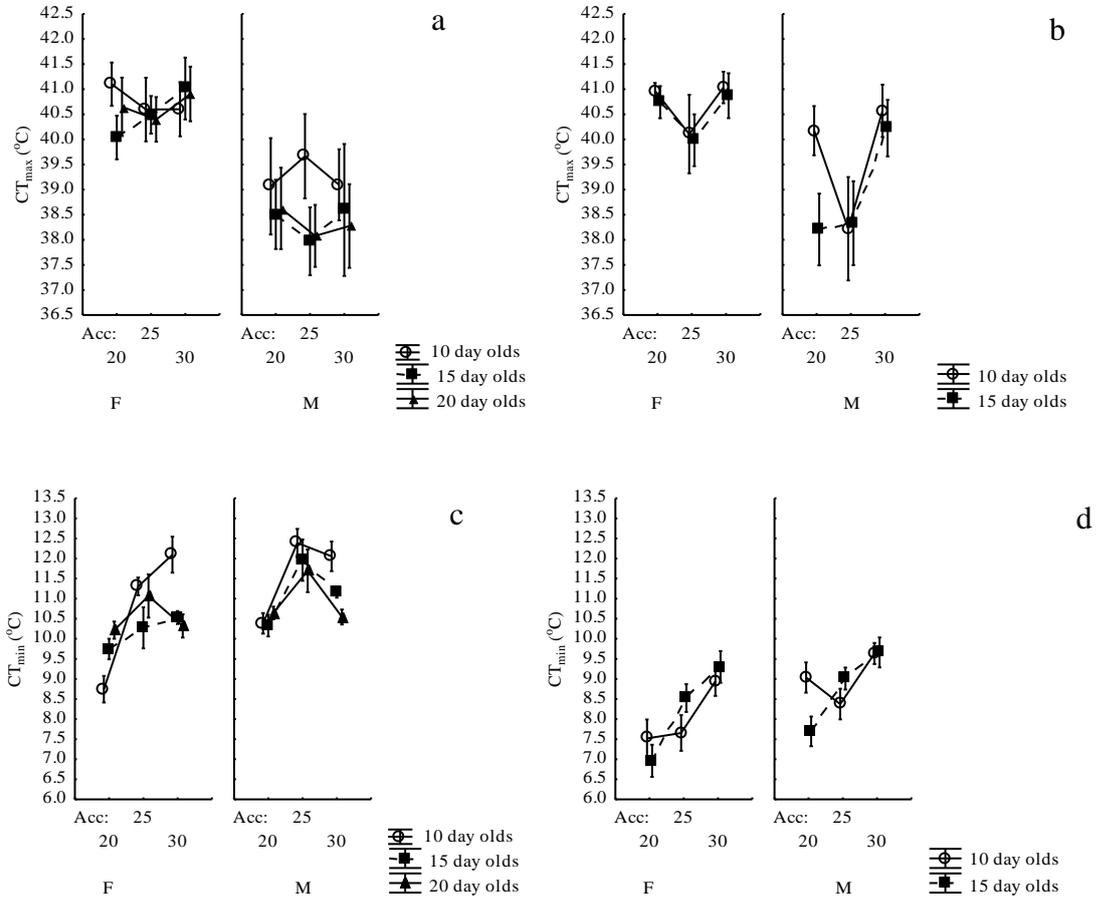


Fig. 4

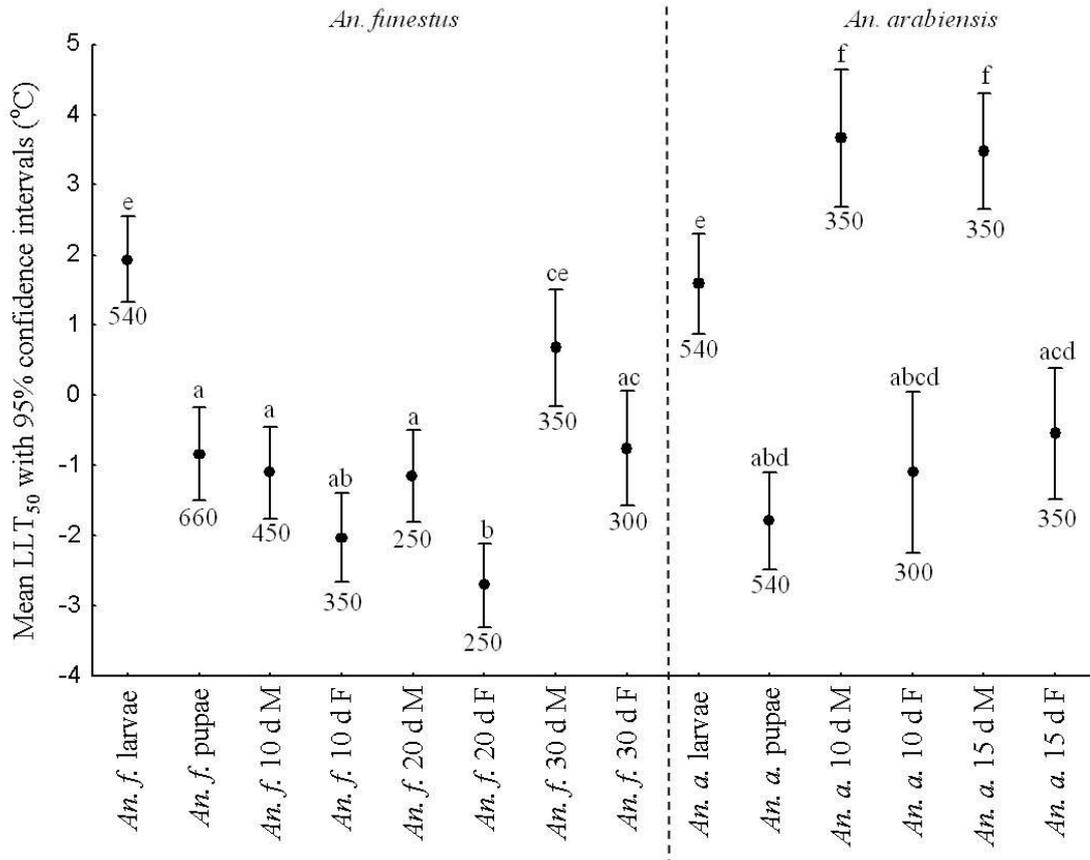
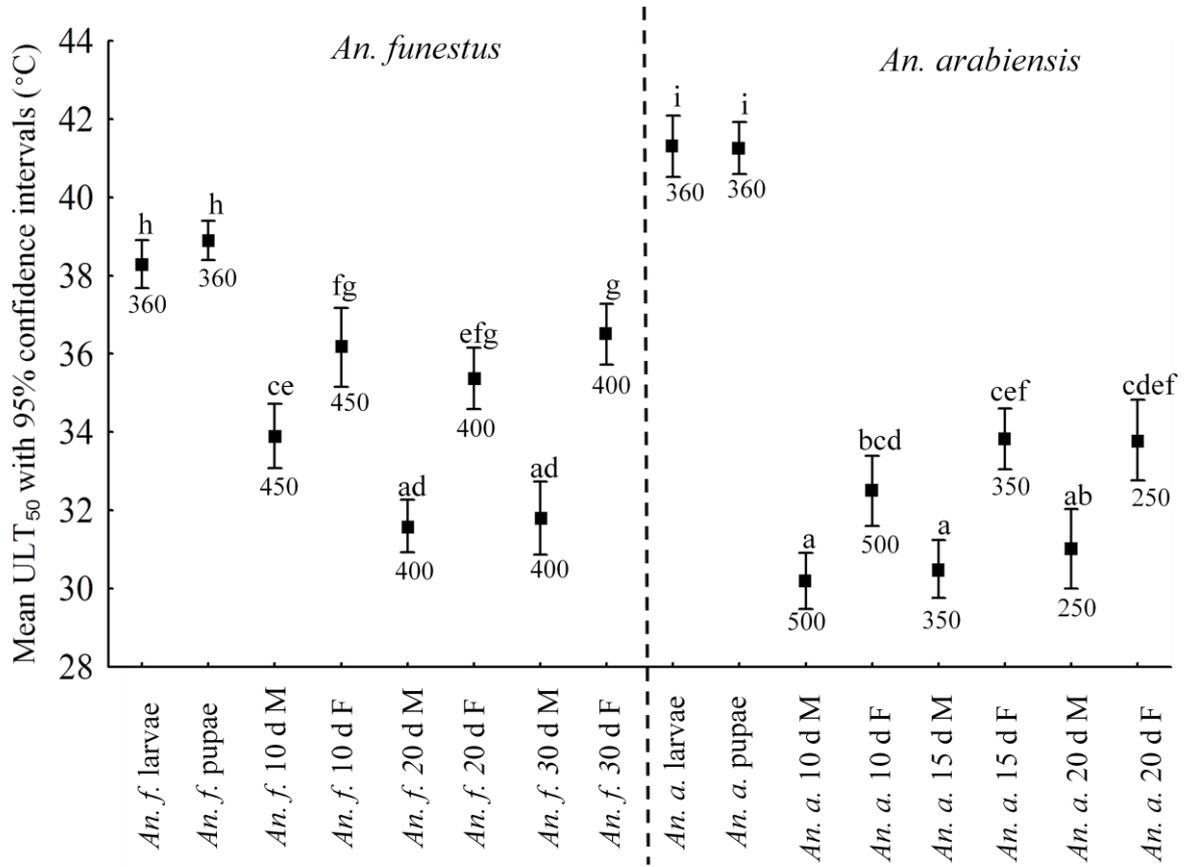


Fig. 5



Age	35.79	1	70.10	< 0.0001
Acclimation	56.80	2	55.64	< 0.0001
Strain*Sex	0.84	1	1.65	0.200
Strain*Age	7.63	1	14.94	< 0.0001
Sex*Age	0.02	1	0.04	0.851
Strain*Acclimation	11.33	2	11.09	< 0.0001
Sex*Acclimation	4.43	2	4.34	0.014
Age*Acclimation	6.26	2	6.13	< 0.0100
Strain*Sex*Age	0.70	1	1.37	0.242
Strain*Sex*Acclimation	2.48	2	2.43	0.089
Strain*Age*Acclimation	0.66	2	0.65	0.523
Sex*Age*Acclimation	0.84	2	0.82	0.441
Strain*Sex*Age*Acclimation	2.98	2	2.92	0.055

Table 2. Outcomes of general linear models examining the effects of sex, age, acclimation temperature and their interactions on CTmax (°C) of laboratory and wild strains of adult *Anopheles funestus*.

Strain	Effect	SS	Df	F	P
Laboratory	Sex	3.45	1	4.16	0.042
F _{17,372} =20.01; P<0.0005	Acclimation	6.53	2	3.94	0.020
	Age	0.74	2	0.45	0.640
	Sex*Acclimation	1.52	2	0.92	0.401
	Sex*Age	8.69	2	5.24	0.006
	Acclimation*Age	3.69	4	1.11	0.351
	Sex*Acclimation*Age	4.92	4	1.48	0.207
Wild	Sex	1.89	1	1.45	0.230
F _{11,258} =13.07; P<0.0005	Age	42.85	1	32.84	< 0.0001
	Acclimation	41.7	2	15.98	< 0.0001
	Sex*Age	4.09	1	3.14	0.078
	Sex*Acclimation	2.36	2	0.90	0.406
	Age*Acclimation	38.18	2	14.63	< 0.0001
	Sex*Age*Acclimation	12.09	2	4.64	0.011

Table 3. Outcomes of general linear models examining the effects of sex, age, acclimation temperature and their interactions on CT_{min} (°C) of laboratory and wild strains of adult *Anopheles funestus*.

Strain	Effect	SS	df	F	P
Laboratory	Sex	16.13	1	42.05	< 0.0001
					F _{17, 392} =119.4;
					P<0.0005
	Acclimation	202.23	2	263.63	< 0.0001
	Age	204.49	2	266.58	< 0.0001
	Sex*Acclimation	4.43	2	5.77	< 0.0100
	Sex*Age	1.09	2	1.41	0.244
	Acclimation*age	84.33	4	54.97	< 0.0001
	Sex*Acclimation*Age	8.43	4	5.49	< 0.0001
Wild	Sex	7.39	1	11.49	< 0.0001
					F _{11, 220} =15.92;
					P<0.0005
	Acclimation	34.57	2	26.88	< 0.0001
	Age	8.62	1	13.39	< 0.0001
	Sex*Acclimation	1.24	2	0.96	0.383
	Sex*Age	1.76	1	2.74	0.099
	Acclimation*Age	10.18	2	7.92	< 0.0001
	Sex*Acclimation*Age	2.54	2	1.97	0.142

Table 4. Outcomes of general linear models examining the effects of strain, sex, age, acclimation temperature and their interactions on CTmax (°C) and CTmin (°C) for wild versus laboratory strains of adult *Anopheles arabiensis*.

Critical Thermal Limit	Effect	SS	Df	F	P
CTmax	Strain	0.14	1	0.06	0.799
F _{23, 500} = 11.29; P < 0.0005	Sex	6.84	1	3.11	0.079
	Acclimation	11.23	2	2.55	0.079
	Age	0.68	1	0.31	0.580
	Strain*Sex	7.33	1	3.33	0.069
	Strain*Acclimation	5.08	2	1.15	0.316
	Sex*Acclimation	13.89	2	3.15	0.044
	Strain*Age	3.38	1	1.54	0.216
	Sex*Age	15.39	1	6.99	0.009
	Acclimation*Age	0.04	2	0.01	0.990
	Strain*Sex*Acclimation	19.85	2	4.51	0.012
	Strain*Sex*Age	13.9	1	6.31	0.012
	Strain*Acclimation*Age	5.4	2	1.23	0.294
	Sex*Acclimation*Age	12.49	2	2.84	0.060
	Strain*Sex*Acclimation*Age	26.43	2	5.99	0.003
CTmin	Strain	15.01	1	24.97	< 0.0001
F _{23, 464} = 82.4; P < 0.0005	Sex	22.95	1	38.19	< 0.0001
	Acclimation	24.57	2	20.45	< 0.0001
	Age	3.14	1	5.22	0.023
	Strain*Sex	0.08	1	0.13	0.719

Strain*Acclimation	32.84	2	27.32	< 0.0001
Sex*Acclimation	4.35	2	3.62	0.028
Strain*age	12.17	1	20.25	< 0.0001
Sex*Age	3.04	1	5.06	0.025
Acclimation*Age	11.17	2	9.29	< 0.001
Strain*Sex*Acclimation	3.35	2	2.78	0.063
Strain*Sex*Age	0.19	1	0.31	0.575
Strain*Acclimation*age	40.69	2	33.85	< 0.0001
Sex*Acclimation*Age	0.86	2	0.71	0.4898
Strain*Sex*Acclimation*Age	2.41	2	2.01	0.1354

Table 7. Differences in biology between *Anopheles arabiensis* and *Anopheles funestus* (Gillies and Coetzee 1987).

	<i>Anopheles arabiensis</i>	<i>Anopheles funestus</i>
Southern African distribution	Present in South Africa in the low-lying north-eastern areas	Absent from South Africa at present, but occurs in southern Mozambique
Habitat type	Arid-adapted, has been found in areas with less than 40% relative humidity, environmental temperatures as high as 50°C	“Tropical species”, requires more humid environment, environmental temperatures up to 40°C
Breeding sites	Shallow, temporary pools < 0.5 m deep e.g. hoof prints, tyre tracks	Swamps, slow-flowing streams, deep and vegetated water bodies
Behaviour	Exophilic and endophilic, feeds on cattle and humans	Endophilic, prefers to feed on humans

Supplementary Materials

Table S1. Results from a Shapiro-Wilk's test for normality and Levene's test for homogeneity of variance for all groups and each group separately for *Anopheles arabiensis* and *Anopheles funestus*.

Species and trait	Group	Shapiro-Wilk's	Group	df	Levene's
<i>An. arabiensis</i>					
CTmin (°C)	ALL	W = 0.99, P = 0.009	ALL	35, 678	F = 3.64, P < 0.0001
	Laboratory strain	W = 0.98, P < 0.0001	Strain	1, 712	F = 0.52, P = 0.4701
	Wild strain	W = 0.97, P < 0.0001			
	Males	W = 0.99, P = 0.1285	Sex	1, 712	F = 0.64, P = 0.4248
	Females	W = 0.99, P = 0.0031			
	20°C acclimation	W = 0.95, P < 0.0001	Acclimation	2, 711	F = 28.17, P < 0.0001
	25°C acclimation	W = 0.98, P = 0.0038			
	30°C acclimation	W = 0.98, P = 0.0016			
	10 day olds	W = 0.97, P = 0.0001	Age	1, 486	F = 14.71, P = 0.0001
	15 day olds	W = 0.98, P = 0.0041			

An. funestus

CTmin (°C)	ALL	W = 0.99, P = 0.0001	ALL	23, 498	F = 3.51, P < 0.0001
	Laboratory strain	W = 0.95, P < 0.0001	Strain	1, 520	F = 37.39, P < 0.0001
	Wild strain	W = 0.98, P = 0.0052			
	Males	W = 0.99, P = 0.0340	Sex	1, 520	F = 0.03, P = 0.8723
	Females	W = 0.98, P = 0.0009			
	20°C acclimation	W = 0.97, P = 0.0008	Acclimation	2, 519	F = 39.68, P < 0.0001
	25°C acclimation	W = 0.99, P = 0.2272			
	30°C acclimation	W = 0.99, P = 0.1657			
	10 day olds	W = 0.95, P < 0.0001	Age	1, 520	F = 0.28, P = 0.5947
	20 day olds	W = 0.97, P = 0.0002			

An. arabiensis

CTmax (°C)	ALL	W = 0.90, P < 0.0001	ALL	23, 500	F = 7.24, P < 0.0001
	Laboratory strain	W = 0.92, P < 0.0001	Strain	1, 522	F = 3.21, P = 0.0736
	Wild strain	W = 0.86, P < 0.0001			
	Males	W = 0.93, P < 0.0001	Sex	1, 522	F = 72.28, P < 0.0001

Females	W = 0.93, P < 0.0001			
20°C acclimation	W = 0.89, P < 0.0001	Acclimation	2, 521	F = 2.65, P = 0.0716
25°C acclimation	W = 0.93, P < 0.0001			
30°C acclimation	W = 0.84, P < 0.0001			
10 day olds	W = 0.87, P < 0.0001	Age	1, 522	F = 4.72, P = 0.0302
15 day olds	W = 0.92, P < 0.0001			

An. funestus

CTmax (°C)	ALL	W = 0.88, P < 0.0001	ALL	23, 516	F = 5.01, P < 0.0001
	Laboratory strain	W = 0.96, P < 0.0001	Strain	1, 538	F = 1.42, P = 0.2339
	Wild strain	W = 0.81, P < 0.0001			
	Males	W = 0.97, P < 0.0001	Sex	1, 538	F = 0.00, P = 0.9958
	Females	W = 0.77, P < 0.0001			
	20°C acclimation	W = 0.69, P < 0.0001	Acclimation	2, 537	F = 1.08, P = 0.3402
	25°C acclimation	W = 0.97, P < 0.0001			
	30°C acclimation	W = 0.96, P < 0.0001			
	10 day olds	W = 0.98, P = 0.0030	Age	1, 538	F = 15.72, P < 0.0001

20 day olds W = 0.84, P < 0.0001

An. arabiensis

CTmin (°C)	ALL	W=0.98, P < 0.0001	ALL	17, 342	F = 5.75, P < 0.0001
Laboratory strain	Males	W=0.95, P < 0.0001	Sex	1, 358	F = 0.33, P = 5673
	Females	W=0.98, P = 0.0029			
	20°C acclimation	W=0.94, P < 0.0001	Acclimation	2, 357	F = 9.65, P < 0.0001
	25°C acclimation	W=0.98, P = 0.0705			
	30°C acclimation	W=0.94, P < 0.0001			
	10 day olds	W=0.97, P = 0.0124	Age	2, 357	F = 17.81, P < 0.0001
	15 day olds	W=0.92, P < 0.0001			
	20 day olds	W=0.87, P < 0.0001			

An. funestus

CTmin (°C)	ALL	W=0.94, P < 0.0001	ALL	17, 392	F=3.67, P < 0.0001
Laboratory strain	Males	W=0.95, P < 0.0001	Sex	1, 408	F=0.66, P = 0.4158
	Females	W=0.90, P < 0.0001			
	20°C acclimation	W=0.91, P < 0.0001	Acclimation	2, 407	F=64.83, P < 0.0001

25°C acclimation	W=0.98, P = 0.1214			
30°C acclimation	W=0.96, P = 0.0002			
10 day olds	W=0.93, P < 0.0001	Age	2, 407	F=31.57, P < 0.0001
20 day olds	W=0.97, P = 0.0068			
30 day olds	W=0.97, P = 0.0117			

An. arabiensis

CTmin (°C)	ALL	W=0.98, P < 0.0010	ALL	11, 236	F=0.94, P = 0.5054
Wild strain	Males	W=0.99, P = 0.2095	Sex	1, 246	F=8.04, P = 0.0050
	Females	W=0.97, P = 0.0033			
	20°C acclimation	W=0.96, P = 0.0123	Acclimation	2, 245	F=4.95, P = 0.0078
	25°C acclimation	W=0.96, P = 0.0107			
	30°C acclimation	W=0.97, P = 0.0274			
	10 day olds	W=0.96, P = 0.0018	Age	1, 246	F=0.05, P = 0.8198
	15 day olds	W=0.97, P = 0.0136			

An. funestus

CTmin (°C)	ALL	W=0.98, P = 0.0052	ALL	11, 220	F=1.82, P = 0.0524
------------	-----	--------------------	-----	---------	--------------------

Wild strain	Males	W=0.96, P = 0.0059	Sex	1, 230	F=0.19, P = 0.6583
	Females	W=0.98, P = 0.1319			
	20°C acclimation	W=0.98, P = 0.2028	Acclimation	2, 229	F=1.48, P = 0.2296
	25°C acclimation	W=0.98, P = 0.4414			
	30°C acclimation	W=0.97, P = 0.1358			
	10 day olds	W=0.95, P = 0.0005	Age	1, 230	F=9.94, P = 0.0018
	20 day olds	W=0.99, P=0.6761			

An. arabiensis

CTmax (°C)	ALL	W=0.93, P < 0.0001	ALL	17, 423	F=4.12, P < 0.0001
Laboratory strain	Males	W=0.95, P < 0.0001	Sex	1, 439	F=41.99, P < 0.0001
	Females	W=0.95, P < 0.0001			
	20°C acclimation	W=0.92, P < 0.0001	Acclimation	2, 438	F=0.84, P = 0.4309
	25°C acclimation	W=0.93, P < 0.0001			
	30°C acclimation	W=0.92, P < 0.0001			
	10 day olds	W=0.89, P < 0.0001	Age	2, 438	F=2.75, P = 0.0653
	15 day olds	W=0.94, P < 0.0001			

	20 day olds	W=0.94, P < 0.0001			
<hr/>					
<i>An. funestus</i>					
CTmax (°C)	ALL	W=0.94, P < 0.0001	ALL	17, 372	F=2.95, P < 0.0001
Laboratory strain	Males	W=0.89, P < 0.0001	Sex	1, 388	F=6.73, P = 0.0098
	Females	W=0.98, P < 0.0072			
	20°C acclimation	W=0.82, P < 0.0001	Acclimation	2, 387	F=0.72, P = 0.4861
	25°C acclimation	W=0.95, P = 0.0003			
	30°C acclimation	W=0.97, P = 0.0009			
	10 day olds	W=0.98, P = 0.0269	Age	2, 387	F=7.75, P = 0.0005
	20 day olds	W=0.96, P = 0.0010			
	30 day olds	W=0.88, P < 0.0001			
<hr/>					
<i>An. arabiensis</i>					
CTmax (°C)	ALL	W=0.85, P < 0.0001	ALL	11, 232	F=7.36, P < 0.0001
Wild strain	Males	W=0.91, P < 0.0001	Sex	1, 242	F=42.20, P < 0.0001
	Females	W=0.83, P < 0.0001			
	20°C acclimation	W=0.82, P < 0.0001	Acclimation	2, 241	F=13.58, P < 0.0001

25°C acclimation	W=0.92, P < 0.0010			
30°C acclimation	W=0.84, P < 0.0001			
10 day olds	W=0.80, P < 0.0001	Age	1, 242	F=0.38, P = 0.5382
15 day olds	W=0.88, P < 0.0001			

An. funestus

CTmax (°C)	ALL	W=0.81, P < 0.0001	ALL	11, 258	F=5.97, P < 0.0001
Wild strain	Males	W=0.97, P = 0.0070	Sex	1, 268	F=0.76, P = 0.3835
	Females	W=0.69, P < 0.0001			
	20°C acclimation	W=0.97, P = 0.0040	Acclimation	2, 267	F=6.24, P = 0.0023
	25°C acclimation	W=0.63, P < 0.0001			
	30°C acclimation	W=0.97, P = 0.0861			
	10 day olds	W=0.97, P = 0.0166	Age	1, 268	F=5.04, P = 0.0257
	20 day olds	W=0.73, P < 0.0001			

Table S2. Percentage deviation from the mean critical thermal minimum (CT_{min}) and maximum (CT_{max}) per group, per strain for *Anopheles funestus* and *Anopheles arabiensis*.

Strain and CT	Group	Mean CT (°C)	% Deviation
<i>An. funestus</i> both strains	10 day olds	40.5	1.0
CT _{max} (°C)	20 day olds	39.7	-1.0
	Males	39.7	-1.0
	Females	40.4	0.7
	20°C acclimation	39.6	-1.2
	25°C acclimation	40	-0.2
	30°C acclimation	40.6	1.2
	wild	39.8	-0.7
	Lab	40.3	0.5
<i>An. funestus</i> both strains	10 day olds	8	-8.0
CT _{min} (°C)	20 day olds	9.1	4.6
	30 day olds	9.1	4.6
	Males	9.1	4.6
	Females	8.4	-3.4
	20°C acclimation	8.1	-6.9
	25°C acclimation	8.5	-2.3
	30°C acclimation	9.6	10.3
	wild	8.2	-5.7
Lab	9.2	5.7	
Laboratory <i>An. funestus</i>	10 day olds	40.7	0.7
CT _{max} (°C)	20 day olds	40.1	-0.7

	30 day olds	40.2	-0.5
	Males	39.7	-1.7
	Females	41	1.5
	20°C acclimation	39.8	-1.5
	25°C acclimation	40.5	0.2
	30°C acclimation	40.8	1.0
<hr/>			
Wild <i>An. funestus</i>	10 day olds	40.2	1.0
CTmax (°C)	20 day olds	39.4	-1.0
	Males	39.6	-0.5
	Females	40	0.5
	20°C acclimation	39.3	-1.3
	25°C acclimation	39.5	-0.8
	30°C acclimation	40.6	2.0
<hr/>			
Laboratory <i>An. funestus</i>	10 day olds	7.8	-15.2
CTmin (°C)	20 day olds	10	8.7
	30 day olds	9.7	5.4
	Males	9.5	3.3
	Females	8.8	-4.3
	20°C acclimation	8.5	-7.6
	25°C acclimation	8.8	-4.3
	30°C acclimation	10.2	10.9
<hr/>			
Wild <i>An. funestus</i>	10 day olds	8.2	0.0
CTmin (°C)	20 day olds	8.2	0.0
	30 day olds	8.2	0.0
	Males	8.5	3.7

	Females	7.9	-3.7
	20°C acclimation	7.8	-4.9
	25°C acclimation	8.3	1.2
	30°C acclimation	8.8	7.3
<hr/>			
<i>An. arabiensis</i> both strains	10 day olds	40.1	1.0
CTmax (°C)	15 day olds	39.6	-0.3
	20 day olds	39.5	-0.5
	Males	38.9	-2.0
	Females	40.6	2.3
	20°C acclimation	39.8	0.3
	25°C acclimation	39.3	-1.0
	30°C acclimation	40.1	1.0
	wild	39.8	0.3
	Lab	39.7	0.0
<hr/>			
<i>An. arabiensis</i> both strains	10 day olds	9.8	0.0
CTmin (°C)	15 day olds	9.6	-2.0
	20 day olds	10.1	3.1
	Males	10.2	4.1
	Females	9.5	-3.1
	20°C acclimation	9.1	-7.1
	25°C acclimation	10	2.0
	30°C acclimation	10.3	5.1
	wild	8.8	-10.2
	Lab	10.9	11.2
<hr/>			
Laboratory <i>An. arabiensis</i>	10 day olds	40.1	1.0

CTmax (°C)	15 day olds	39.5	-0.5
	20 day olds	39.5	-0.5
	Males	38.7	-2.5
	Females	40.6	-2.3
	20°C acclimation	39.7	0.0
	25°C acclimation	39.6	-0.3
	30°C acclimation	39.7	0.0
<hr/>			
Laboratory <i>An. arabiensis</i>	10 day olds	11.2	2.8
CTmin (°C)	15 day olds	10.7	-1.8
	20 day olds	10.8	-0.9
	Males	11.2	2.8
	Females	10.5	-3.7
	20°C acclimation	10	-8.3
	25°C acclimation	11.5	5.5
	30°C acclimation	11.1	1.8
<hr/>			
Wild <i>An. arabiensis</i>	10 day olds	40.2	1.0
CTmax (°C)	15 day olds	39.7	-0.3
	20 day olds	39.5	-0.8
	Males	39.1	-1.8
	Females	40.5	1.8
	20°C acclimation	40	0.5
	25°C acclimation	39	-2.0
	30°C acclimation	40.5	1.8
<hr/>			
Wild <i>An. arabiensis</i>	10 day olds	8.5	-3.4
CTmin (°C)	15 day olds	8.5	-3.4

20 day olds	9.4	6.8
Males	9.1	3.4
Females	8.5	-3.4
20°C acclimation	8.3	-5.7
25°C acclimation	8.6	-2.3
30°C acclimation	9.5	8.0

Chapter 3

Stable and fluctuating temperature effects on the development rate and survival of two malaria vectors,

Anopheles arabiensis and *Anopheles funestus*



Introduction

Malaria is one of Africa's most significant vector-borne diseases of humans, accounting for millions of clinical cases and many deaths per year. Although several factors affect malaria prevalence, including the efficacy of control interventions, it depends significantly on the entomological inoculation rate (EIR): the average number of infectious mosquito bites one person receives in a year (Smith *et al.* 2007). The EIR is, in turn, dependent on the human biting rate, which is a product of the number of mosquitoes per human and the number of bites per mosquito. The number of mosquitoes in a population depends on the number of adults entering and leaving the population (Patz *et al.* 2000; Small *et al.* 2003), both of which are affected significantly by environmental temperature. Low temperatures tend to preclude immature development and adult activity, while extremely high temperatures lead to substantial mortality (Love and Whelchel 1957; Bayoh and Lindsay 2003; Lyons *et al.* 2012). In the intermediate temperature range, development rate, feeding rate and adult survival increase with temperature, as is true of most ectotherms (Hoffmann 2010), often leading, in the case of vector-borne disease, to an increase in disease prevalence (Patz *et al.* 2000).

Given these relatively straightforward relationships between temperature and significant population parameters (Worner 1992; Patz *et al.* 2000; Small *et al.* 2003), it is perhaps not surprising that forecasts of increasing malaria burden with climate change have been made (Tanser *et al.* 2003). However, these forecasts are controversial for several reasons. First, despite claims that overall the disease burden will increase, several analyses have suggested that in some areas incidence will decrease and in others increase, leading to overall stasis (Rogers and Randolph 2000). When coupled with human intervention, the outcome should be a decline in disease prevalence. Second, much of the focus has been on changes in mean annual temperature. However, climate change involves more than a change in mean temperatures. Rather, extremes are changing too, with extreme high temperatures

being more common and extreme low temperatures less common than in the past (Hansen *et al.* 2012). Moreover, fluctuating temperatures can result in substantially different likelihoods of malaria transmission than constant temperatures (Pascual *et al.* 2009; Paaijmans *et al.* 2009) and the predicted temperature for optimal transmission has also been estimated at lower than previously thought (Mordecai *et al.* 2012). In consequence, much attention is now being given to developing spatially accurate and biologically more realistic forecasts of changes in malaria prevalence (Moffett *et al.* 2007; Reid *et al.* 2010), reflecting a general trend in the field of climate change impact forecasting for vectors and other species (Martens *et al.* 1997; Kearney *et al.* 2009; Williams *et al.* 2010).

Although several approaches for forecasting population-level consequences of climate change are available (Moffett *et al.* 2007; Phillips *et al.* 2008; Elith and Graham 2009; Kearney *et al.* 2009), much of the recent focus has been on developing mechanistic models that may be capable of forecasting outside of current climate envelopes into non-analogous situations (Kearney *et al.* 2009; Kearney and Porter 2009). Mechanistic models complement the more traditional environmental niche models, which are based on some form of modeling of the relationships between distribution/abundance data and current climates (see Araújo and Peterson 2012), and provide a means to include significant nuances such as the likely influence of evolutionary change and variation among species, populations and genotypes (Kearney and Porter 2009; Chown *et al.* 2010). Moreover, although considered data intensive, often the inclusion of just a few key physiological parameters can result in accurate forecasts of species ranges and their change into the future (Crozier 2004; Crozier and Dwyer 2006; Kearney and Porter 2009; Chown *et al.* 2010; Buckley *et al.* 2011; Richardson *et al.* 2011). Such models are therefore of considerable significance for vector-borne diseases such as malaria because of the variety of vectors that are involved, their varying habitat requirements, and their change in relative abundance across the range of the disease (Gillies

and Coetzee 1987; Coetzee *et al.* 2000; Kirby and Lindsay 2009). Nonetheless, they are dependent on the availability of basic physiological data which, though relatively straightforward to collect, are often missing for vectors.

For the anopheline mosquitoes that transmit malaria, temperature and water availability are key factors influencing both the adult and immature stages, and therefore demography, as is the case for many other insect species (Gullan and Cranston 1994; Githetko *et al.* 2000; Kearney and Porter 2009). Whilst much information is available on temperature effects on major life cycle components of *Anopheles gambiae* (Bayoh and Lindsay 2003, 2004; Kirby and Lindsay 2009; Rocca *et al.* 2009), an important vector of *Plasmodium falciparum*, the major cause of malaria-associated mortality in Africa, much less is known about the thermal biology of the two other major vectors, *An. arabiensis* and *An. funestus* (Kirby and Lindsay 2004). These species are also major vectors of *falciparum* malaria in south-eastern Africa, an area for which environmental niche models suggest an increase in disease prevalence with climate change (Small *et al.* 2003). Although recent work has provided comprehensive information on extreme tolerance limits for these species (Lyons *et al.* 2012), the effects of temperature on development and intrinsic survival, from egg to adult, have not been as comprehensively investigated (Kirby and Lindsay 2009). Furthermore, the influence of fluctuations in temperature on development, representing exposure to more extreme conditions, have not been extensively examined for African malaria vectors (see Huffaker 1944 for an important early approach), despite the fact that fluctuating temperatures clearly influence other aspects of malaria transmission (Pascual *et al.* 2009; Paaijmans *et al.* 2009), and may also significantly affect development rates in other insect species (Hagstrum and Milliken 1991).

In this chapter, I therefore examined the effects of constant and fluctuating temperatures on the development and survival of two malaria vectors, *An. arabiensis* and *An.*

funestus. These kinds of data have been shown to be useful in estimating population level responses to temperature treatments, based on developmental and growth parameters of species (e.g. Parham *et al.* 2012). The main purpose is therefore, to contribute to the information that is required for mechanistic forecasts of likely changes in mosquito population density and, ultimately to provide experimental data for the EIR associated with climate change across southern Africa, which is taking place and forecast to be substantial into the future (Cox *et al.* 2000; New *et al.* 2006; Sanderson *et al.* 2011).

Methods

Colony maintenance and egg collection

Eggs were collected from two existing colonies at the Vector Control Reference Unit in Johannesburg, South Africa. *Anopheles arabiensis* eggs were collected from the KGB-strain, originally established from individuals collected in Zimbabwe in 1975, and *An. funestus* eggs were collected from the FUMOZ-strain, originally established from individuals collected in Mozambique in 2000 (Hunt *et al.* 2005). Although the colonies have shown some laboratory adaptation in thermal responses, it has typically not been pronounced (Lyons *et al.* 2012).

Adult mosquitoes of each colony were maintained at the insectary temperature of 25°C (\pm 2°C) and relative humidity of 80% (checked with a Masons thermohygrometer, Brannan, UK). Adults were housed in 25 L plastic buckets with a nylon mesh lid and a handling entrance cut out of the side of the bucket. The handling entrance was sealed with nylon meshing and tied to prevent escapees when handling was not required. Adults were provided with a 10% sugar water solution *ad libitum*. In addition, females were provided with a blood meal every alternative day. *Anopheles arabiensis* usually requires at least two blood meals to produce eggs, while *An. funestus* requires at least three (Clements 1963). Hence, only females that had received at least three blood meals were used for egg collections.

Female mosquitoes of each colony were given no longer than half of one dark cycle (six hours) in which to lay eggs. This six hour period was chosen to allow the chorion of the mosquito eggs to harden before being disturbed (see Clements 1963). Because mosquitoes are known to prefer dark backgrounds when ovipositing (Clements 1963), females were provided with darkened plastic petri dishes (10 mm depth; 70 mm diameter) filled with distilled water for oviposition. Following this six hour period, eggs were collected and separated into 25, 200 ml bowls (filled with distilled water) of between 20 to 30 eggs each. These 25 replicates were the basic sample unit used for assessment of development rate at each of several temperatures (i.e. n=25 per temperature): constant temperatures of 15, 18, 20, 22, 25, 28, 30, 32 and 35°C; and two fluctuating temperature regimes: 15°C to 35°C, and 20°C to 30°C, each with a mean temperature of 25°C and the lowest temperature representing the temperature during the 12 hour scotophase of a 12L:12D cycle. These temperatures were chosen to represent the temperatures within which development to adulthood is known to occur in other *Anopheles* species (e.g. Bayoh and Lindsay 2003; Kirby and Lindsay 2009). Temperatures were maintained to $\pm 0.5^\circ\text{C}$ through the use of PTC-1 Peltier portable temperature control cabinets (Sable Systems, Las Vegas, Nevada, USA) or through the use of an incubator (SANYO, MIR-154, SANYO Electric Co. Ltd., Osaka, Japan) and were checked using a mercury thermometer. Photoperiod was maintained through non-heating fluorescent tubes connected to a timer. Eggs were maintained under these conditions until eclosion. To prevent eggs from sticking to the sides of replicate bowls, they were washed down using a wash bottle containing distilled water of the same temperature as each relevant treatment. Larval food comprised a mixture of finely ground dog biscuits and yeast extract. Larvae were fed once or twice daily depending on instar, and adults were left without food to die following eclosion.

Development rate

All temperature treatments were checked every 8-12 hours depending on stage of development, for any developmental change. Because *Anopheles* larvae breathe at the water surface (Clements 1963), oxygen saturation was not a concern. Experiments per temperature were randomized with regard to temperature and positions in the incubators. The length of time that 50% of the population in each replicate took to reach each life stage, and total time to adulthood (again 50% of the population) was recorded for each of the 25 replicates per temperature treatment and for each species. The 50% criterion was used because of several substantial outliers (long time to development) and therefore the potential to skew substantially the value for each replicate. Rate-temperature curves were plotted for each species using 1/mean time to larva/pupa/adult emergence per temperature. The full suite of development could be assessed this way. Using the linear part of the curve for each species (between 15°C and 32°C for *An. arabiensis* and between 15°C and 30°C for *An. funestus*), ordinary least squares linear regression was applied to estimate the lower developmental threshold (LDT: $-\text{slope}/\text{intercept}$ in °C) and the sum of effective temperatures (SET: $1/\text{slope}$ in degree-days) for each life stage change (i.e. egg, larva, pupa), and for overall development from egg to adult (Honěk and Kocourek 1990; Honěk 1996; Trudgill *et al.* 2005). To compare overall development rates between the two fluctuating temperature treatments and their constant mean of 25°C, an analysis of variance (ANOVA) was used, as implemented in R (v. 2.15.1) (R Foundation for Statistical Computing, Vienna, Austria) for each species. Normality and homogeneity of variance were first checked using Shapiro-Wilk's and Levene's tests, respectively (Table S1). In some cases deviations from normality were observed, but generally, few deviations occurred and the model assumptions were met, allowing use of a parametric ANOVA which is reasonably robust and insensitive to deviations from normality provided designs are balanced (Quinn and Keough 2002). Mean

development time in days for each stage and overall across all 11 temperature treatments are shown in Table S2. To compare development rates (the reciprocal of time, days⁻¹) of each stage and for overall development between species, general linear models using normal distribution of errors and identity link functions were implemented in R (v. 2.15.1) for each stage comparison and overall egg to adult development using temperature and species as categorical predictors in the model and development rates as response variables. Deviations from normality occurred in some instances, but model assumptions were generally met (Quinn and Keough 2002; Faraway 2005) (Figures S1 and S2).

To determine the optimum development temperature (T_{opt}) and the maximum development rate associated with this temperature (μ_{max}) (see Gilchrist 1996), a non-linear curve-fitting approach was adopted using TableCurve 2D (v. 5.01, SYSTAT Software Inc., 2002, San Jose, California, USA) (Figures S3-S5) (see Janion *et al.* 2010). T_{opt} and μ_{max} were determined from the equations for the best fit curve, which differed among stages and between species (Table 1, Tables S3 and S4). To compare T_{opt} and μ_{max} of *An. arabiensis* to that of *An. funestus*, one replicate for each temperature treatment was selected at random (without replacement) to provide 25 separate curves for overall development rate for each species and for each life stage. The equations used to obtain T_{opt} and μ_{max} for overall development and development of each stage across all 25 replicates are presented in Table S5. Except in a few cases (pupal development rates) these equations all had r^2 values above 0.90. The same equations for all 25 replicates were chosen to minimize discrepancies when comparing T_{opt} and μ_{max} between species. T_{opt} and μ_{max} were then compared, for overall development and for each life stage, between the species using t-tests implemented in R (v. 2.15.1).

Survival

Although development rate generally increases with increasing temperature up to the optimum (Clements 2000; Chown and Nicolson 2004; Kingsolver *et al.* 2004), high development rates are often accompanied by mortality and reduced population output (Clements 2000; Bayoh and Lindsay 2003; Régnière *et al.* 2012). In consequence, overall survival from egg to adult was recorded as the proportion of eggs that emerged as adults (expressed as a percentage). This % survival was recorded for all 25 replicates per temperature treatment. To assess differences in survival between the fluctuating temperature treatments and their constant mean (25°C), a generalized linear model with a binomial distribution of errors and logit link function was implemented in R (v. 2.15.1) (R Foundation for Statistical Computing, Vienna, Austria). To compare the effect of temperature on survival of each species, mean percentage survival (\pm standard error) was plotted at each constant temperature and in a comparison between the two fluctuating temperatures and constant mean of 25°C.

Results

Development rate

Total development rate from egg to adult of *Anopheles arabiensis* and *An. funestus* increases between 18°C and 32°C and between 18°C and 30°C, respectively in a linear fashion (Figure 1). At 15°C and 35°C, no development from egg to adult occurs for either species (Figure 1). Although experimentally no development occurred at 15°C and 35°C, lower developmental thresholds for *An. arabiensis* and *An. funestus* were estimated at ~13°C and 14°C for complete development of each respective species (Table 1).

Overall development rates at constant and fluctuating treatments differed significantly for both species, with fluctuating temperatures leading to significantly lower development

rates for *An. funestus* (ANOVA: *An. funestus* df=2, F=395.3, P<0.001; Figure 2). Development rates of each stage and from egg to adult across all temperatures (including fluctuating temperatures) differed significantly between species, with *An. arabiensis* showing consistently faster development rates when compared with *An. funestus* (Table 2). Development rate of *An. arabiensis* at 25°C did not differ significantly from development rate at 20°C to 30°C, but both these temperature treatments differed significantly from the 15°C to 35°C treatment (ANOVA: *An. arabiensis* df=2, F=25.5, P<0.0001; Figure 2). Species comparisons of μ_{\max} and T_{opt} revealed significantly higher T_{opt} and μ_{\max} for overall development and for larval development in *An. arabiensis* than in *An. funestus* (Table 1, Table 3), but no significant difference in T_{opt} or μ_{\max} for pupal development and only significantly different μ_{\max} for egg development, although T_{opt} for this stage was similar between species (Table 3).

Survival

Survival (%) from egg to adult was highest at 32°C for *An. arabiensis* and at 25°C for *An. funestus* (Figure 3). No survival occurred at 15°C or 35°C for *An. arabiensis* and *An. funestus* (Fig. 3). Mean percentage survival at the constant mean of 25°C was higher than at the fluctuating temperatures for both species (Figure 4). Mean survival of *An. arabiensis* at 20°C to 30°C was estimated at c. 69%, whereas mean survival at 15°C to 35°C was estimated at c. 59%, compared with c. 68% at 25°C. *Anopheles funestus* had a mean survival of c. 33% at 20°C to 30°C, c. 29% at 15°C to 35°C and c. 62% at 25°C constant (Figure 4).

Discussion

The two species examined here, along with *Anopheles gambiae*, are the three most significant vectors of *Plasmodium falciparum* malaria in Africa (Gillies and Coetzee 1987; Collins and

Besansky 1994). How their population size fluctuates under different thermal conditions is of particular significance because this determines the entomological inoculation rate (EIR) (Hay *et al.* 2000). Malaria incidence and prevalence are likely to be impacted by climate change through their effect on the EIR.

Based on the breeding habits of these three vector species and general life history information available (Dukeen and Omer 1986; Gillies and Coetzee 1987), it is expected that their development rates in the linear parts of their rate-temperature curves will differ significantly. Results indicate that development rate of *An. arabiensis* is indeed faster than that of *An. funestus*, and optimum temperatures for development (T_{opt}) are significantly higher for *An. arabiensis* than for *An. funestus*. Furthermore, although the estimated lower developmental thresholds are similar for these two vectors and although experimentally determined lower developmental temperatures of ~ 15°C-16°C have been found for all three African malaria vectors (Bayoh and Lindsay 2004), the number of heat units required to complete one generation (SET) is also less in *An. arabiensis* than *An. funestus* (Table 1). For the puddle-breeding *An. arabiensis*, the steepness of the slope of the rate-temperature relationship is greater than for *An. funestus* and *An. gambiae*, and development rate of this species is therefore significantly faster than for *An. gambiae* or *An. funestus*. Additionally, *An. arabiensis* also has a shorter total lifespan than *An. funestus* (Hunt *et al.* 2005; Munhenga *et al.* 2011).

Comparisons of development rates across all temperature treatments between stages, between species, showed significantly faster development of *An. arabiensis* eggs, larvae, pupae and overall egg to adult when compared to *An. funestus* (Table 2). However, maximum development rates (μ_{max}) of pupal stages over only the constant temperature range, as determined from non-linear equations, did not differ significantly between species (Table 3). This is likely a result of the massive changes in structural complexity associated with

metamorphosis from the larval to pupal stage (Gullan and Cranston 1994; Chown and Nicolson 2004). The shorter lifespan and the use of transient breeding sites by *An. arabiensis* (Gillies and Coetzee 1987) and therefore, the risk of evaporation of the ephemeral breeding sites, explains a faster development rate for this species when compared to *An. funestus*.

The EIR depends on the number of surviving adults that emerge from breeding sites that are able to impart an infective bite on humans (Hay *et al.* 2000). Survival of each of these three vectors was also different across constant temperature treatments. *Anopheles arabiensis* showed highest survival at 32°C, while *An. funestus* showed highest survival at 25°C. *Anopheles gambiae*, however, displays highest survival at 24°C (Bayoh and Lindsay 2004). This information suggests that warming temperatures will reduce the time from egg to adult for all three species, but will only increase or maintain high survival or emergence of adults in *An. arabiensis*. *Anopheles funestus* and *An. gambiae* are likely to show reduced population output based on these survival estimates under constant temperatures. These changes to the vector populations will mean changes in both positive and negative directions to the ratio of mosquitoes to humans, a crucial aspect to the EIR in any malarious area (Hay *et al.* 2000). How malaria will be influenced in turn, depends on where each of these species is the primary vector of malaria and what the influence of temperature on vector competence might be (Mordecai *et al.* 2012). Increases in malaria in areas where the population of *An. arabiensis* supercedes that of other *Anopheles* vectors might be expected, but in areas where *An. funestus* or *An. gambiae* are the primary vectors, reductions in the numbers of emerging mosquitoes may lead to an overall reduction in malaria incidence in these regions.

However, climate change is predicted to lead not only to changes in mean temperatures, but to changes in temperature variability and changes in the frequency and magnitude of extreme weather events (New *et al.* 2006; Hansen *et al.* 2012). The responses of development to fluctuating temperatures for *An. arabiensis* and *An. funestus* resulted in

similar development rates for *An. arabiensis* and lower development rates for *An. funestus*. Generally insects develop significantly faster under fluctuating temperatures than they do under constant temperatures (Hagstrum and Hagstrum 1970; Hagstrum and Leach 1972), although this trend differs between species and is dependent on the amplitude of fluctuating temperatures and fluctuation about the mean (Hagstrum and Milliken 1991). Proposed reasons for differences observed between fluctuating and constant temperatures in insects include those associated with the rate summation effect and the non-linearity of the development rate-temperature relationship (Worner 1992). Under thermally fluctuating environments within the more linear part of a species developmental temperature range, the difference between constant and fluctuating temperatures is less pronounced (or development is accelerated) (Worner 1992). Under more thermally variable conditions, incorporating the upper bounds to development, development rate may be retarded (Worner 1992), as shown for both vectors in this study.

Survival under fluctuating temperatures compared to constant temperatures also differed for *An. arabiensis* and *An. funestus*, with *An. arabiensis* showing similar survival under constant temperatures and *An. funestus* showing significantly lower survival under fluctuating temperatures when compared to constant temperatures. Survival of these vectors under fluctuating temperatures closely corresponds to that for other insect species (Hagstrum and Hagstrum 1970; Hagstrum and Leach 1972; Behrens *et al.* 1983) including mosquitoes in the laboratory and under field conditions (Huffaker 1944; Afrane *et al.* 2007). Under fluctuating temperatures, injurious effects of unfavourable temperatures (e.g. chill injuries) are reduced, lowering mortality (Colinet *et al.* 2007). However, for a species such as *An. funestus*, fluctuating temperatures may be more detrimental to development due to the more thermally stable breeding sites used by this species (Gillies and Coetzee 1987). Hence, the nett effect of temperature changes associated with climate change on these three vectors'

populations is likely to be an increase for *An. arabiensis*, a decrease for *An. funestus* and a possible decrease for *An. gambiae*.

These changes in mosquito population sizes agree with predictions of the impacts of climate change on malaria in certain regions. Increases in malaria as a result of increasing temperature trends have been proposed for the highland regions of Africa (Pascual *et al.* 2006; Omumbo *et al.* 2011) and might result from the potential increase in vector populations associated with these temperature increases in these areas. For southern Africa, these results suggest that populations of vectors are likely to increase, leading to an increase in the potential for malaria in these areas. However, whether or not this is the case, also depends on changes in rainfall which are predicted to occur for southern Africa (Hewitson and Crane 2005) and therefore, also need to be considered when trying to assess the likely impacts of climate change on mosquito populations. Whether or not malaria is likely to increase, also depends not only on vector numbers but also vector competence, for which a lower than previously assumed optimum temperature has recently been determined (Mordecai *et al.* 2012). Nonetheless, the new estimate combines data across several species to build a general model, leaving taxon-specific estimates wanting. Moreover, their model needs to be disaggregated to understand how changing climates might affect vector competence. It also says little about effects of relative abundance changes among the primary vector species. Thus, from the perspective of the vectors adopted here, it is likely that malaria cases will increase in southern Africa, owing to increased vector populations. In contrast, in areas like southern Mozambique, where the main vector is *An. funestus*, an overall decrease in malaria cases might occur (Kloke *et al.* 2011). To determine the exact extent of climate change on malaria, a full mechanistic model will be required to elucidate these changes. For now, a complete thermal picture for *An. arabiensis* and *An. funestus* exists, which will go a long way

in informing mechanisms behind mosquito population responses to climate change (see Chapter 5, Figure 1).

References

- Afrane, Y.A., Zhou, G., Lawson, B.W., Githeko, A.K. and Yan, G. 2007. Life-table analysis of *Anopheles arabiensis* in western Kenya highlands: effects of land covers on larval and adult survivorship. *American Journal of Tropical Medicine and Hygiene* **77**: 660-666.
- Araújo, M.B. and Peterson, A.T. 2012. Uses and misuses of bioclimatic envelope modelling. *Ecology* **93**: 1527-1539.
- Bayoh, M.N. and Lindsay, S.W. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Bulletin of Entomological Research* **93**: 375-381.
- Bayoh, M.N. and Lindsay, S.W. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Medical and Veterinary Entomology* **18**: 174-179.
- Behrans, W., Hoffmann, K-H., Kempa, S., Gößler, S. and Merkel-Wallner, G. 1983. Effects of diurnal thermoperiods and quickly oscillating temperatures on the development and reproduction of crickets, *Gryllus bimaculatus*. *Oecologia* **59**: 279-287.
- Brittain, J.E. and Campbell, I.C. 1991. The effect of temperature on egg development in the Australian mayfly genus *Coloburiscoides* (Ephemeroptera: Coloburiscidae) and its relationship to distribution and life history. *Journal of Biogeography* **18**: 231-235.
- Buckley, L.B., Waaser, S.A., MacLean, H.J. and Fox, R. 2011. Does including physiology improve species distribution model predictions of responses to recent climate change? *Ecology* **92**: 2214-2221.

- Chown, S.L. and Nicolson, S.W. 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, Oxford, UK.
- Chown, S.L., Hoffmann, A.A., Kristensen, T.N., Angilletta Jr., M.J., Stenseth, N.C. and Pertoldi, C. 2010. Adapting to climate change: a perspective from evolutionary physiology. *Climate Research* **43**: 3-15.
- Clements, A.N. 1963. *The Physiology of Mosquitoes*. The MacMillan Company, New York, USA.
- Clements, A.N. 2000. *The Biology of Mosquitoes: Development, Nutrition and Reproduction*. CABI Publishing, Oxfordshire, UK.
- Coetzee, M., Craig, M. and le Sueur, D. 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today* **13**: 149-151.
- Colinet, H., Nguyen, T.T.A., Cloutier, C., Michaud, D. and Hance, T. 2007. Proteomic profiling of a parasitic wasp exposed to constant and fluctuating cold exposure. *Insect Biochemistry and Molecular Biology* **37**: 1177-1188.
- Collins, F.H. and Besansky, N.J. 1994. Vector biology and the control of malaria in Africa. *Science* **264**: 1874-1875.
- Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A. and Totterdell, I.J. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* **408**: 184-187.
- Crozier, L. 2004. Warmer winters drive butterfly range expansion by increasing survivorship. *Ecology* **85**: 231-241.
- Crozier, L. and Dwyer, G. 2006. Combining population-dynamic and ecophysiological models to predict climate-induced insect range shifts. *American Naturalist* **167**: 853-866.

- Davidson, J. 1944. On the relationship between temperature and rate of development of insects at constant temperatures. *Journal of Animal Ecology* **13**: 26-38.
- Dukeen, M.Y.H. and Omer, S.M. 1986. Ecology of the malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae) by the Nile in northern Sudan. *Bulletin of Entomological Research* **76**: 451-467.
- Elith, J. and Graham, C.H. 2009. Do they? How do they? Why do they differ? On finding reasons for differing performances of species distribution models. *Ecography* **32**: 66-77.
- Faraway, J.J. 2005. *Linear Models with R*. Chapman and Hall, London, UK.
- Gilchrist, G.W. 1996. A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* **50**: 1560-1572.
- Gillies, M.T. and Coetzee, M. 1987. *A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region)*. Publications of the South African Institute of Medical Research, Johannesburg, South Africa.
- Githetko, A.K., Lindsay, S.W., Confalonieri, U.E. and Patz, J.A. 2000. Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organization* **78**: 1136-1147.
- Gullan, P.J. and Cranston, P.S. 1994. *The Insects: An Outline of Entomology*. First Edition. Chapman and Hall, London, UK.
- Hagstrum, D.W. and Hagstrum, W.R. 1970. A simple device for producing fluctuating temperatures, with an evaluation of the ecological significance of fluctuating temperatures. *Annals of the Entomological Society of America* **63**: 1385-1389.
- Hagstrum, D.W. and Leach, C.E. 1972. Role of constant and fluctuating temperatures in determining development time and fecundity of three species of stored-products Coleoptera. *Annals of the Entomological Society of America* **66**: 407-410.

- Hagstrum, D.W. and Milliken, G.A. 1991. Modelling differences in insect developmental times between constant and fluctuating temperatures. *Annals of the Entomological Society of America* **84**: 369-379.
- Hansen, J., Sato, M. and Ruedy, R. 2012. Perception of climate change. Proceedings of the National Academy of Sciences of the U.S.A.: Early Edition: pnas.org/cgi/doi/10.1073/pnas.1205276109.
- Hay, S.I., Rogers, D.J., Toomer, J.F. and Snow, R.W. 2000. Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, internet access and review. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**: 113-127.
- Hewitson, B.C. and Crane, R.G. 2005. Consensus between GCM climate change projections with empirical downscaling: precipitation downscaling over South Africa. *International Journal of Climatology* **26**: 1315-1337.
- Hoffmann, A.A. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. *Journal of Experimental Biology* **213**: 870-880.
- Honěk, A. 1996. Geographical variation in thermal requirements for insect development. *European Journal of Entomology* **93**: 303-312.
- Honěk, A. and Kocourek, F. 1990. Temperature and development time in insects: a general relationship between thermal constants. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* **117**: 401-439.
- Huffaker, C.B. 1944. The temperature relations of the immature stages of the malarial mosquito *Anopheles quadrimaculatus* Say, with a comparison of the developmental power of constant and variable temperatures in insect metabolism. *Annals of the Entomological Society of America* **37**: 1-27.

- Hunt, R.H., Brooke, B.D., Pillay, C., Koekemoer, L.L. and Coetzee, M. 2005. Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector *Anopheles funestus*. *Medical and Veterinary Entomology* **19**: 271-275.
- Janion, C., Leinaas, H.P., Terblanche, J.S. and Chown, S.L. 2010. Trait means and reaction norms: the consequences of climate change/invasion interactions at the organism level. *Evolutionary Ecology* **24**: 1365-1380.
- Jarošík, V., Honěk, A. and Dixon, A.F.G. 2002. Developmental rate isomorphy in insects and mites. *American Naturalist* **160**: 497-510.
- Kearney, M. and Porter, W. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters* **12**: 334-350.
- Kearney, M., Porter, W.P., Williams, C., Ritchie, S. and Hoffmann, A.A. 2009. Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: the dengue mosquito *Aedes aegypti* in Australia. *Functional Ecology* **23**: 528-538.
- Kingsolver, J.G., Ragland, G.J. and Shlichta, J.G. 2004. Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. *Evolution* **58**: 1521-1529.
- Kirby, M.J. and Lindsay, S.W. 2004. Responses of adult mosquitoes of two sibling species, *Anopheles arabiensis* and *An. gambiae* s.s. (Diptera: Culicidae), to high temperatures. *Bulletin of Entomological Research* **94**: 441-448.
- Kirby, M.J. and Lindsay, S.W. 2009. Effect of temperature and inter-specific competition on the development and survival of *Anopheles gambiae sensu stricto* and *An. arabiensis* larvae. *Acta Tropica* **109**: 118-123.

- Kloke, R.G., Nhamahanga, E., Hunt, R.H. and Coetzee, M. 2011. Vectorial status and insecticide resistance of *Anopheles funestus* from a sugar estate in southern Mozambique. *Parasites and Vectors* **4**: 16.
- Lindsay, S.W. and Bayoh, M.N. 2004. Mapping members of the *Anopheles gambiae* complex using climate data. *Physiological Entomology* **29**: 204-209.
- Love, G.J. and Whelchel, J.G. 1957. Lethal effects of high temperatures on the immature stages of *Anopheles quadrimaculatus*. *Ecology* **38**: 570-576.
- Lyons, C.L., Coetzee, M., Terblanche, J.S. and Chown, S.L. 2012. Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*. *Malaria Journal* **11**: 226.
- Martens, W.J.M., Jetten, T.H. and Focks, D.A. 1997. Sensitivity of malaria, schistosomiasis and dengue to global warming. *Climatic Change* **35**: 145-156.
- Moffett, A., Shackelford, N. and Sarkar, S. 2007. Malaria in Africa: vector species' niche models and relative risk maps. *PLoS ONE* **2**: e824.doi:10.1371/journal.pone.0000824.
- Mordecai, E.A., Paaijmans, K.P., Johnson, L.R., Balzer, C., Ben-Horin, T., Moor, E., McNally, A., Pawar, S., Ryan, S.J., Smith, T.C. and Lafferty, K.D. 2012. Optimal temperature for malaria transmission is lower than previously predicted. *Ecology Letters*: doi: 10.1111/ele.12015.
- Munhenga, G., Brooke, B.D., Chirwa, T.F., Hunt, R.H., Coetzee, M., Govender, D. and Koekemoer, L.L. 2011. Evaluating the potential of the sterile insect technique for malaria control: relative fitness and mating compatibility between laboratory colonized and a wild population of *Anopheles arabiensis* from the Kruger National Park, South Africa. *Parasites and Vectors* **4**: 208.

- New, M., Hewitson, B., Stephenson, D.B., Tsiga, A., Kruger, A., Manhique, A., Gomez, B., Coelho, C.A.S., Masisi, D.N., Kululanga, E., Mbambalala, E., Adesina, F., Saleh, H., Kanyanga, J., Adosi, J., Bulane, L., Fortunata, L., Mdoka, M.L. and Lajoie, R. 2006. Evidence of trends in daily climate extremes over southern and west Africa. *Journal of Geophysical Research* **111**: D14102.doi:10.1029/2005JD006289.
- Omumbo, J.A., Lyon, B., Waweru, S.M., Connor, S.J. and Thomson, M.C. 2011. Raised temperatures over the Kericho tea estates: revisiting the climate in the East African highlands malaria debate. *Malaria Journal* **10**: 12.
- Paaijmans, K.P., Read, A.F. and Thomas, M.B. 2009. Understanding the link between malaria risk and climate. *Proceedings of the National Academy of Sciences of the USA* **106**: 13844-13849.
- Parham, P.E., Pople, D., Christiansen-Jucht, C., Lindsay, S., Hinsley, W. and Michael, E. 2012. Modeling the role of environmental variables on the population dynamics of the malaria vector *Anopheles gambiae* sensu strict. *Malaria Journal* **11**: 271.
- Pascual, M., Ahumada, J.A., Chaves, L.F., Rodó, X. and Bouma, M. 2006. Malaria resurgence in the East African highlands: temperature trends revisited. *Proceedings of the National Academy of Sciences of the U.S.A.* **103**: 5829-5834.
- Pascual, M., Dobson, A.P. and Bouma, M.J. 2009. Underestimating malaria risk under variable temperatures. *Proceedings of the National Academy of Sciences of the U.S.A.* **106**: 13645-13646.
- Patz, J.A., Graczyk, T.K., Geller, N. and Vittor, A.Y. 2000. Effects of environmental temperature on emerging parasitic diseases. *International Journal for Parasitology* **30**: 1395-1405.
- Phillips, B.L., Chipperfield, J.D. and Kearney, M.R. 2008. The toad ahead: challenges of modeling the range and spread of an invasive species. *Wildlife Research* **35**: 222-234.

- Quinn, G.P. and Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, UK.
- Régnière, J., Powell, J., Bentz, B. and Nealis, V. 2012. Effects of temperature on development, survival and reproduction of insects: experimental design, data analysis and modelling. *Journal of Insect Physiology* **58**: 634-647.
- Reid, H., Haque, U., Clements, A.C.A., Tatem, A.J., Vallely, A., Masud Ahmed, S., Islam, A. and Haque, R. 2010. Mapping malaria risk in Bangladesh using Bayesian geostatistical models. *American Journal of Tropical Medicine and Hygiene* **84**: 861-867.
- Richardson, K., Hoffmann, A.A., Johnson, P., Ritchie, S. and Kearney, M.R. 2011. Thermal sensitivity of *Aedes aegypti* from Australia: empirical data and predictions of effects on distribution. *Journal of Medical entomology* **48**: 914-923.
- Rocca, K.A.C., Gray, E.M., Constantini, C. and Besansky, N.J. 2009. 2La chromosomal inversion enhances thermal tolerance of *Anopheles gambiae* larvae. *Malaria Journal* **8**: 147.
- Rogers, D.J. and Randolph, S.E. 2000. The global spread of malaria in a future, warmer world. *Science* **289**: 1763-1766.
- Sanderson, M.G., Hemming, D.L. and Betts, R.A. 2011. Regional temperature and precipitation changes under high-end ($\geq 4^{\circ}\text{C}$) global warming. *Philosophical Transactions of the Royal Society A* **369**: 85-98.
- Small, J., Goetz, S.J. and Hay, S.I. 2003. Climatic suitability for malaria transmission in Africa, 1911-1995. *Proceedings of the National Academy of Sciences of the U.S.A.* **100**: 15341-15345.
- Smith, D.L., McKenzie, F.E., Snow, R.W. and Hay, S.I. 2007. Revisiting the basic reproductive number for malaria and its implications for malaria control. *PLoS Biology* **5**: e42. doi:10.1371/journal.pbio.0050042.

- Tanser, F.C., Sharp, B. and le Sueur, D. 2003. Potential effects of climate change on malaria transmission in Africa. *Lancet* **362**: 1792-1798.
- Trudgill, D.L., Honěk, A., Li, D. and van Straalen, N.M. 2005. Thermal time – concepts and utility. *Annals of Applied Biology* **146**: 1-4.
- Williams, C.R., Bader, C.A., Kearney, M.R., Ritchie, S.A. and Russell, R.C. 2010. The extinction of dengue through natural vulnerability of its vectors. *PLoS Neglected Tropical Diseases* **4**: e922.doi:10.1371/journal.pntd.0000922.
- Worner, S.P. 1992. Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. *Environmental Entomology* **21**: 689-699.

Figure legends

Figure 1. Mean development rate (days^{-1}) per constant temperature (ranging from 15°C to 35°C) for *Anopheles arabiensis* (blue \blacklozenge), *An. funestus* (red \blacksquare) and *An. gambiae* (green \blacktriangle) (data for *An. gambiae* obtained from Bayoh and Lindsay 2003). Lines linking data points are not fitted and are for reference only. 95% confidence intervals are shown for *An. arabiensis* and *An. funestus*, but are typically obscured by the data points. For the full range of temperatures, the development rate of each species is typically non-linear. For each species, there exists a linear part to this curve, which differs between species.

Figure 2. Development rate (days^{-1}) of *Anopheles arabiensis* (left) and *Anopheles funestus* (right) at the two fluctuating temperature regimes and the constant mean of 25°C. Differences in lower case letters indicate significant differences in development rates (within each species) between the two fluctuating temperature regimes of 20 to 30°C and 15 to 35°C, and their constant mean of 25°C (ANOVA: *An. arabiensis* $df=2, 72, F=25.5, P<0.0001$; *An. funestus* $df=2, 72, F=395.3, P<0.001$). Development at 25°C was significantly faster than at fluctuating temperatures for *An. funestus* but did not differ markedly between treatments for *An. arabiensis*.

Figure 3. Mean percentage survival per constant temperature for *Anopheles arabiensis* (blue), *An. funestus* (red) and *An. gambiae* (green) (data for *An. gambiae* obtained from Bayoh and Lindsay 2003). Error bars are shown for *An. arabiensis* and *An. funestus*. Survival of *An. gambiae* is highest at the lower end of the temperature range, while survival of *An. arabiensis* is highest towards the upper end of this temperature scale. *An. funestus* displays lower survival at all temperatures when compared to the other two vector species. No development

and hence, no survival occurred at 15°C and 35°C for *An. funestus* and *An. arabiensis*, while *An. gambiae* did not develop at 16°C and 34°C.

Figure 4. Mean percentage survival for *Anopheles arabiensis* (left) and *Anopheles funestus* (right) between the two fluctuating temperature regimes and constant mean of 25°C. Differences in lower case letters indicate significant differences in survival between temperature treatments within each species (GLZ with binomial distribution and logit link: *An. arabiensis* df=2, 72, chi-squared=15.4, P<0.001; *An. funestus* df=2, 72, chi-squared=164.9, P<0.0001). Survival of *An. arabiensis* was only negatively affected at the most variable temperature treatment. *Anopheles funestus* experienced severely lowered survival at the two fluctuating temperature treatments when compared to the constant 25°C treatment.

Figures and Tables

Fig. 1

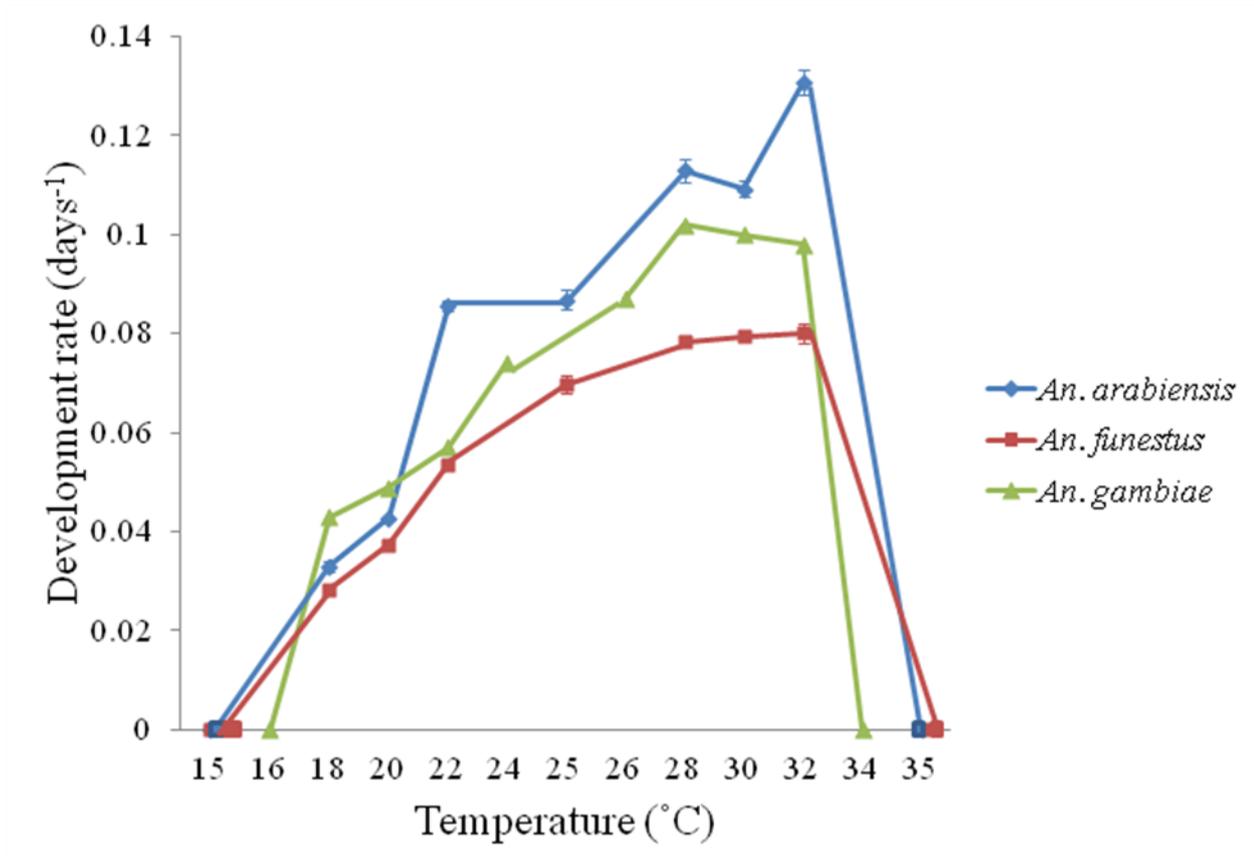


Fig. 2

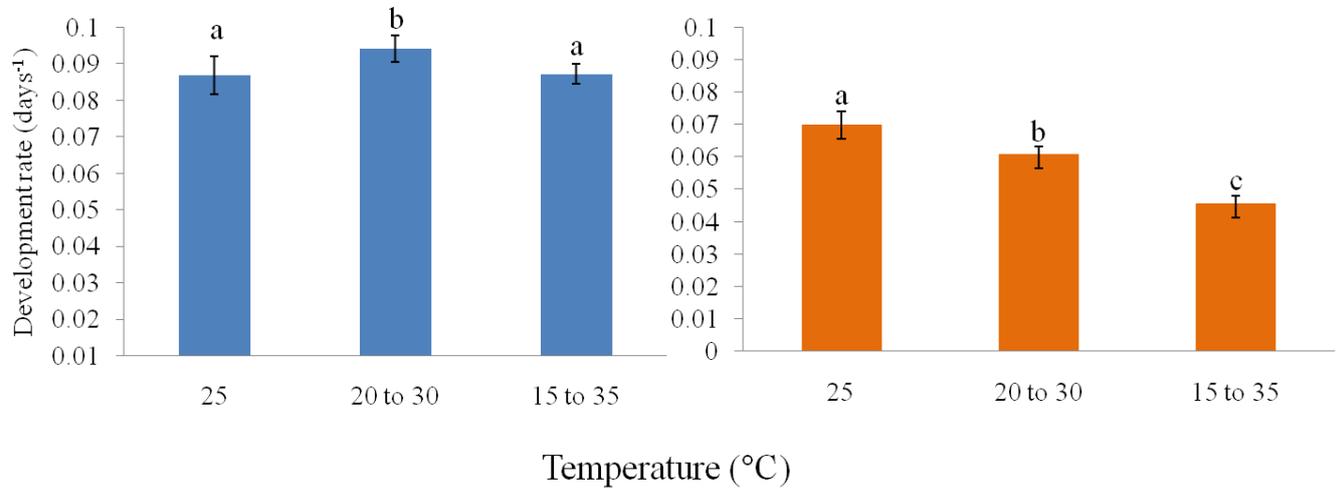


Fig. 3

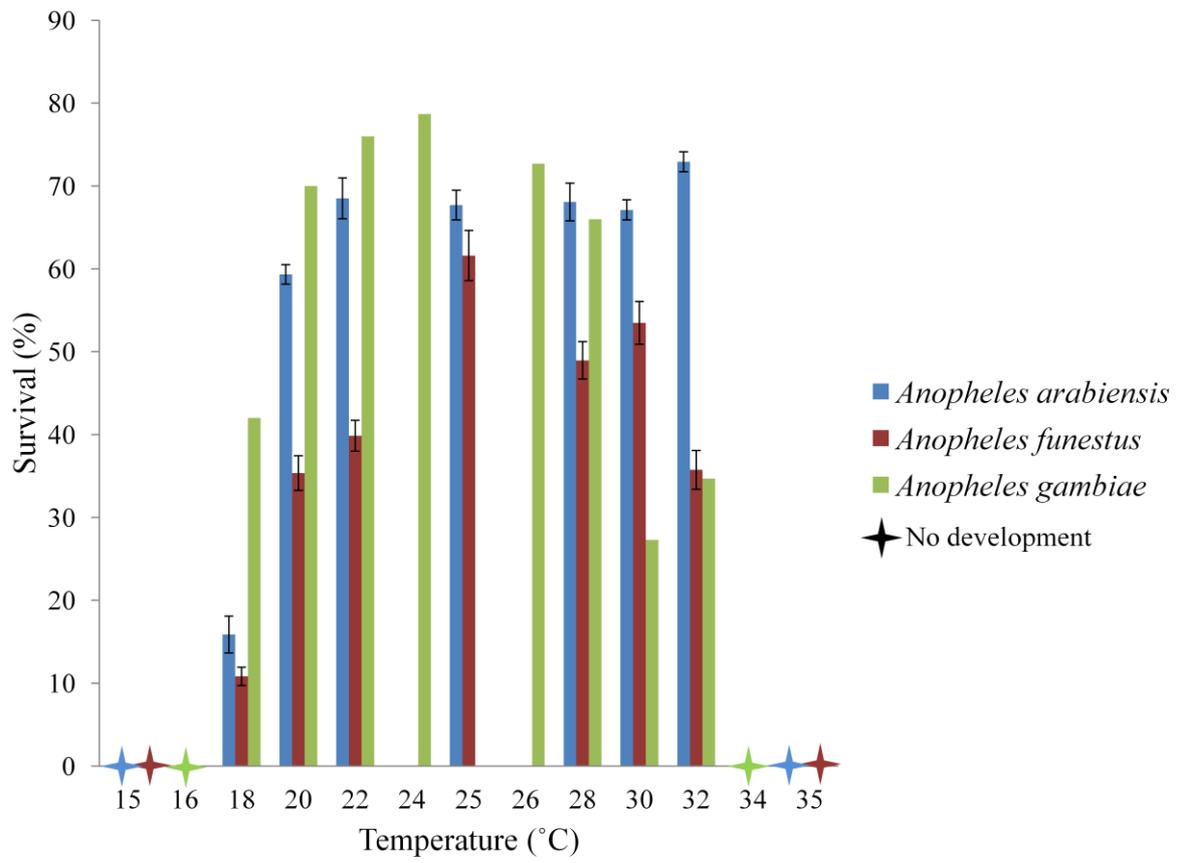


Fig. 4

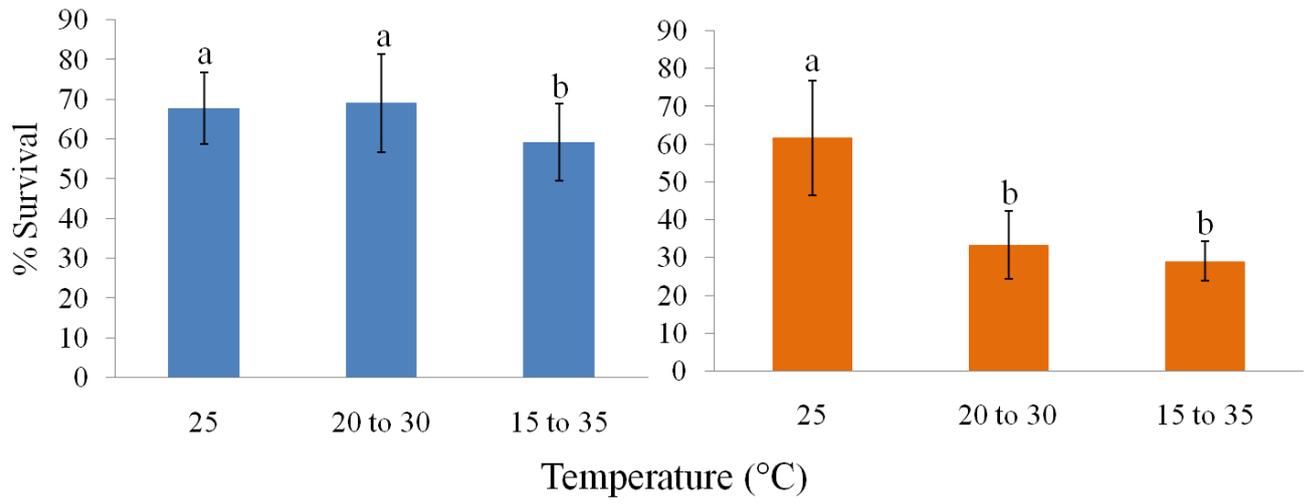


Table 1. Lower developmental threshold (LDT), sums of effective temperatures (SET), T_{opt} , μ_{max} for *Anopheles arabiensis* and *Anopheles funestus*. LDT and SET were estimated from standard methods using the linear part of the development rate-temperature curve, while T_{opt} and μ_{max} were obtained by the best curve fit for each stage (see Table S4 for equations).

Species	Life stage	T_{opt} (°C)	μ_{max} (days⁻¹)	LDT	SET
<i>An. arabiensis</i>	Eggs	31.1	0.7727	13.1	25.4
	Larvae	31.1	0.2080	14.3	75.8
	Pupae	28.7	1.6109	14.0	13.8
	Overall	31.5	0.1292	13.4	137.0
<i>An. funestus</i>	Eggs	31.0	0.5772	12.7	35.6
	Larvae	30.9	0.1357	13.8	116.3
	Pupae	27.3	0.9052	14.4	16.3
	Overall	31.1	0.0813	14.0	166.7

Table 2. Results from general linear models comparing development rates (days^{-1}) of each stage and from egg to adult, between species, as a function of temperature and species. Model results are shown in the left hand column under each stage comparison.

Stage	Predictor	df	SS	F	P-value
Eggs	Temperature	8	2.63	80.39	< 0.0001
$F_{17,432}=184.8;$ $P<0.0005$	Species	1	0.86	209.45	< 0.0001
	Temperature*Species	8	1.51	46.29	< 0.0001
Larvae	Temperature	8	0.24	213.33	< 0.0001
$F_{17,432}=460.5;$ $P<0.0005$	Species	1	0.03	234.73	< 0.0001
	Temperature*Species	8	0.05	52.12	< 0.0001
Pupae	Temperature	8	8.29	8.07	< 0.0001
$F_{17,432}=20.51;$ $P<0.0005$	Species	1	7.75	60.34	< 0.0001
	Temperature*Species	8	8.62	8.39	< 0.0001
Egg to adult	Temperature	8	0.08	361.88	< 0.0001
$F_{17,432}=795.9;$ $P<0.0005$	Species	1	0.02	766.33	< 0.0001
	Temperature*Species	8	0.02	110.25	< 0.0001

Table 3. Results from two-sample t-tests comparing T_{opt} and μ_{max} of each stage between *Anopheles arabiensis* and *Anopheles funestus*.

Life stage	t-value	Df	P-value
T_{opt}			
Eggs	-0.06	48	0.9492
Larvae	-2.03	48	0.0475
Pupae	-1.86	48	0.0694
Overall	-3.97	48	0.0002
μ_{max}			
Eggs	-16.34	48	< 0.0001
Larvae	-18.86	48	< 0.0001
Pupae	-1.98	48	0.0537
Overall	-33.71	48	< 0.0001

Supplementary Materials

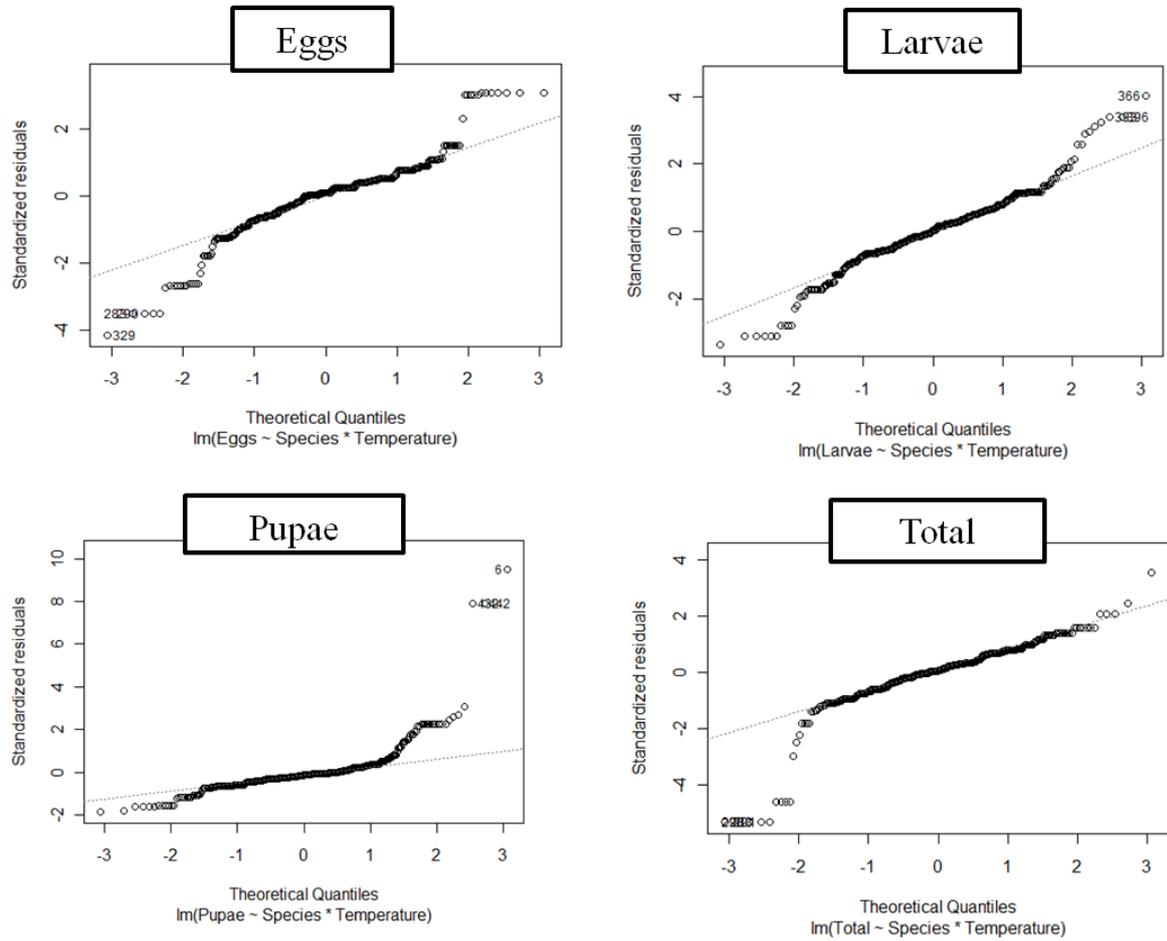


Figure S1. Normal QQ residual plots for comparisons between stages (eggs, larvae, pupae and total development) of species that meet model assumptions.

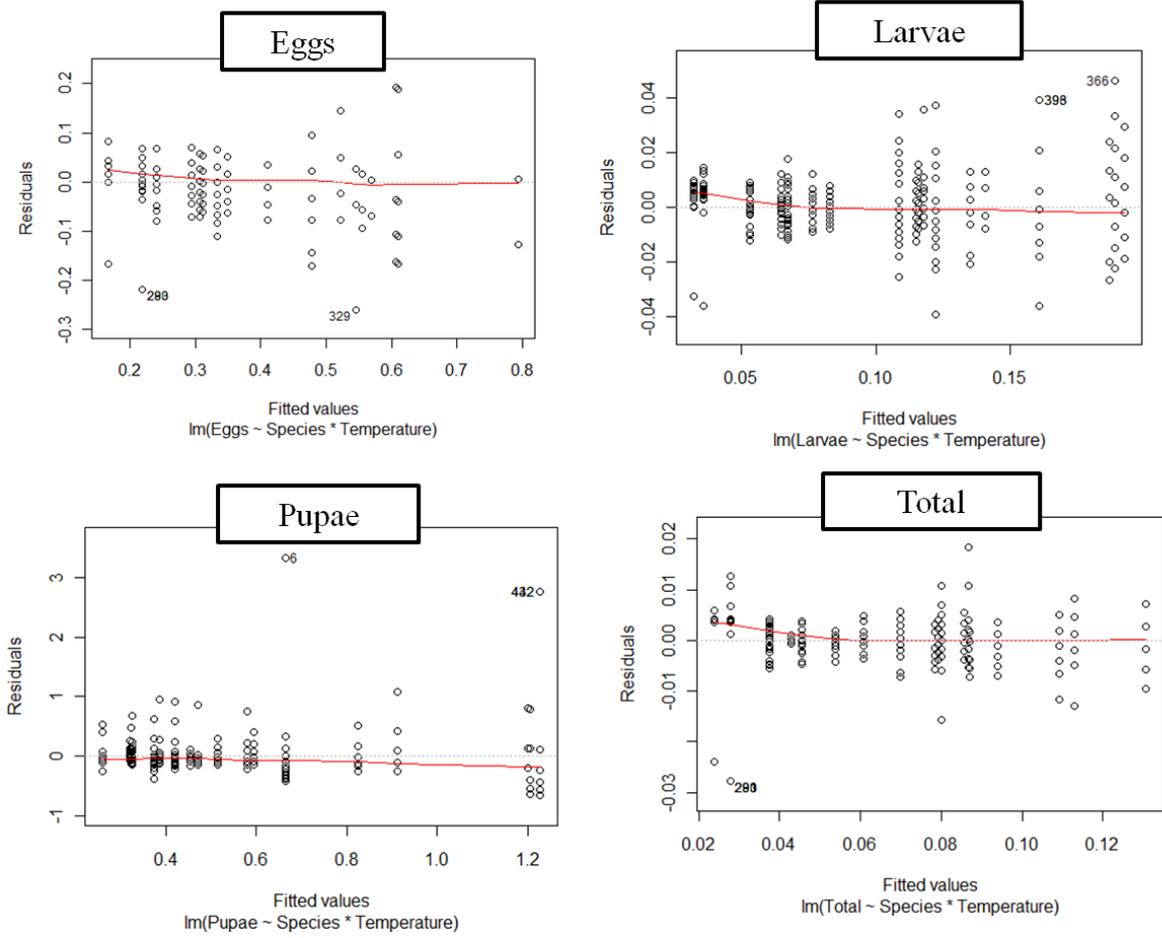


Figure S2. Fitted vs. residual plots of development rates of eggs, larvae, pupae and total development between species.

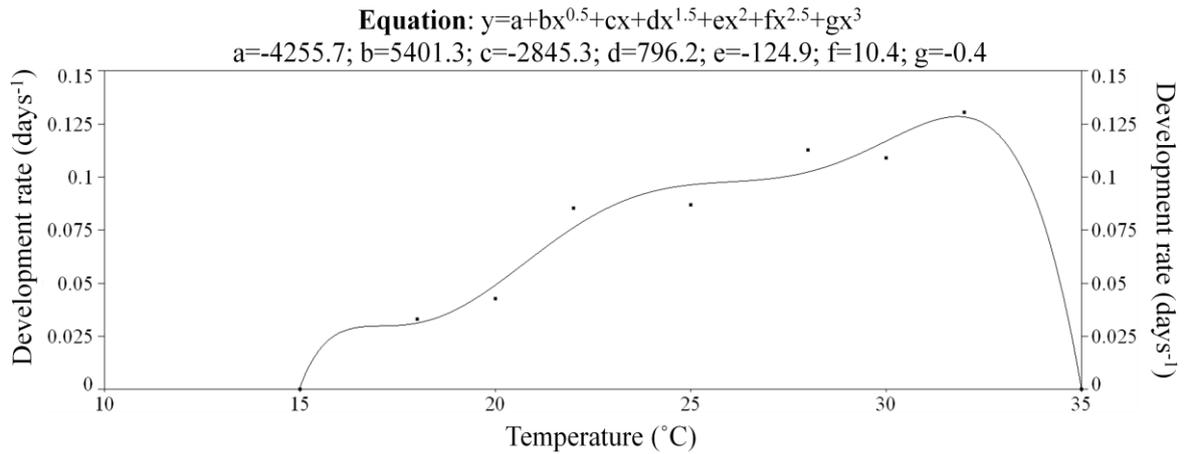


Figure S3. Rate-temperature relationship for overall development from egg to adult of *Anopheles arabiensis*. The best-fit equation and estimates are shown in the figure title ($r^2=0.986$). Development rate for *Anopheles arabiensis* is typically non linear across the full range of temperatures from 15°C to 35°C. No development occurred at 15°C or 35°C for this species.

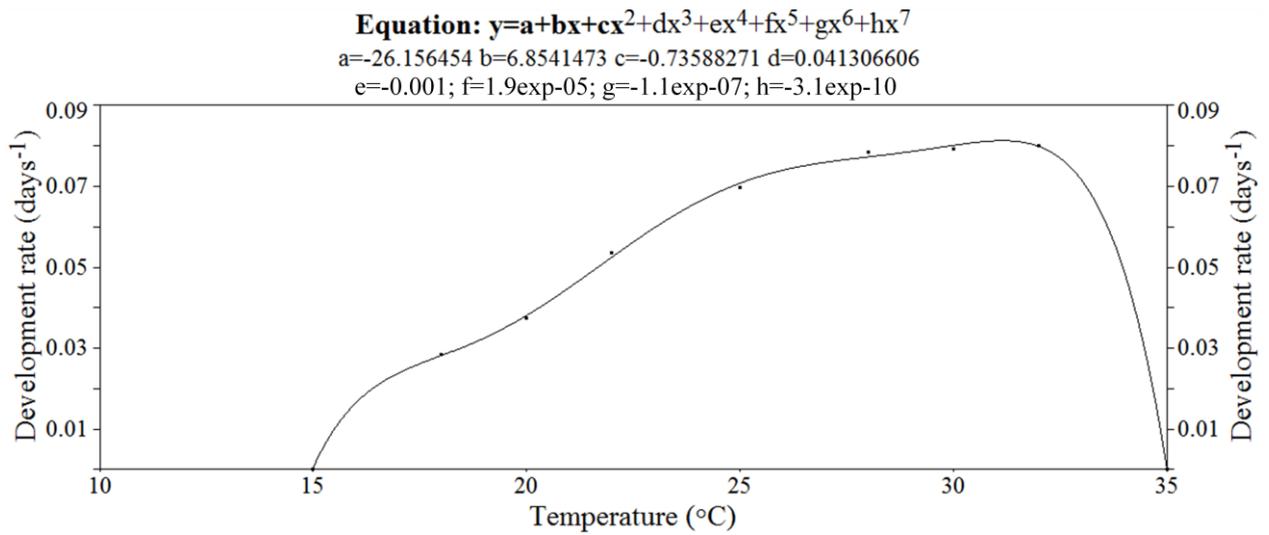


Figure S4. Rate-temperature relationship for overall development from egg to adult of *Anopheles funestus*. The best-fit equation and estimates are shown in the figure title ($r^2=0.999$). *Anopheles funestus* does not develop at 15°C or 35°C and development rate generally increases in a non linear fashion from 18°C to 30°C.

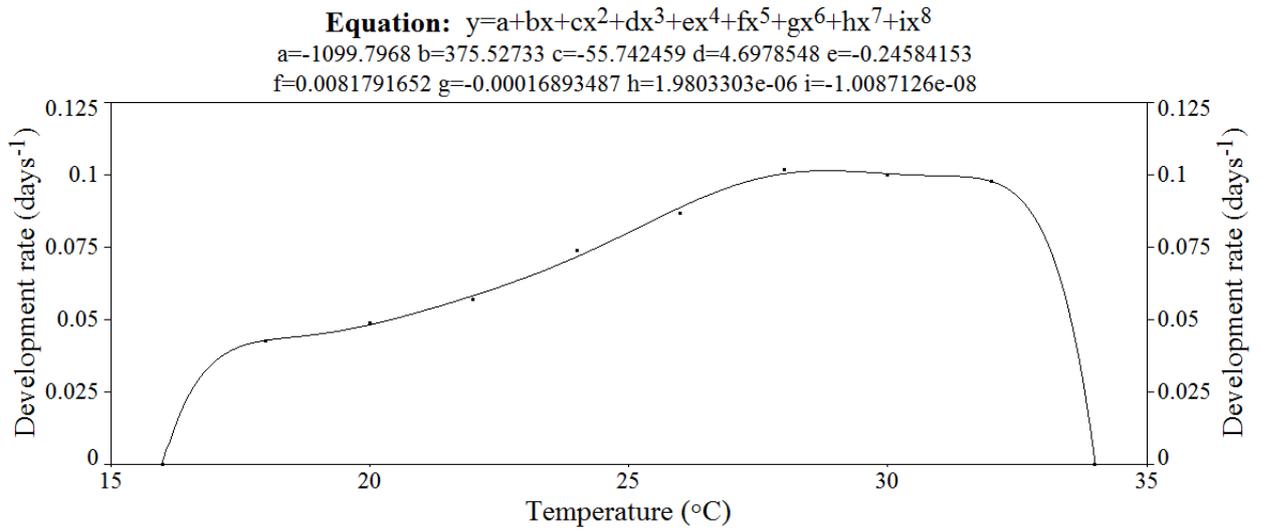


Figure S5. Non-linear curve fit for *Anopheles gambiae* (data from Bayoh and Lindsay 2003) ($r^2=0.999$). *Anopheles gambiae* does not develop at 16°C and 34°C and typically shows a linear increase in development rate with temperature, up to approximately 28°C.

Table S1. Results for normality and homogeneity of variance tests from Shapiro-Wilk's and Levene's tests, respectively, for development rate at the constant temperature treatment of 25°C and the two fluctuating temperature treatments of 20°C to 30°C and 15°C to 35°C for *Anopheles arabiensis* and *Anopheles funestus*.

Species	Temperature treatment	Shapiro-Wilk's test	Levene's test
<i>Anopheles arabiensis</i>	25°C	W=0.77, P<0.0001	
	20°C to 30°C	W=0.78, P<0.0010	
	15°C to 35°C	W=0.68, P<0.0001	
	Constant vs. fluctuating groups		df=2, 72, F=2.12, P=0.1269
<i>Anopheles funestus</i>	25°C	W=0.92, P=0.0551	
	20°C to 30°C	W=0.91, P=0.0296	
	15°C to 35°C	W=0.94, P=0.1540	
	Constant vs. fluctuating groups		df=2, 72, F=8.11, P=0.0010

Table S2. Average development time (days \pm SD) for each life stage to the next and overall from egg to adult, for each species, *Anopheles arabiensis* and *Anopheles funestus* and average % survival (\pm S.E.) at each of 11 temperature treatments. No development to the adult stage occurred at 15°C or 35°C for either species.

Species	Temperature (°C)	Eggs (days)	Larvae (days)	Pupae (days)	Overall (days)	% Survival (\pm S.E.)
<i>Anopheles arabiensis</i>	15	0	0	0	0	0
	18	3.8 (\pm 0.3)	23.2 (\pm 1.8)	3.4 (\pm 1.6)	31 (\pm 1.55)	15.7(\pm 1.7)
	20	4.3 (\pm 0.8)	15.2 (\pm 1.5)	4.3 (\pm 1.3)	23.4 (\pm 0.1)	59.1 (\pm 2.1)
	22	1.8 (\pm 0.1)	8.6 (\pm 0.4)	1.4 (\pm 0.3)	11.7 (\pm 0.3)	64.9 (\pm 1.9)
	25	3.3 (\pm 0.5)	5.4 (\pm 0.4)	3.0 (\pm 0.5)	11.7 (\pm 0.4)	68.0 (\pm 3.5)
	28	1.7 (\pm 0.4)	6.3 (\pm 0.4)	0.9 (\pm 0.1)	8.8 (\pm 0.44)	66.8 (\pm 2.3)
	30	1.7 (\pm 0.3)	5.3 (\pm 0.3)	2.2 (\pm 0.3)	9.1 (\pm 0.3)	67.3 (\pm 2.6)
	32	1.3 (\pm 0.0)	5.4 (\pm 0.5)	1.1 (\pm 0.3)	7.7 (\pm 0.4)	72.9 (\pm 2.4)
	35	0	0	0	0	0
	20 to 30	1.8 (\pm 0.0)	7.1 (\pm 0.5)	1.8 (\pm 0.2)	10.7 (\pm 0.4)	69.0 (\pm 12.3)
	15 to 35	1.8 (\pm 0.1)	8.7 (\pm 0.5)	1.0 (\pm 0.4)	11.4 (\pm 0.3)	59.2 (\pm 9.6)

<i>Anopheles funestus</i>	15	0	0	0	0	0
	18	5.1 (± 0.3)	25.5 (± 1.4)	4.5 (± 1.6)	35.2 (± 1.2)	10.9 (± 2.5)
	20	4.6 (± 0.4)	19 (± 2.3)	3.4 (± 0.6)	26.9 (± 2.1)	35.3 (± 1.2)
	22	3.3 (± 0.4)	13.2 (± 0.7)	2.3 (± 0.3)	18.5 (± 0.3)	39.2 (± 3.7)
	25	2.9 (± 0.3)	9.4 (± 1.0)	2.0 (± 0.3)	14.4 (± 0.9)	59.8 (± 1.8)
	28	2.8 (± 0.2)	8.6 (± 0.4)	1.2 (± 0.2)	12.8 (± 0.5)	48.9 (± 3.0)
	30	2.0 (± 0.2)	7.4 (± 0.5)	3.3 (± 0.4)	12.6 (± 0.3)	53.5 (± 1.2)
	32	2.1 (± 0.3)	8.3 (± 0.9)	2.1 (± 0.7)	12.5 (± 0.6)	36.8 (± 1.2)
	35	0	0	0	0	0
	20 to 30	2.5 (± 0.3)	12.1 (± 0.6)	2.0 (± 0.5)	16.6 (± 0.5)	33.3 (± 8.9)
	15 to 35	3.5 (± 0.5)	15.6 (± 1.2)	3.2 (± 1.1)	21.9 (± 1.0)	29.0 (± 5.2)

Table S3. Equations best describing the non-linear relationship between development rate of each stage and overall development from egg to adult, for *Anopheles arabiensis* and *Anopheles funestus* (parameter values shown in Table S4).

Species	Stage	Equation
<i>Anopheles</i>		
<i>arabiensis</i>	Total	$y=a+b/\ln x+c/(\ln x)^2+d/(\ln x)^3+e/(\ln x)^4+f/(\ln x)^5+g/(\ln x)^6+h/(\ln x)^7$
	Eggs	$y=a+b/x+c/x^2+d/x^3+e/x^4+f/x^5+g/x^6+h/x^7$
	Larvae	$y=a+bx^2+cx^4+dx^6+ex^8+fx^{10}+gx^{12}+hx^{14}$
	Pupae	$y^{-1}=a+bx+cx^2+dx^3+ex^4+fx^5$
<i>Anopheles</i>		
<i>funestus</i>	Total	$y=a+bx+cx^2+dx^3+ex^4+fx^5+gx^6+hx^7$
	Eggs	$y=a+bx^2+cx^4+dx^6+ex^8+fx^{10}+gx^{12}+hx^{14}$
	Larvae	$y=a+bx^2+cx^4+dx^6+ex^8+fx^{10}+gx^{12}+hx^{14}$
	Pupae	$y=a+bx^2+cx^4+dx^6+ex^8+fx^{10}+gx^{12}+hx^{14}$

Table S4. Parameter estimates for non-linear curve fits for development rate from one stage to the next and from egg to adult (total) for *Anopheles arabiensis* and *Anopheles funestus* (equations in Table S3).

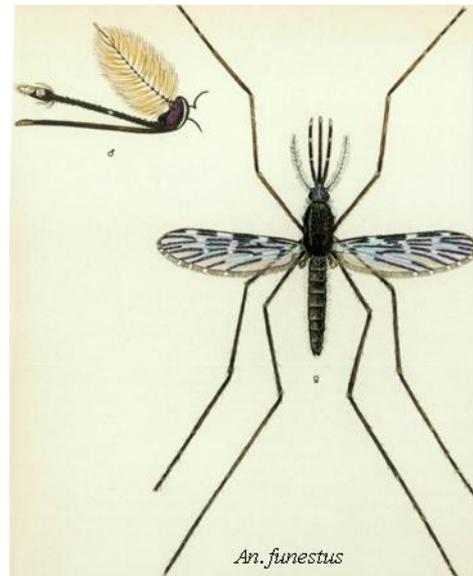
Species	Stage	Parameters
<i>Anopheles arabiensis</i>	Total	a=-553026; b=11605820; c=-1exp+08; d=5.17exp+08; e=-1.5exp+09; f=2.73exp+09; g=-2.7exp+09; h=1.13exp+09
	Eggs	a=1172.6; b=-255851; c=22175477; d=-1exp+09; e=2.66exp+10; f=-4exp+11; g=3.32exp+12; h=- 1.1exp+13
	Larvae	a=-7.1; b=0.09; c=-0.0006; d=1.63exp-06; e=-2.7exp- 09; f=2.5exp-12; g=-1.3exp-15; h=2.6exp-19
	Pupae	a=-7306.9; b=1586.9; c=-136.6; d=5.8; e=-0.12; f=0.001
<i>Anopheles funestus</i>	Total	a=-26.2; b=6.9; c=-0.74; d=0.04; e=-0.001; f=1.9exp-05; g=-1.1exp-07; h=-3.1exp-10
	Eggs	a=-17.4; b=0.24; c=-0.001; d=3.9exp-06; e=-6.6exp-09; f=6.3exp-12; g=-3.2exp-15; h=6.8exp-19
	Larvae	a=-2.6; b=0.04; c=-0.0002; d=5.4exp-07; e=-9.1exp-10; f=8.6exp-13; g=-4.3exp-16; h=8.7exp-20
	Pupae	a=51.3; b=-0.8; c=0.004; d=-1.4exp-05; e=2.4exp-08; f=-2.3exp-11; g=1.2exp-14; h=-2.6exp-18

Table S5. Equations used for comparisons between T_{opt} and μ_{max} of *Anopheles arabiensis* and *Anopheles funestus* obtained from 25 separate non-linear curves for overall development, and development of each stage.

Life stage	Equation
Egg	$y=a+b/x+c/x^2+d/x^3+e/x^4+f/x^5+g/x^6+h/x^7$
Larva	$y=a+b/x+c/x^2+d/x^3+e/x^4+f/x^5+g/x^6+h/x^7$
Pupa	$y=a+bx^2+cx^4+dx^6+ex^8+fx^{10}+gx^{12}+hx^{14}$
Total	$y=a+bx+cx^2+dx^3+ex^4+fx^5+gx^6+hx^7$

Chapter 4

Intrinsic and extrinsic factors interact to affect desiccation tolerance in adult mosquitoes: implications for malaria vector competence



Images taken from “Lighton’s Insects of Medical Importance. Original paintings of *Diptera* by Norman C.K. Lighton” by M. Coetzee, D. MacFadyen and R. Hunt. Published by the National Health Laboratory Service, Sandringham, South Africa. 2009.

Introduction

Persistence of any population not being continually rescued by immigration is dependent on survival and reproduction. Within the adult cohort, survival between reproductive bouts is especially significant for iteroparous species (Calow 1979). Survival is dependent both on density-dependent factors, such as resource availability, disease and predation, and largely density-independent factors such as tolerance of abiotic conditions, although the two may interact (Menge and Sutherland 1987).

For insects, ambient temperature and water availability are the two key extrinsic factors influencing survival, with the latter being significant especially for smaller species (Stone and Willmer 1989; Gibbs *et al.* 1997; Chown 2002; Terblanche *et al.* 2006; Stillwell *et al.* 2010). Much is now known about the effects of temperature and water availability on adult survival, and the responses insects mount to improve that survival (Gibbs *et al.* 1997; Le Lagadec *et al.* 1998; Hoffmann *et al.* 2001; Marron *et al.* 2003; Chown and Nicolson 2004; Chown *et al.* 2011). By contrast, less is known about how survival changes with sex and age in iteroparous adults (see Bowler and Terblanche 2008 for review).

In vector species, such as mosquitoes or tsetse, which have multiple reproductive bouts and feed between them, understanding intrinsic survival and its change with age and sex is important for developing life tables and the likely change in their components under different environmental conditions. Differential impacts of conditions on the sexes and at different ages are likely to have major roles on the extrinsic factors determining vector competence. That is, on the factors that govern the probability that a vector will encounter a host (Hardy *et al.* 1983).

Particularly important for small ectothermic species is how such survival may be influenced by interactions between temperature and water availability. Investigations of survival of dry conditions are typically undertaken at a given water content of the air and at a

specific temperature to obtain an indication of the resistance of a given species or population, often to understand relative resistance or tolerance (Hoffmann and Parsons 1989; Hoffmann 1990; Gibbs and Markow 2001; Gray and Bradley 2005). However, as is widely acknowledged, under most natural conditions, various environmental factors change simultaneously, and this is true especially for temperature and water availability that so substantially affect small ectotherms such as insects (Stone and Willmer 1989; Chown and Nicolson 2004). Nonetheless, investigations of the effects of such interactions are uncommon, and indeed, are more generally considered one of the most significant challenges both in physiology (Benoit and Denlinger 2010; Hoffmann 2010; Kleynhans and Terblanche 2011) and in a broader macrophysiological context (Gaston *et al.* 2009). For vectors they are just as unusual, even though understanding these interactions is not only significant for estimating vector competence and therefore disease prevalence under current conditions, but also how these may change into the future as climates across the globe change.

Whilst much of the focus of current research on climate change impacts on vectors and other species is rightly on temperature change, especially given forecasts of rapid changes in means and extremes (Hansen *et al.* 2012), the likely impacts of changing water availability are also important. Current forecasts and records indicate changing precipitation regularity, event size and overall quantity (Fung *et al.* 2011). How such changes will play out geographically is currently uncertain, as most work in the area acknowledges. However, in ascertaining how the changing situation will affect populations of significant species, such as the vectors of human diseases, uncertainty can be reduced overall by establishing more certain links between the abiotic environment and animal responses (see discussion in Lafferty 2009). Even if the climate model outputs remain inexact, the range of uncertainty may be reduced by including more explicit information in downstream aspects of the

modelling process. Indeed, calls for such improved understanding are widespread (Thuiller *et al.* 2008) and growing (Hansen *et al.* 2012; Buckley and Kingsolver 2012).

For these reasons, the influence of sex, age, temperature and water availability on the desiccation tolerance of two of the most significant vectors of *Plasmodium falciparum* malaria in southern Africa, *Anopheles arabiensis* and *An. funestus* are investigated. Although only the females are vectors of the *Plasmodium* parasite, population persistence is dependent both on male and on female survival. Moreover, disease prevalence is dependent on multiple meals taken by the female (parasite acquisition), which in turn requires survival between feeding bouts. These two species were selected specifically also because of their different life histories and habitat preferences (Gillies and Coetzee 1987), and because of the three vector species of malaria in south-eastern Africa, they are least investigated. The third vector, *An. gambiae*, is more widespread and better known (e.g. Bayoh and Lindsay 2003, 2004; Rocca *et al.* 2009).

Methods

Study populations

Anopheles arabiensis mosquitoes from the KGB-strain, originally established from individuals caught in Zimbabwe in 1975, and *Anopheles funestus* mosquitoes from the FUMOS-strain, originally established from individuals caught in Mozambique in 2000, were used for desiccation tolerance and starvation resistance experiments. Prior to experiments, colonies were maintained at insectary conditions ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $\pm 80\%$ RH), checked with a Masons Thermohygrometer, Brannan, UK). All mosquitoes were provided with a 10% sugar water solution *ad libitum* and females were offered a blood meal three times weekly. Given the importance of age in stage-structured population models (Kareiva *et al.* 1990), and the possible effect of senescence or loss of tolerance with increasing age (Gibbs and Markow

2001; Bowler and Terblanche 2008; Lyons *et al.* 2012), three age groups for each species were used during desiccation resistance experiments. Age groups for *An. arabiensis* mosquitoes were 10-, 15- and 20-day olds, while ages for *An. funestus* mosquitoes were 10-, 20- and 30-day olds. Ages chosen differed between species because of the species' biology (Hunt *et al.* 2005; Munhenga *et al.* 2011), with *An. funestus* being the longer-lived of the two.

Desiccation tolerance trials

Between 18 and 20 individual males and females from each of these age groups were exposed to different combinations of three relative humidity (RH) treatments and three different experimental temperatures. The lowest humidity, *c.*5%, was maintained through the use of silica gel, the *c.*55% treatment through saturated $\text{Mg}(\text{NO}_3)_2$ solution (Winston and Bates 1960), and the *c.*100% humidity treatment through the use of double-distilled water. Temperature was controlled using PTC-1 cabinets (Sable Systems, Las Vegas, Nevada, USA) or a SANYO incubator (MIR-154, SANYO Electric Co. Ltd. Osaka, Japan). Temperature and humidity were checked using hygchron i-buttons (DS 1923-F5, Maxim/Dallas Semiconductor, Sunnyvale, CA, USA). Each mosquito was anaesthetized by brief CO_2 exposure (< 10 s) so that initial mass could be obtained (Mettler Toledo UMX2 microbalance, Greifensee, Switzerland) to the nearest 0.0001 mg. Following weighing, each individual mosquito was placed into a clear, double open-ended 10 ml vial, closed on either end with 1 mm gauze mesh. Each of the twenty vials containing an individual mosquito was placed into a clear container (230 mm X 160 mm X 100 mm) containing the silica gel, $\text{Mg}(\text{NO}_3)_2$ solution, or distilled water. The entire container was then sealed and placed at one of three temperatures (20°C, 25°C, 30°C) with a 12L:12D cycle. Mosquitoes were checked every 2-3 hours for the first 24 hours and then every 6 hours until death occurred. The container was opened to remove mosquitoes that were visibly knocked down and showed

signs of desiccation stress, with care taken to remove mosquitoes in under 30 seconds to ensure humidity within the containers was kept as constant as possible. Knocked down or dead mosquitoes were weighed immediately after removal from experimental conditions (Mettler Toledo UMX2 microbalance) to obtain a wet mass at death for each individual. The difference between initial mass and wet mass at death provided an indication of mass lost from desiccation. Dividing this difference by the time each mosquito took to die, provided an indication of water loss rate (WLR in mg/h). In total, 1120 and 1060, *An. funestus* and *An. arabiensis* individuals, respectively, were used in the desiccation trials.

Influence of mass, age, sex, relative humidity and temperature on desiccation tolerance

Several insect species show a relationship between sex and mass, with females often, though not always, being heavier than males (Studier and Sevick 1992; Chown and Gaston 2010; Stillwell *et al.* 2010). To determine the influence of sex, mass (mg), relative humidity (RH %), age (days) and temperature (°C), on desiccation tolerance (response), measured as survival time (hours), a generalized linear model with quasipoisson error distribution (to correct for overdispersion (Crawley 2007)) and log link function was implemented in R (v. 2.15.1) (R Foundation for Statistical Computing, Vienna, Austria), for each species (Table 1). Because data were zero-bounded on the left and showed left-skewed distributions, the poisson error distribution was chosen for initial analyses (Quinn and Keough 2002). These models showed a high degree of overdispersion for reasons explained by Crawley (2007) and the quasipoisson family function was fit to all subsequent models. The highest order interactions were removed from the model sequentially if they were not significant, so results present the minimal adequate model (Crawley 2007). To determine the relative effect size change of temperature and humidity on survival time, the mean survival time for each

temperature at each humidity was calculated and the difference between the minimum time and maximum time resulting from temperature or humidity was determined.

Organisms can survive desiccation by several means, two of which are increased body mass/size and reduced water loss rates (Gibbs *et al.* 1997). To determine the relative contributions of water loss rate and initial mass to desiccation tolerance of *An. arabiensis* and *An. funestus*, a generalized linear model with quasipoisson distribution of errors (to correct for overdispersion (Crawley 2007)) and log link function, using survival time of each group as the dependent data and WLR and initial mass as independent data, was implemented in R (v.2.15.1). This analysis was performed for only the 5% relative humidity treatment and was performed per temperature, per age and per sex category. Only one humidity treatment was chosen to provide an indication of possible mechanisms behind desiccation resistance in these species. The estimates from these models indicate how the response (time) changes with each parameter (WLR or mass), with negative indicating a negative correlation between time (dependent) and the relative parameter. The higher/lower estimate value indicates a greater contribution of that parameter to influencing the dependent variable, time.

To graphically present the influence of age and temperature on desiccation tolerance (survival time) of each species, a distance-weighted least squares 3D contour plot using the residuals from a regression of time on mass, against temperature and age was plotted for each species. No residuals were used for statistical analyses, only for graphical representations.

To graphically present the influence of temperature and humidity on survival time for both species, the residuals from a mass *vs.* time regression of the oldest groups for both species (20-day olds for *An. arabiensis* and 30-day old for *An. funestus*) were plotted on a distance-weighted least squares 3D contour plot against temperature and relative humidity. Owing to the seasonality of malaria in certain areas, the ability of these older ages to survive desiccation could be key to their overwintering ability (Lehmann *et al.* 2010).

Species comparison

How desiccation tolerance (measured as survival time) compared between species was determined through the use of a generalized linear model with quasipoisson distribution of errors (Crawley 2007) and log link function implemented in R (v. 2.15.1). Only one age group (the oldest for each species) was chosen for this statistical comparison, because of different longevities experienced by these species (Hunt *et al.* 2005; Munhenga *et al.* 2011) and because these older ages are likely to be the groups most likely to overwinter during the dry season. Initial mass (mg) was included as the continuous predictor in the model owing to substantially different masses between these species. Sexes were analysed separately and relative humidity and temperature were input as ordered factors.

Results

Generalized linear model results for *An. funestus* indicated independent effects of mass and sex on survival time (Table 1). Females survived on average, longer than males, and larger individuals survived for longer than smaller individuals. Temperature, relative humidity and age all significantly influence survival of this species, with increases in temperature leading to reduced survival, increases in humidity resulting in longer survival, and increases in age resulting in shorter survival time. Three significant two-way interactions were also evident in the model (Table 1). The Sex*RH interaction suggests that the response of each sex is different across humidities with males dying faster than females at all humidities. The significant RH*Age interaction suggests that the relationship between survival time and RH differs between age groups, with younger ages surviving longer than older age groups across all humidities. The RH*Temperature interaction suggests that the slopes of survival against temperatures, differs between humidity treatments, with lower temperatures and high humidity showing the highest survival.

Mass, sex, relative humidity, temperature and age all significantly influence desiccation resistance in *Anopheles arabiensis* (Table 1). As well as significant main effects in the models, there are also significant two-way interactions, between Sex*Mass, RH*Temperature, Sex*Age, Temperature*Age, Sex*RH and RH*Age for *An. arabiensis* (Table 1). The Sex*Mass interaction show differing responses among individuals of different mass in the two sexes (i.e. different slopes of the time-mass relationship). The RH*Temperature interaction showed higher survival across all temperatures at 100% humidity relative to 55% or 5% RH treatments and higher survival at lower temperatures. Survival showed a steady decline with increase in temperature across all humidity treatments. The RH*Age interaction for *An. arabiensis* again indicates a different relationship between RH and survival for different age groups, with survival of younger age groups being higher across all humidities, but in different ways. This model also had significant three and four-way interactions between variables, which were not readily interpretable.

At 20°C, changes in humidity from 5% to 100% resulted in a difference in survival of c. 35 hours, while at 25°C, this difference was c. 18 hours, and at 30°C, this difference was c. 21 hours. From 20°C to 30°C, survival at 5% RH changed by c. 6 hours; at 55% RH differences in survival time from 20°C to 30°C were c. 8 hours; and at 100% RH, the differences in survival time from 20°C to 30°C temperatures were c. 16 hours. For *An. arabiensis*, differences across humidities at 20°C resulted in a 21 hour difference in survival, at 25°C and 30°C this difference was c. 16 hours. Differences as a result of humidity showed that at 5% RH, temperature changes from 20°C to 30°C resulted in a c. 4 hour difference in survival time. At 55% RH, temperature changes from 20°C to 30°C resulted in survival changes of c. 5 hours. At 100% RH, temperature changes resulted in a change in survival time of c. 8 hours. Higher temperatures and lower humidities had the shortest survival times.

The 3D contour plot of the residuals from a regression of survival time on mass, as a function of temperature and age for *An. arabiensis*, shows that at 5% RH, temperature is more important for survival, with no real difference occurring between ages (Figure 1). However, at 100% RH, age becomes more important for survival and temperature plays a smaller role. At 55% RH, the shift of importance changes from temperature-oriented to age-oriented (Figure 1).

In the age by temperature comparison of survival time at 5% RH, male and female *An. funestus* appear to show similar survival in response to temperature (Figure 2). At temperatures between 22°C and 26°C, no effect of age is observed. However, below and above these temperatures, older age groups die more rapidly than younger ones. At 55% RH, survival is extended at low temperatures, particularly for individuals less than 20-days old. Younger males at 55% RH seem more tolerant of high temperatures than older ages. Above ~28°C, there is a sharp fall in survival time of older ages compared to younger groups. Across all temperatures, older males at 55% RH die more rapidly than younger ones. At 100% RH, the response of males and females is similar across ages and temperatures (Figure 2). Lower temperatures seem to prolong survival, especially for younger individuals (Figure 2).

For 20-day old female *An. arabiensis*, optimum temperatures seem to exist between ~21°C and 27°C, with higher survival occurring above humidities of approximately 60% (Figure 3). Males of this species show preference for lower temperatures, with survival decreasing rapidly above temperatures of approximately 26°C (Figure 3). For *An. funestus* males and females, humidity seems to influence survival more than temperature, with temperature becoming most important only at the higher end of the scale (Figure 4). Higher temperatures appear to decrease *An. arabiensis* survival more than they do survival of *An. funestus*. Survival of species across humidities, however, seems similar (Figures 3 and 4).

Effects of WLR and mass on survival within species

Results from the generalized linear model of the effect of water loss rate (WLR) and mass on the dependent variable, time, indicate that WLR and mass both significantly influence survival time (desiccation tolerance) across all sexes, ages and temperatures for *An. funestus* at 5% RH (Table 2). WLR was most often more important in contributing to survival, with lower WLR significantly increasing survival time. The positive estimate values for mass, indicate that increased survival is associated with increased mass (Table 2).

For *An. arabiensis*, WLR again contributed most significantly to survival across sexes, ages and temperatures at the 5% RH treatment (Table 3). Reduced WLR led to significantly longer survival (negative estimate value) while increased mass (when significant) led to increased survival (positive estimate) (Table 3).

Differences in survival between species

Results from the generalized linear model for the effects of species, mass, RH and temperature on survival time of the oldest groups of each species, showed that mass significantly influenced survival time of males and females between species (Table 4) although not in the direction predicted. *Anopheles arabiensis* and *An. funestus* showed significantly different survival times between females, with *An. funestus* surviving on average, longer than *An. arabiensis* across all treatments (Table 4). The interactions between RH*Species, RH*Temperature, and Species*RH*Temperature were also significant in influencing survival times of females. The RH*Species interaction suggests that slopes of survival time vs. RH differ between species with a steeper slope observed in *An. arabiensis*. Survival of females at different RH*Temperature combinations was highest at low temperatures and high humidities and became steeper at high temperature and low humidity. The three-way interaction between species*RH*Temperature was not clearly interpretable

but suggests different survival times of species at different RH and temperature combinations.

When comparing survival times of males between species, no significant species effect was observed (Table 4). However, mass, RH and temperature all significantly influenced survival. Higher RH and lower temperatures increased survival for males. The two-way interactions between Mass*Species, RH*Temperature, Species*Temperature and Species*RH were also significant in the model. The significant Mass*Species interaction indicates that mass/time slopes are different between males of each species. The Species*Temperature and Species*RH interactions show that the species respond differently to RH and temperature in terms of their survival times, with *An. arabiensis* males dying faster than *An. funestus* males under high temperatures and low humidities.

Discussion

Aestivation by adult mosquitoes over the dry season is one mechanism by which mosquitoes are thought to survive in areas where malaria has a seasonal transmission (Omer and Cloudsley-Thompson 1970; Huestis *et al.* 2011). Understanding how mosquitoes respond to different combinations of temperature and humidity, across ages and sexes can shed some light on their ability to tolerate dry season conditions. Unsurprisingly, the results from this study show an increase in survival at high humidity and low temperature across all ages and for both sexes, and both vectors. Differences between different age groups indicate a decrease in desiccation tolerance with an increase in age, similar to findings on *An. arabiensis* and *An. gambiae* (Gray and Bradely 2005) and those of other insect species such as *Drosophila* (Nghiem *et al.* 2000; Gibbs and Markow 2001). This reduced tolerance of older age groups suggests a senescence response in terms of desiccation stress, similar to senescence observed

in thermal tolerance traits of *Anopheles arabiensis* and *An. funestus* (Lyons *et al.* 2012) and in other insect species (Bowler and Terblanche 2008).

Survival and persistence of malaria vector populations is determined not only by surviving females but also by the presence of males. However, in most mark-recapture studies, females are often the sex shown to persist over several months (Omer and Cloudsley-Thompson 1970). Results from this study clearly demonstrate a pronounced sex effect on desiccation tolerance, in addition to the effects of mass in both *An. arabiensis* and *An. funestus*. In both cases, females survive for significantly longer than males under all combinations of temperature and humidity. Hence, this evidence suggests that it is more than likely the females which overwinter during the dry season and that they probably do so in a nulliparous state, in the absence of oviposition (Omer and Cloudsley-Thompson 1970). Furthermore, these females probably reduce blood feeding to lower metabolic demands and thus, increase survival (Huestis *et al.* 2011). Survival of females in the field during the dry season can exceed several months (Omer and Cloudsley-Thompson 1970), substantially longer than survival of any individual in this lab-based trial. The individuals used in this trial were provided constant access to sugar water and are offered blood three times weekly. Hence, their metabolic rates may exceed those normally expected of overwintering females which could lead to significantly faster death under desiccating conditions (Huestis *et al.* 2011). Furthermore, the reduced resilience of males when compared to females may be explained by the necessity of females to leave breeding sites in search of blood (e.g. Charlwood *et al.* 2000) and the fact that males do not partake in this behaviour and hence, may not have built up enough resistance to desiccation as what females may have.

Survival time of *An. arabiensis* was most often lower than that of *An. funestus*, with *An. arabiensis* surviving only for *c.* 28 hours at 20°C and 100% RH and *An. funestus* surviving for *c.* 42 hours at the same humidity and temperature. At lower humidities, survival

of *An. funestus* was still higher than that for *An. arabiensis*. However, *An. arabiensis* is traditionally thought of as an arid-adapted species (Gillies and Coetzee 1987; Lindsay *et al.* 1998; Gray and Bradley 2005) and it would be expected that for this reason, this species should tolerate desiccation better than *An. funestus*. However, *An. funestus* is typically a very flexible species and occurs in a wide range of habitats and climatic conditions (Sinka *et al.* 2010). It is a highly anthropophilic species, although it does exhibit behavioural changes to this pattern in some regions (Wanji *et al.* 2003; Muriu *et al.* 2008; Sinka *et al.* 2010). These differences in behaviour between populations of the same species may have led to differences in desiccation tolerance observed for these species. Additionally, *Anopheles funestus* adults have also been shown to be more tolerant of high temperatures than *An. arabiensis* adults (Lyons *et al.* 2012). The requirement of *An. funestus* to inhabit areas close to ephemeral pools, and then to travel from these sources in search of blood meals (Charlwood *et al.* 2000), could also account for their increased desiccation resistance when compared to *An. arabiensis*. In this study, *An. funestus* survived for longer under desiccation trials than *An. arabiensis*, largely because of its reduced water loss rate (Gibbs *et al.* 1997; Gibbs 2002). In some regions, *An. funestus* is thought to be more important in the persistence of malaria throughout a dry season than what *An. arabiensis* or *An. gambiae* are (Charlwood *et al.* 2000). Hence, the need for them to leave relatively moist refugia (e.g. on the banks of permanent water stores) may account for their increased desiccation tolerance as found in this study.

A further reason for these species differences might be the influence of laboratory adaptation on desiccation tolerance, with a larger effect on *An. arabiensis* than on *An. funestus*. Certain physiological traits are known to be affected by laboratory conditions more than others (Hoffmann *et al.* 2001; Huho *et al.* 2007), and perhaps desiccation tolerance of *An. arabiensis* is one such trait (but see Terblanche *et al.* 2006). The presence of *An.*

arabiensis in a laboratory colony for several decades could have altered their resistance to desiccation, although thermal tolerance traits of this species seem to be less affected under laboratory conditions when compared to wild strains (Lyons *et al.* 2012). However, species are also known to show different phenotypes and different stress resistance among different populations (Hoffmann and Harshman 1999; Sinka *et al.* 2010). For example, in *Drosophila*, individuals from higher latitude regions display greater cold resistance, while those that are more desiccation tolerant, inhabit arid regions (Hoffmann and Harshman 1999). *Anopheles gambiae s.s.* the sister taxon to *An. arabiensis*, exhibits polymorphic inversions, one of which confers an advantage on the species in arid environments (Gray *et al.* 2009). *Anopheles funestus* has recently also shown chromosomal inversions, with different forms occurring in different regions of the African continent (Sinka *et al.* 2010). Furthermore, *An. arabiensis* in Sudan have been shown to survive during the dry season where temperatures spiked to over 50°C (Omer and Cloudsley-Thompson 1970) in contrast to lethal temperature estimates of the southern African strain of this species used in this and previous studies, which sit at only ~ 34°C (Lyons *et al.* 2012). Hence, it is possible that different phenotypes of *An. arabiensis* exist, and indeed, to account for the seasonality of malaria in certain regions (Hay *et al.* 1998; Tanser *et al.* 2003) and the overwintering nature of females in some populations (Taylor *et al.* 1993; Huestis *et al.* 2011) some individuals need to be more desiccation tolerant than others.

So what do these results mean for the field situation? As an example, at Krokodilbrug in Mpumalanga, a location on the eastern side of South Africa where both species have been collected, average relative humidity and temperature over the last ten years have been measured as, *c.* 65% and 22°C during the dry season (data obtained from the Agricultural Research Council, South Africa). Under these conditions, *An. arabiensis* and *An. funestus* are likely to survive for approximately 20 hours and 30 hours, respectively. However, such a conclusion is entirely at odds with the persistence of these species. In consequence, it is clear

that both species must seek out refuges, and that additional water loss downregulation is likely to occur. The former is in keeping with what is known of the behaviour of *Anopheles* species (Kessler and Guerin 2008) and suggests that a trapping method based on humidity manipulation might be developed, or at the very least where other control methods might be targeted. The latter provides grounds for further investigations of whether such downregulation takes place, whether it can be induced under laboratory and/or field situations. To date, investigations of overwintering have largely met with little success, but promising data are now starting to appear (Lehmann *et al.* 2010; Huestis *et al.* 2011). A key new set of work should investigate whether an aestivation response (see e.g. Storey 2002; Košťál 2006; Hahn and Denlinger 2011) can be elicited, what physiological mechanisms might be involved, and what the population dynamics consequences thereof might be. Alternatively, pockets of individuals displaying a greater tolerance for desiccation may also be able to persist through the dry season, and indeed, this seems to be the case, especially given the seasonality of malaria in some areas (Dukeen and Omer 1986; Hay *et al.* 1998; Patz *et al.* 1998; Charlwood *et al.* 2000; Tanser *et al.* 2003).

References

- Bayoh, M.N. and Lindsay, S.W. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Bulletin of Entomological Research* **93**: 375-381.
- Bayoh, M.N. and Lindsay, S.W. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Medical and Veterinary Entomology* **18**: 174-179.
- Benoit, J.B. and Denlinger, D.L. 2010. Meeting the challenges of on-host and off-host water balance in blood-feeding arthropods. *Journal of Insect Physiology* **56**: 1366-1376.

- Bowler, K. and Terblanche, J.S. 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biological Reviews* **83**: 339-355.
- Buckley, L.B. and Kingsolver, J.G. 2012. Functional and phylogenetic approaches to forecasting species' responses to climate. *Annual Review of Ecology, Evolution and Systematics* **43**: 205-226.
- Calow, P. 1979. The cost of reproduction – a physiological approach. *Biological Reviews* **54**: 23-40.
- Charlwood, J.D., Viji, R. and Billingsley, P.F. 2000. Dry season refugia of malaria-transmitting mosquitoes in a dry savannah zone of East Africa. *American Journal of Tropical Medicine and Hygiene* **62**: 726-732.
- Chown, S.L. 2002. Respiratory water loss in insects. *Comparative Biochemistry and Physiology Part A* **133**: 791-804.
- Chown, S.L. and Nicolson, S.W. 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford: Oxford University Press, UK.
- Chown, S.L. and Gaston, K.J. 2010. Body size variation in insects: a macroecological perspective. *Biological Reviews of the Cambridge Philosophical Society* **85**: 139-169.
- Chown, S.L., Sørensen, J.G. and Terblanche, J.S. 2011. Water loss in insects: an environmental change perspective. *Journal of Insect Physiology* **57**: 1070-1084.
- Crawley, M.J. 2007. *The R Book*. John Wiley & Sons, Ltd., England, UK.
- Dukeen, M.Y.,H. and Omer, S.M. 1986. Ecology of the malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae) by the Nile in northern Sudan. *Bulletin of Entomological Research* **76**: 451-467.
- Fung, F., Lopez, A. and New, M. 2011. Water availability in +2°C and +4°C worlds. *Philosophical Transactions of the Royal Society A* **369**: 99-116.

- Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., Clusella-Trullas, S., Ghalambor, C.K., Konarzewski, M., Peck, L.S., Porter, W.P., Pörtner, H.O., Rezende, E.L., Schulte, P.M., Spicer, J.I., Stillman, J.H., Terblanche, J.S. and van Kleunen, M. 2009. Macrophysiology: a conceptual reunification. *American Naturalist* **174**: 595-612.
- Gibbs, A.G., Chippindale, A.K. and Rose, M.R. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology* **200**: 1821-1832.
- Gibbs, A.G. and Markow, T.A. 2001. Effects of age on water balance in *Drosophila* species. *Physiological and Biochemical Zoology* **74**: 520-530.
- Gibbs, A.G. 2002. Water balance in desert *Drosophila*: lessons from non-charismatic microfauna. *Comparative Biochemistry and Physiology A* **133**: 781-789.
- Gillies, M.T. and Coetzee, M. 1987. *A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region)*. Publications of the South African Institute of Medical Research, Johannesburg, South Africa.
- Gray, E.M. and Bradley, T.J. 2005. Physiology of desiccation resistance in *Anopheles gambiae* and *Anopheles arabiensis*. *American Journal of Tropical Medicine and Hygiene* **73**: 553-559.
- Gray, E.M., Rocca, K.A.C., Constantini, C. and Besansky, N.J. 2009. Inversion 2La is associated with enhanced desiccation resistance in *Anopheles gambiae*. *Malaria Journal* **8**: 215.
- Hahn, D.A. and Denlinger, D.L. 2011. Energetics of insect diapause. *Annual Review of Entomology* **56**: 103-121.

- Hansen, J., Sato, M. and Ruedy, R. 2012. Perception of climate change. *Proceedings of the National Academy of Sciences of the U.S.A.: Early Edition*: pnas.org/cgi/doi/10.1073/pnas.1205276109.
- Hardy, J.L., Houk, E.J., Kramer, L.D. and Reeves, W.C. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. *Annual Review of Entomology* **28**: 229-262.
- Hay, S.I., Snow, R.W. and Rogers, D.J. 1998. Predicting malaria seasons in Kenya using multitemporal meteorological satellite sensor data. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**: 12-20.
- Hoffmann, A.A. and Parsons, P.A. 1989. Selection for increased desiccation resistance in *Drosophila melanogaster*: additive genetic control and correlated responses for other stresses. *Genetics* **122**: 837-845.
- Hoffmann, A.A. 1990. The influence of age and experience with conspecifics on territorial behaviour in *Drosophila melanogaster*. *Journal of Insect Behaviour* **3**: 1-12.
- Hoffmann, A.A. and Harshman, L.G. 1999. Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and interpopulation levels. *Heredity* **83**: 637-643.
- Hoffmann, A.A., Hallas, R., Sinclair, C. and Mitrovski, P. 2001. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance and associated traits. *Evolution* **55**: 1621-1630.
- Hoffmann, A.A. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. *Journal of Experimental Biology* **213**: 870-880.

- Huestis, D.L., Yaro, A.S., Traoré, A.I., Adamou, A., Kassogué, Y., Diallo, M., Timbiné, S., Dao, A. and Lehmann, T. 2011. Variation in metabolic rate of *Anopheles gambiae* and *A. arabiensis* in a Sahelian village. *Journal of Experimental Biology* **214**: 2345-2353.
- Huho, B.J., Ng'habi, K.R., Killeen, G.F., Nkwengulila, G., Knols, B.G.J. and Ferguson, H.M. 2007. Nature beats nurture: a case study of the physiological fitness of free-living and laboratory-reared male *Anopheles gambiae s.l.* *Journal of Experimental Biology* **210**: 2939-2947.
- Hunt, R.H., Brooke, B.D., Pillay, C., Koekemoer, L.L. and Coetzee, M. 2005. Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector *Anopheles funestus*. *Medical and Veterinary Entomology* **19**: 271-275.
- Kareiva, P., Mullen, A. and Southwood, R. 1990. Population dynamics in spatially complex environments: Theory and data [and discussion]. *Philosophical Transactions of the Royal Society B* **330**: 175-190.
- Kessler, S. and Guerin, P.M. 2008. Responses of *Anopheles gambiae*, *Anopheles stephensi*, *Aedes aegypti* and *Culex pipiens* mosquitoes (Diptera: Culicidae) to cool and humid refugium conditions. *Journal of Vector Ecology* **33**: 145-149.
- Kleynhans, E. and Terblanche, J.S. 2011. Complex interactions between temperature and relative humidity on water balance of adult tsetse (Glossinidae, Diptera): implications for climate change. *Frontiers in Invertebrate Physiology* **2**: doi: 10.3389/fphys.2011.00074.
- Košťál, V. 2006. Eco-physiological phases of insect diapause. *Journal of Insect Physiology* **52**: 113-279.
- Lafferty, K.D. 2009. Calling for an ecological approach to studying climate change and infectious diseases. *Ecology* **90**: 932-933.

- Le Lagadec, M.D., Chown, S.L. and Scholtz, C.H. 1998. Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): the influence of body size and habitat. *Journal of Comparative Physiology B* **168**: 112-122.
- Lehmann, T., Dao, A., Yaro, A.S., Adamou, A., Kassogue, Y., Diallo, M., Sékou, T. and Coscaron-Arias, C. 2010. Aestivation of the African malaria mosquito, *Anopheles gambiae* in the Sahel. *American Journal of Tropical Medicine and Hygiene* **83**: 601-606.
- Lindsay, S.W., Parson, L. and Thomas, C.J. 1998. Mapping the range and relative abundance of the two principal African malaria vectors, *Anopheles gambiae sensu stricto* and *An. arabiensis*, using climate data. *Proceedings of the Royal Society of London B* **265**: 847-854.
- Lyons, C.L., Coetzee, M., Terblanche, J.S. and Chown, S.L. 2012. Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*. *Malaria Journal* **11**: 226.
- Marron, N., Dreyer, E., Boudouresque, E., Delay, D., Petit, J.M., Delmotte, F.M. and Brignolas, F. 2003. Impact of successive drought and re-watering cycles on growth and specific leaf area of two *PopulusXcanadensis* (Moench) clones, 'Dorkamp' and 'Luisa Avanzo'. *Tree Physiology* **23**: 1225–1235.
- Menge, B.A. and Sutherland, J.P. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *American Naturalist* **130**: 730-757.
- Munhenga, G., Brooke, B.D., Chirwa, T.F., Hunt, R.H., Coetzee, M., Govender, D. and Koekemoer, L.L. 2011. Evaluating the potential of the sterile insect technique for malaria control: relative fitness and mating compatibility between laboratory colonized

- and a wild population of *Anopheles arabiensis* from the Kruger National Park, South Africa. *Parasites and Vectors* **4**: 208.
- Muriu, S.M., Muturi, E.J., Shililu, J.I., Mbogo, C.M., Mwangangi, J.M., Jacob, B.G., Irungu, L.W., Mukabana, R.W., Githure, J.I. and Novak. R.J. 2008. Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya. *Malaria Journal* **7**: 43.
- Nghiem, D., Gibbs, A.G., Rose, M.R. and Bradley, T.J. 2000. Postponed aging and desiccation resistance in *Drosophila melanogaster*. *Experimental Gerontology* **35**: 957-969.
- Patz, J.A., Strzepek, K., Lele, S., Hedden, M., Greene, S., Noden, B., Hay, S.I., Kalkstein, L. and Beier, J. C. 1998. Predicting key malaria transmission factors, biting and entomological inoculation rates, using modelled soil moisture in Kenya. *Tropical Medicine and International Health* **3**: 818-827.
- Omer, S.M. and Cloudsley-Thompson, J.L. 1970. Survival of female *Anopheles gambiae* Giles through a 9-month dry season in Sudan. *Bulletin of the World Health Organization* **42**: 319-330.
- Quinn, G.P. and Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press, UK.
- Rocca, K.A.C., Gray, E.M., Constantini, C. and Besansky, N.J. 2009. 2La chromosomal inversion enhances thermal tolerance of *Anopheles gambiae* larvae. *Malaria Journal* **8**: 147.
- Sinka, M.E., Bangs, M.J., Manguin, S., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Okara, R.M., Van Boeckel, T., Godfray, H.C.J., Harbach, R.E. and Hay, S.I. 2010. The dominant *Anopheles* vectors of

- human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites and Vectors* **3**: 117.
- Stillwell, R.C., Blanckenhorn, W.U., Teder, T., Davidowitz, G. and Fox, C.,W. 2010. Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annual Review of Entomology* **55**: 227-245.
- Stone, G.N. and Willmer, P.G. 1989. Endothermy and temperature regulation in bees: a critique of 'grab and stab' measurement of body temperature. *Journal of Experimental Biology* **143**: 211-223.
- Storey, K.B. 2002. Life in the slow lane: molecular mechanisms of aestivation. *Comparative Biochemistry and Physiology A* **133**: 733-754.
- Studier, E.H. and Sevick, S.H. 1992. Live mass, water content, nitrogen and mineral levels in some insects from south-central lower Michigan. *Comparative Biochemistry and Physiology* **103A**: 579-595.
- Tanser, F.C., Sharp, B. and le Sueur, D. 2003. Potential effect of climate change on malaria transmission in Africa. *Lancet* **362**: 1792-1798.
- Taylor, C.E., Toure, Y.T., Coluzzi, M and Petrarca, V. 1993. Effective population size and persistence of *Anopheles arabiensis* during the dry season in West Africa. *Medical and Veterinary Entomology* **7**: 351-357.
- Terblanche, J.S., Klok, J.C., Krafur, E.S. and 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. *American Journal of Tropical Medicine and Hygiene* **74**: 786-794.
- Thuiller, W., Albert, C., Araújo, M.B., Berry, P.M., Cabeza, M., Guisan, A., Hickler, T., Midgley, G.F., Paterson, J., Schurr, F.M., Sykes, M.T. and Zimmermann, N.E. 2008.

Predicting global change impacts on plant species' distributions: future challenges.

Perspectives in Plant Ecology, Evolution and Systematics **9**: 137-152.

Wanji, S., Tanke, T., Atanga, S.N., Ajonina, C., Nicholas, T. and Fontenille, D. 2003.

Anopheles species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates. *Tropical Medicine and International Health* **8**: 643-649.

Winston, P.W. and Bates, D.H. 1960. Saturated salt solutions for the control of humidity in biological research. *Ecology* **41**: 232-237.

Figure Legends

Figure 1. Contour plot of the relationship between the residuals of a mass (mg) vs. survival time (hours) regression, and temperature (°C) and age (days) for each of the three humidity treatments, for *Anopheles arabiensis*. Green indicates high survival while red indicates low survival.

Figure. 2. Contour plot of the relationship between the residuals of a mass (mg) vs. survival time (hours) regression, and temperature (°C) (x-axis) and age (days) (y-axis) for each of the three humidity treatments, for *Anopheles funestus* males (top row) and females (bottom row). Green indicates high survival while red indicates low survival.

Figure 3. Contour plot of the relationship between the residuals of a mass (mg) vs. survival time (hours) regression, and temperature (°C) and humidity (RH %) for each of the three humidity treatments, for 20-day old *Anopheles arabiensis* females (left) and males (right). Green indicates high survival while red indicates low survival.

Figure 4. Contour plot of the relationship between the residuals of a mass (mg) vs. survival time (hours) regression, and temperature (°C) and humidity (RH %) for each of the three humidity treatments, for 30-day old *Anopheles funestus* females (left) and males (right). Green indicates high survival while red indicates low survival.

Figures and Tables

Fig. 1

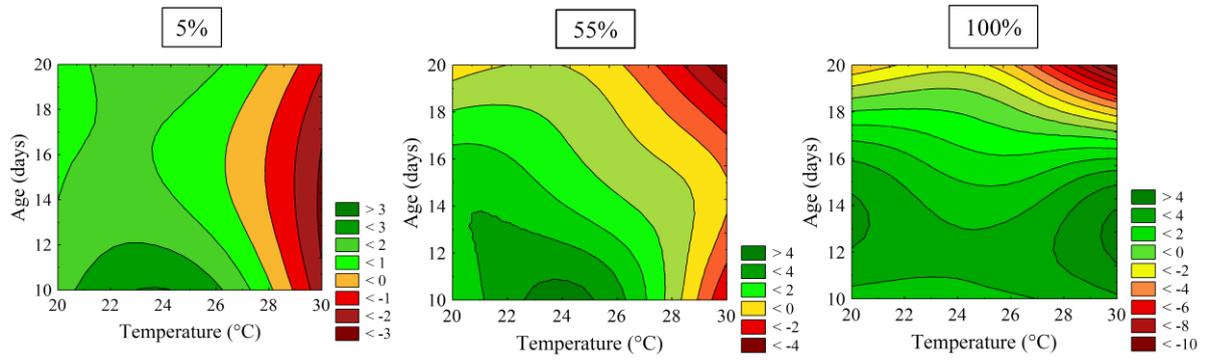


Fig. 2

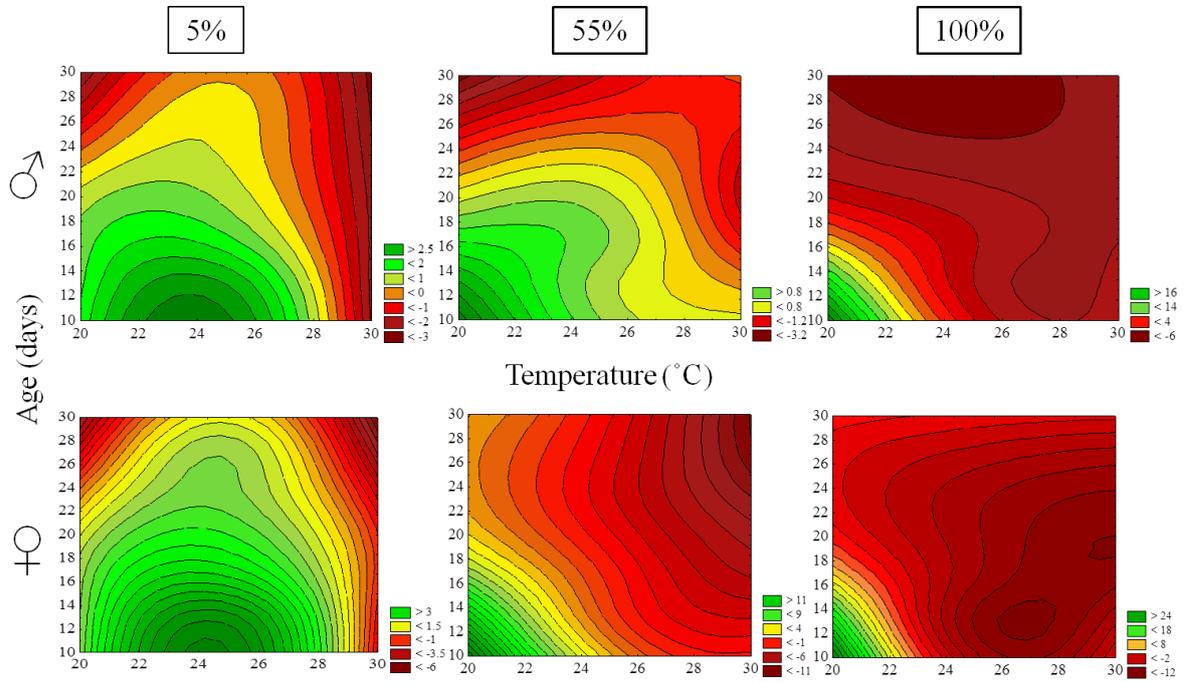


Fig. 3

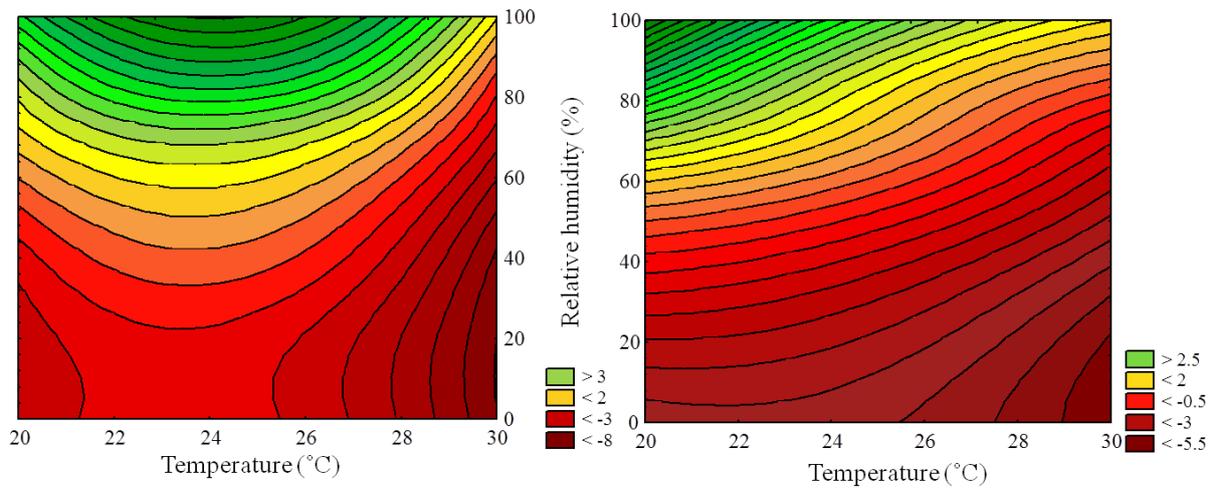


Fig. 4

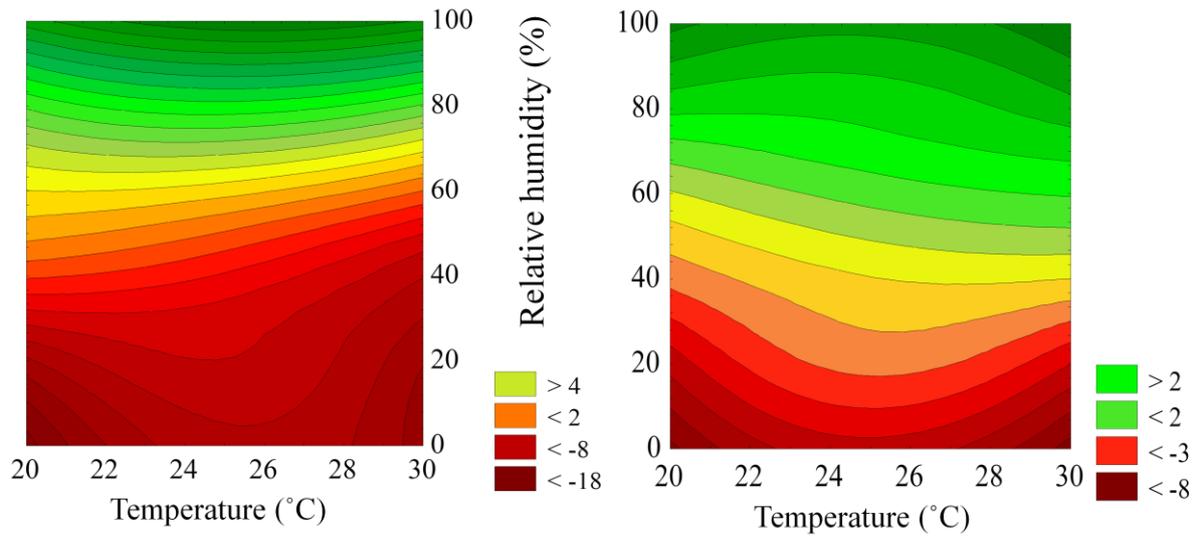


Table 1. Results from a generalized linear model with quasipoisson distribution of errors (to correct for overdispersion) and log link function for desiccation tolerance (time) as a function of mass (mg), sex, relative humidity (RH), temperature (°C) and age (days) for *Anopheles funestus* and *Anopheles arabiensis*.

Species	Predictors	Df	F	P-value
<i>Anopheles funestus</i>	Mass	1	34.87	< 0.0001
	Sex	1	10.31	0.0014
	RH	2	445.92	< 0.0001
	Temperature	2	33.09	< 0.0001
	Age	2	26.92	< 0.0001
	Mass*Sex	1	1.47	0.2252
	Sex*RH	2	3.31	0.0367
	Sex*Temperature	2	0.52	0.5964
	RH*Temperature	4	15.28	< 0.0001
	RH*Age	4	6.89	< 0.0001
	Temperature*Age	4	4.59	0.0011
	Sex*Age	2	1.01	0.3658
	Sex*RH*Temperature	4	4.39	0.0016
	RH*Temperature*Age	8	3.36	0.0011

	Sex*Temperature*Age	4	1.86	0.1151
	Sex*RH*Age	4	3.34	0.0099
	Residuals	1072		
<hr/>				
<i>Anopheles arabiensis</i>	Mass	1	88.71	< 0.0001
	Sex	1	12.87	0.0004
	RH	2	530.10	< 0.0001
	Temperature	2	44.46	< 0.0001
	Age	2	15.48	< 0.0001
	Mass*Sex	1	5.85	0.0157
	Sex*RH	2	3.29	0.0375
	Sex*Temperature	2	0.55	0.5757
	RH*Temperature	4	15.66	< 0.0001
	Sex*Age	2	4.73	0.0090
	RH*Age	4	10.74	< 0.0001
	Temperature*Age	4	4.40	0.0016
	Sex*RH*Temperature	4	5.98	< 0.0001
	Sex*RH*Age	4	3.61	0.0063
	Sex*Temperature*Age	4	3.93	0.0036

RH*Temperature*Age	8	4.79	< 0.0001
Sex*RH*Temperature*Age	8	4.27	< 0.0001
Residuals	1004		

Table 2. Results from a generalized linear model with quasipoisson distribution of errors and log link function, showing the relative contributions of water loss rate (WLR in mg/h) and initial mass (mg) to desiccation tolerance (time) of each group of *Anopheles funestus* at each temperature (Temp) and at the 5% relative humidity treatment. Model degrees of freedom (df) are shown under each group category.

Temp	Group	Parameter	N	F	P	Estimate	S.E.
20°C	10 d ♀	WLR	20	9.70	0.0063	-16.01	6.48
	Df=17	Mass		5.13	0.0369	1.15	0.51
	10 d ♂	WLR	20	42.16	< 0.0001	-22.68	4.33
	Df=17	Mass		4.25	0.0549	1.49	0.73
	20 d ♀	WLR	40	35.92	< 0.0001	-20.23	4.12
	Df=37	Mass		22.34	< 0.0001	0.86	0.18
	20 d ♂	WLR	40	102.13	< 0.0001	-40.03	4.72
	Df=37	Mass		21.32	< 0.0001	2.27	0.49
	30 d ♀	WLR	20	124.36	< 0.0001	-6.17	0.62
	Df=17	Mass		5.16	0.0363	1.05	0.47
	30 d ♂	WLR	20	10.57	0.0050	40.01	12.36
	Df=17	Mass		20.42	0.0003	14.93	3.32
		Mass*WLR		14.90	0.0014	-88.74	23.10

25°C	10 d ♀	WLR	20	20.15	0.0003	-18.33	4.34
	Df=17	Mass		6.62	0.0198	0.76	0.29
	10 d ♂	WLR	20	10.79	0.0044	-23.59	8.26
	Df=17	Mass		8.94	0.0082	2.55	0.85
	20 d ♀	WLR	20	12.42	0.0026	-18.08	5.48
	Df=17	Mass		8.09	0.0112	0.56	0.19
	20 d ♂	WLR	20	9.18	0.0076	-21.46	7.59
	Df=17	Mass		2.14	0.1622	2.76	1.84
	30 d ♀	WLR	20	47.81	< 0.0001	-31.35	4.65
	Df=17	Mass		14.91	0.0013	1.01	0.27
	30 d ♂	WLR	20	22.26	0.0002	-24.97	6.12
	Df=17	Mass		2.33	0.1455	1.32	0.88
30°C	10 d ♀	WLR	20	260.32	< 0.0001	-7.91	0.61
	Df=17	Mass		21.28	0.0002	1.24	0.27
	10 d ♂	WLR	20	25.58	< 0.0001	-6.85	1.41
	Df=17	Mass		12.66	0.0024	4.63	1.22
	20 d ♀	WLR	20	56.33	< 0.0001	-15.22	2.53
	Df=17	Mass		13.53	0.0019	1.73	0.47

20 d ♂	WLR	20	8.68	0.0090	-4.70	1.68
Df=17	Mass		0.09	0.7697	-0.54	1.89
30 d ♀	WLR	20	32.33	< 0.0001	-4.54	0.93
Df=17	Mass		2.59	0.1258	0.45	0.28
30 d ♂	WLR	20	22.26	0.0002	-3.59	0.75
Df=17	Mass		3.02	0.1001	1.07	0.61

Table 3. Results from generalized linear model with quasipoisson distribution of errors and log link function for *Anopheles arabiensis* showing the influence of mass (mg) and water loss rate (WLR in mg/h) on desiccation tolerance (time in hours) for each age and sex group at each temperature (Temp) for the 5% relative humidity treatment. Model degrees of freedom (df) are shown under each group category.

Temp	Group	Parameter	N	F	P	Estimate	S.E.
20°C	10 d ♀	WLR	20	45.94	< 0.0001	-7.24	1.21
	Df=17	Mass		8.49	0.0097	0.35	0.11
	10 d ♂	WLR	20	25.88	< 0.0001	-6.81	1.67
	Df=17	Mass		0.21	0.6538	0.27	0.59
	15 d ♀	WLR	20	82.75	< 0.0001	-7.16	0.89
	Df=17	Mass		5.90	0.0265	0.30	0.12
	15 d ♂	WLR	20	29.88	< 0.0001	-6.62	1.4
	Df=17	Mass		0.11	0.7487	0.15	0.44
	20 d ♀	WLR	20	8.87	0.0089	-29.00	9.88
	Df=17	Mass		1.49	0.2401	-0.54	0.45
		Mass*WLR		4.99	0.0401	14.69	6.62
	20 d ♂	WLR	20	41.47	< 0.0001	-6.26	1.10
	Df=17	Mass		13.05	0.0022	2.61	0.73

25°C	10 d ♀	WLR	19	0.27	0.6121	-1.18	2.33
	Df=16	Mass		4.44	0.0510	0.23	0.11
	10 d ♂	WLR	21	5.20	0.0350	-6.39	2.84
	Df=18	Mass		4.57	0.0464	1.30	0.60
	15 d ♀	WLR	20	17.68	0.0006	-12.93	3.13
	Df=17	Mass		8.99	0.0081	0.44	0.14
	15 d ♂	WLR	20	30.22	< 0.0001	-3.93	0.72
	Df=17	Mass		0.78	0.3910	0.61	0.67
	20 d ♀	WLR	20	16.39	0.0008	-7.77	1.97
	Df=17	Mass		17.47	0.0006	0.43	0.10
	20 d ♂	WLR	20	8.84	0.0085	-14.16	5.19
	Df=17	Mass		0.67	0.4245	1.06	1.34
30°C	10 d ♀	WLR	20	22.10	0.0002	-5.92	1.59
	Df=17	Mass		0.08	0.7829	0.05	0.17
	10 d ♂	WLR	20	8.19	0.0108	-3.36	1.38
	Df=17	Mass		0.00	0.9750	-0.03	0.95
	15 d ♀	WLR	20	49.78	< 0.0001	-2.46	0.40
	Df=17	Mass		2.95	0.1038	0.37	0.23

15 d ♂	WLR	20	116.82	< 0.0001	-3.81	0.34
Df=17	Mass		14.27	0.0015	1.23	0.32
20 d ♀	WLR	20	9.34	0.0072	-3.90	1.27
Df=17	Mass		3.05	0.0987	0.26	0.14
20 d ♂	WLR	20	13.15	0.0021	-4.04	1.14
Df=17	Mass		0.33	0.5725	-0.32	0.55

Table 4. Generalized linear model results using a quasipoisson distribution of errors and log link function to determine the influence of mass (mg), temperature (°C), humidity (RH) and species differences on desiccation tolerance (time in hrs) of *Anopheles arabiensis* and *Anopheles funestus* females and males separately. Significant interactions between factors are also shown.

Sex	Parameters	df	F	P-value
Females	Mass	1	13.30	0.0003
	Species	1	4.23	0.0404
	RH	2	289.29	< 0.0001
	Temperature	2	16.55	< 0.0001
	Mass*Species	1	0.85	0.3559
	Species*RH	2	25.24	< 0.0001
	Species*Temperature	2	0.34	0.7121
	RH*Temperature	4	11.56	< 0.0001
	Species*RH*Temperature	4	5.13	0.0005
	Residuals	340		
Males	Mass	1	19.92	< 0.0001
	Species	1	0.09	0.7667
	RH	2	226.42	< 0.0001

Temperature	2	6.91	0.0011
Mass*Species	1	5.92	0.0154
RH*Temperature	4	7.89	< 0.0001
Species*Temperature	2	21.59	< 0.0001
Species*RH	2	5.46	0.0046
Residuals	344		

Supplementary Materials

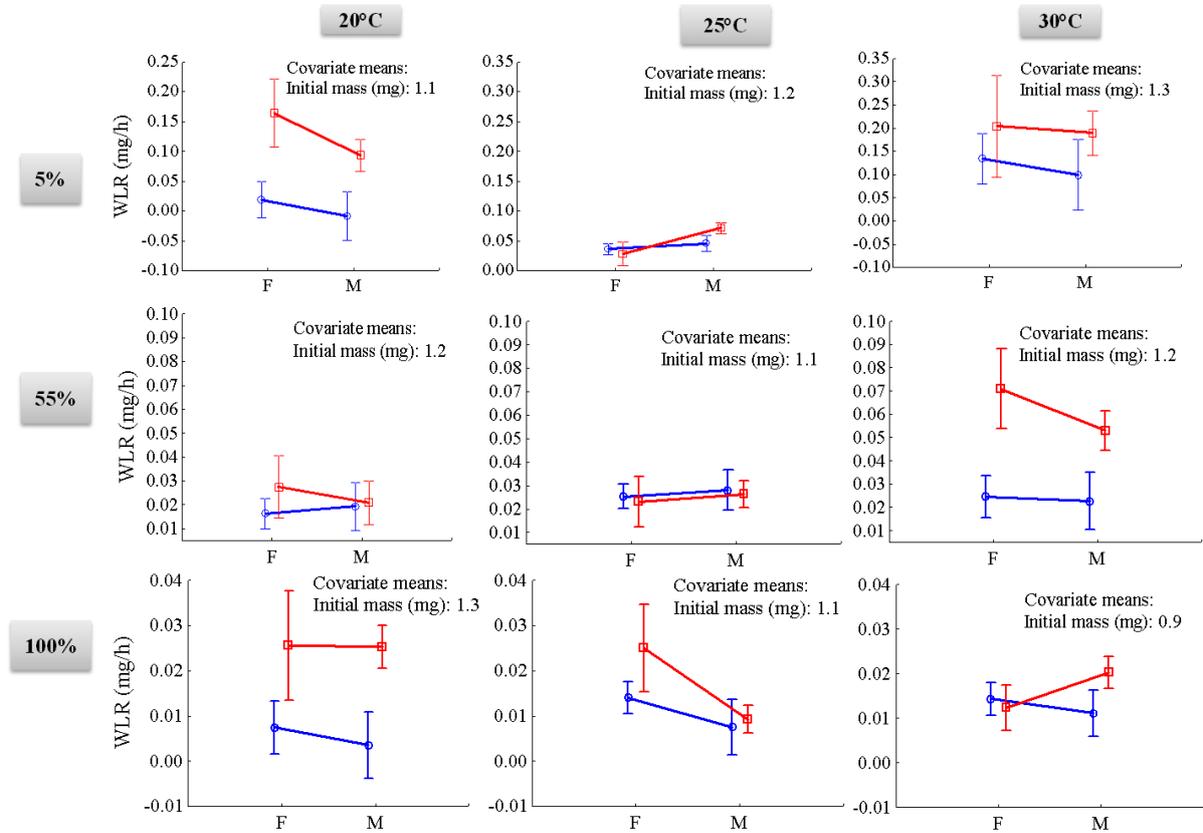


Figure S1. Water loss rate (WLR in mg/h) for male and female *Anopheles arabiensis* (red) and *An. funestus* (blue), at each of three humidity treatments (5%, 55%, 100%) and at each of three temperature treatments (20°C, 25°C, 30°C). In most cases, WLR of *An. funestus* is lower than WLR of *An. arabiensis*.

Chapter 5

Future distributions of *Anopheles arabiensis* and *Anopheles funestus* in Africa



www.scientificamerican.com

Introduction

Climate change-associated range shifts have been observed in several animal and plant species over the past several decades (Parmesan and Yohe 2003; Parmesan 2006). Many of these species have shown changes at their range margins and in a poleward direction (Parmesan 2006). One of the major concerns associated with climate change is how vector-borne diseases such as malaria, dengue, filariasis (Martens *et al.* 1997) and African sleeping sickness will be impacted by changing temperatures and rainfall patterns. Transmitted by arthropods, these diseases are strongly linked to climate through its effects on the invertebrate vectors of the organisms causing the disease (Molyneux 2009). Several arboviruses have already shown range shifts in response to global warming and further changes are predicted (Martens *et al.* 1997; Lafferty 2009).

To understand how these species might respond to climate change, an understanding of the vectors responsible for their transmission is crucial. Information on species current distributions can be used in correlative species distribution models to infer the species' response to environmental conditions associated with its known distribution (Phillips *et al.* 2006; Elith and Leathwick 2009). In this way, predictions or projections of a species future distribution can be made. These kinds of modeling approaches are often said to be concerned with determining the species realized niche (Elith and Leathwick 2009; Buckley and Kingsolver 2012). Alternatively, in the absence of species distribution records, and with species-specific information such as physiological tolerances or developmental temperature thresholds, mechanistic distribution modeling techniques can be employed to assess a species' response to changing climatic or environmental conditions (Kearney *et al.* 2009; Kearney and Porter 2009). These kinds of models most often require species-specific physiological tolerance data, which can be used to determine the species potential niche or fundamental niche (Kearney *et al.* 2009; Buckley and Kingsolver 2012). Both types of

modeling approaches have been used extensively in the past to determine the potential response of a species to climate change (Phillips *et al.* 2006; Kearney *et al.* 2008; Kearney *et al.* 2009; Buckley and Kingsolver 2012). There are also several constraints associated with each modeling approach (Pearson and Dawson 2003; Araújo and Guisan 2006; Elith *et al.* 2010) and several cautionary notes applicable to the use of each (Pearson and Dawson 2003; Jiménez-Valverde *et al.* 2008; Elith *et al.* 2010; Araújo and Peterson 2012). However, both have been used with differing degrees of success (Robertson *et al.* 2003; Kearney *et al.* 2008; Pattison and Mack 2008; Kearney *et al.* 2009; Elith *et al.* 2010) and the use of species distribution modeling as a whole provides a useful tool when determining species invasion (Sutherst *et al.* 1996; Kearney *et al.* 2008; Pattison and Mack 2008; Poutsma *et al.* 2008; Sutherst and Bourne 2009; Lozier and Mills 2011; de Villiers *et al.* 2012) or extinction risks (Midgley *et al.* 2002). It can also provide a useful tool when determining the risk or potential spread of a disease such as malaria.

In Africa, malaria still poses a significant threat to most of the continent, with an estimated 800 000 deaths attributable to the disease on an annual basis (WHO 2010). Because of the continuing infections and need to plan for the future, there is ongoing interest in how climate change might affect the distribution of the disease. However, thus far results have been contradictory. Some studies have proposed that malaria will increase at its current range margins (Martens *et al.* 1997; Tanser *et al.* 2003; van Lieshout *et al.* 2004) whilst others predict that there will be no net affect as a result of warming temperatures (Rogers and Randolph 2000; Hay *et al.* 2002). However, few of these studies have incorporated species-specific physiological information of the kind collected in this thesis. Despite the importance of malaria in sub-Saharan Africa, very little information exists on the physiology of two of the three most important *Plasmodium falciparum* vectors, *Anopheles arabiensis* and *An.*

funestus (Gillies and Coetzee 1987). However, for *An. gambiae*, the third vector, more information is available (e.g. Bayoh and Lindsay 2003, 2004; Rocca *et al.* 2009).

In this chapter, I therefore use the physiological data obtained by work reported in previous chapters to determine the potential distribution for *An. arabiensis* and *An. funestus* under two potential climate change scenarios – one of severe climate change and the other more moderate change. Because the range of temperatures across which development can take place is narrower and lies within the thermal tolerance limits of the species, the focus is primarily on developmental temperature. Future work will incorporate the desiccation data, which at present are indicative of expectations, but cannot be incorporated into the modeling approach adopted.

Methods

For this modeling exercise, CLIMEX (Hearne Scientific Software, Pty Ltd., Australia), a process-based model that uses species traits or distribution data to model projected ranges under historical or future climate scenarios (Sutherst and Maywald 1985; Sutherst *et al.* 2007) was used. Biological information, such as the thermal limits of development and diapause duration, are incorporated and used deductively to simulate a species' response to environmental factors such as temperature and soil moisture (Sutherst *et al.* 2007; Sutherst and Bourne 2009). The species' response to climate is generated through weekly stress (SI) and growth indices (GI) which are then combined into annual SI and GI for the species in each location (Sutherst *et al.* 2007). These indices are then combined to provide an Ecoclimatic Index (EI) (scaled from 0 to 100), a measure of overall habitat suitability for species occupation and propagation (Sutherst and Maywald 1985; Sutherst *et al.* 1996; Sutherst *et al.* 2007; Sutherst and Bourne 2009). An EI of close to 100 indicates a highly favourable habitat for species survival and persistence (Sutherst *et al.* 2007).

Model fitting

To create the CLIMEX model, records of the species current known distribution and biological parameters, such as its response to temperature and moisture, are obtained from experimental data or from extensive literature searches. The distribution limits of species are determined by the stresses they face in their environments (Sutherst *et al.* 2007). The stress functions are therefore usually fitted to limit the species range to the known geographical distribution, minimizing the apparent false positives and thereby maximizing the model specificity. However, when fitting the stress functions, knowledge of the species ability to survive inclement conditions such as cold or drought can also be used to inform the parameter choices. For example, knowledge of a species' inability to tolerate frosts might suggest that the cold stress temperature threshold be set near to zero. The temperature and soil moisture growth limits are informed by the stress thresholds (a species population cannot simultaneously grow and diminish under the same conditions (Kriticos *et al.* 2005)). The most useful information for informing the choice of parameter values for the growth indices however usually comes from direct experimental observations.

To assess whether moisture or temperature were most limiting to the distribution of *An. arabiensis* and *An. funestus*, the moisture index and temperature index for the African continent were plotted in the "compare locations" function within CLIMEX using the CliMond CM101975HV1.1 dataset (Kriticos *et al.* 2012). Shape files of the species' distribution records were then overlaid onto these maps. Based on the mapped outputs and the relative climatic conditions associated with known species distributions, stress indices in the "compare locations" simulation were altered for each species. Parameters used to simulate the distribution of *An. arabiensis* included cold stress, wet stress and the temperature and moisture indices (Table S1). The temperature index incorporates the developmental data

obtained from experimental studies. For *An. funestus*, parameters used to fit the species distribution included temperature and moisture indices and cold stress (Table S2). In addition to these parameter estimates, an irrigation scenario of “top-up” irrigation of 3.6 mm per day during the summer months was included when mapping habitat suitability. This was largely as a result of outlying points of the species current distributions not being suitable under only climatic conditions, and the incorporation of this irrigation scenario made these habitats at least “marginally suitable”. Using these parameter values, the EI of each vector was mapped using “compare locations” in CLIMEX. The models were validated by comparing known phenology data such as seasonality of malaria transmission in several regions on the boundary of current range projections.

The species parameters incorporated in the CLIMEX model were developmental parameters obtained from chapter 3 (Table S1-3). Although thermal tolerance information of adults, larvae and pupae (chapter 2), can be combined with these developmental data (chapter 3) and with desiccation tolerance data (chapter 4) in a future planned comprehensive mechanistic distribution model, the thermal range for development is narrower than that for survival or activity (Figure 1). Development from egg to adult can only take place between ~14°C and 35°C, and hence, these vectors should be constrained to habitats where temperatures allow development and completion of at least one generation to take place.

Climate change scenarios

CLIMEX allows the user to determine potential species distributions under climate change scenarios defined by the user (Sutherst *et al.* 2007). According to the Intergovernmental Panel on Climate Change (IPCC), global average temperatures are expected to increase by approximately 2°C within the next 50 to 100 years (Boko *et al.* 2007). Additionally, changes

in rainfall intensity and rainfall patterns are also expected (Fay *et al.* 2008; Solomon *et al.* 2009).

To understand how habitat suitability for each of these vectors may be altered by climate change, two different climate change scenarios were applied in the “compare locations” function within CLIMEX. The first of these was a moderate climate change scenario of temperature increases of 0.1°C per degree of latitude and a 20% reduction or increase rainfall, in winter and summer, respectively. The second climate change scenario was a more severe temperature increase of 3°C applied to minimum and maximum temperatures in winter, summer and the equatorial zones. This scenario also included a 20% reduction in winter rainfall and a 20% increase in summer rainfall (Sutherst *et al.* 2007).

Results therefore present the potential distributions of these species based on biological and environmental data, both in the absence of climate change and under two climate change scenarios. Distribution data for *Anopheles arabiensis* and *An. funestus* throughout Africa were obtained from the Malaria Atlas Project (MAP) (<http://www.map.ox.ac.uk/>). These distribution data were used in all projections of species distributions. In total, more than 1100 records were included in distribution data for each species. Unfortunately, as with all species distribution records, biases do exist, largely as a result of collection effort. However, results from these CLIMEX projections may be used in hypothesis testing of suitability for sites where there are currently no distribution records present (Sutherst *et al.* 2007).

Results

The moisture index map of Africa suggests that *Anopheles arabiensis* is limited from occupying the central African region as a result of high rainfall (Figure S1). The temperature index map suggests that this species occupies habitats of wide ranging temperatures, but that

its distribution within South Africa is likely limited because of low temperatures (Figure S2). Similarly, *An. funestus* seems constrained by low temperatures in South Africa and in the east African highland regions (Figure S3). However, this species is less constrained by high moisture conditions as distribution data for the tropical central African region indicate (Figure S4). The northern limits for this species fall just within the low moisture regions of Mali and Cameroon, and distribution points do not extend as far north as those for *An. arabiensis* do. Using the parameter values in Table S1, distribution of *An. arabiensis* without climate change, and with two climate change scenarios were mapped (Figures 2A – 2C). Incorporating the irrigation scenario of 3.6 mm/day during summer made the northern limits for *An. arabiensis* more suitable for growth than in the absence of irrigation (Figure 2A). Populations in these regions displayed strong seasonal growth in response to moisture and temperature. Similarly, populations in Namibia and Botswana fell just outside of the projected suitable habitat range, largely because these populations are low temperature-limited during winter and only exist during the warmer, summer months.

Under the first climate change scenario (Figure 2B), habitat suitability for *An. arabiensis* moves southwards, with northern regions becoming less favourable for population growth and southerly limits becoming more favourable for year-round growth. Under the second climate change scenario, this situation is exacerbated, with an even greater region in the current northern limits of the species range becoming more unsuitable, and an even greater extension of suitable habitats in the south, particularly, the north west coast of Namibia and the east coast of South Africa and Mozambique (Figure 2C). Under both climate change scenarios, the previously unsuitable (too wet) central African region increases in suitability for *An. arabiensis* population growth (Figures 2B and 2C).

The current distribution of *An. funestus* indicates that this species occupies regions of relatively high moisture and that it is only limited by extended periods of very low

temperatures in South Africa (Figure 3A). Areas modelled as marginally suitable for this species (in Namibia and Botswana) indicate a seasonal pattern of population growth, limited in the winter months by cold temperatures.

Under the first climate change scenario (Figure 3B) the central African region becomes even more suitable for *An. funestus*, while its northern limits are likely to shift south as a result of drier conditions. Areas where this species shows a seasonal distribution, become more favourable for population growth (western Namibia and Botswana). Furthermore, habitat suitability on the east coast of South Africa is increased. The biggest change under the second climate change scenario (Figure 3C) is evident in the northern limits of current habitat suitability for *An. funestus*. These areas become less favourable for growth and population persistence, largely because of reductions in moisture. A greater portion of Namibia, Zambia and eastern South Africa become suitable for this species under this extreme climate change scenario.

Discussion

Climate change is likely to increase the available habitat for both *An. arabiensis* and *An. funestus* on the eastern and southern margins of current species distributions in Africa. These increases are likely to be associated with increased rainfall and temperatures predicted to occur with climate change (Davis 2011). However, because of the breeding biology of *Anopheles funestus* and its use of semi-permanent to permanent water bodies for breeding (Gillies and Coetzee 1987; Charlwood *et al.* 2000), whether or not climate change will have any influence on this species depends largely on what the effects on these breeding sites may be.

In the northern range of the current species distributions, areas are likely to become less favourable for both species, probably as a result of reduced moisture availability in these

areas as predicted to occur with climate change (Boko *et al.* 2007; IFAD 2011), although again, for *An. funestus*, if permanent water bodies remain regardless of reduced overall rainfall, there may be a lag in the climate change effects on this species in these areas. This southerly shift in species range is also in line with distribution shifts observed in plant and other animal taxa in response to recent climate warming (Parmesan and Yohe 2003; Parmesan 2006). Areas where malaria currently occurs only on a seasonal basis (e.g. Namibia, Mali, Cameroon (www.mara.org.za/pdfmaps; Tanser *et al.* 2003; Roca-Feltrer *et al.* 2009) will increase in habitat suitability, likely resulting in an increased transmission season. Other areas (e.g. Zambia) that also could previously only support seasonal mosquito populations (www.mara.org.za/pdfmaps; Roca-Feltrer *et al.* 2009) may become more favourable for a resident population and hence, year-long malaria transmission.

Areas in the east African highlands are also predicted to increase in habitat suitability. These areas are currently limiting to mosquito population expansion, largely as a result of cool temperatures associated with high altitude regions (Cox *et al.* 1999). However, this area has been the centre of controversy with regard to malaria transmission and how climate change may impact on the disease in this region (Cox *et al.* 1999; Hay *et al.* 2002; Pascual *et al.* 2006; Alonso *et al.* 2010; Stern *et al.* 2011). Several authors have, however, conceded that warming has indeed taken place in this area (Omumbo *et al.* 2011; Stern *et al.* 2011) and that, combined with other factors (Omumbo *et al.* 2011; Himeidan and Kweka 2012) this warming may be responsible for increased cases of malaria, through increased mosquito populations. Furthermore, in situations where mosquito insecticide resistance is present, these potential changes to mosquito distributions could severely impact on the number of annual malaria cases.

Although these mapped projections should be used with caution because they do not include biotic interactions (Sutherst *et al.* 2007; Lozier and Mills 2011; de Villiers *et al.*

2012), and because it is problematic to model potential species habitat suitability for a permanent water breeding species such as *An. funestus*, CLIMEX is likely to provide a conservative estimate of species ranges and range changes in response to climate change, at least for *An. arabiensis*. This conservative approach has been highlighted elsewhere (Lozier and Mills 2011; de Villiers *et al.* 2012). CLIMEX has been used successfully to predict establishment potential of several invasive and pest species (Robinson and Hoffmann 2001; Thomson *et al.* 2011; Lozier and Mills 2011; Macfadyen and Kriticos 2012) and therefore, these projections are likely to provide a good starting point on which to base future planned malaria control interventions. However, results are not meant to provide an exact prediction of where malaria is likely to spread under future climate change scenarios. As a disease, malaria is highly complex and much of its current range is not only limited by climatic conditions in those areas, but rather, by the success of several control methods which have seen the reduction or elimination of vectors from areas where they should otherwise be able to persist (e.g. Maharaj *et al.* 2005). Furthermore, as highlighted previously, the nature of *An. funestus* breeding habitats, complicates modelling efforts for this species, although incorporating fine scale hydrology maps and field-based studies to assess the propensity for these sites to act as sources of this vector, may help to increase the predictive capacity of models for *An. funestus* spread or range change.

Here, only the developmental thresholds for growth of both species from the egg to adult stage were incorporated in model parameters. Although these kinds of data have shown success in predicting the distribution of known breeding sites of the third African malaria vector, *An. gambiae* (Bayoh and Lindsay 2003) the incorporation of other species-specific traits and physiological tolerances, should provide a more accurate estimate of species ranges and potential range changes. Furthermore, *Anopheles* mosquitoes are highly adaptable and their distributions are closely linked to human habitation (Mbogo *et al.* 1993; Githeko *et al.*

1994; Muriu *et al.* 2008). Their ability to switch host (Wanji *et al.* 2003; Sinka *et al.* 2010) and the presence of different genotypes adapted to different habitats (Gray and Bradley 2005; Rocca *et al.* 2009) makes predictions of climate change impacts on the disease difficult. The *Anopheles* mosquitoes also show a level of phenotypic plasticity in thermal traits (Lyons *et al.* 2012) which may indicate that they are able to alter or evolve tolerance mechanisms to counter hot or dry conditions associated with changing climates (Hoffmann and Sgró 2011). In addition, their use of indoor-resting behaviour and microclimates may counteract the effects of climate change on these species (Paaijmans and Thomas 2011). However, if nothing else, development of these vectors may be constrained by climate change and these kinds of models may provide a good indication of areas suitable for population growth.

References

- Alonso, D., Bouma, M.J. and Pascual, M. 2010. Epidemic malaria and warmer temperature in recent decades in an East African highland. *Proceedings of the Royal Society B* **278**: 16661-16669.
- Araújo, M.B. and Guisan, A. 2006. Five (or so) challenges for species distribution modeling. *Journal of Biogeography* **33**: 1677-1688.
- Araújo, M.B. and Peterson, A.T. 2012. Uses and misuses of bioclimatic envelope modeling. *Ecology* **93**: 1527-1539.
- Bayoh, M.N. and Lindsay, S.W. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Bulletin of Entomological Research* **93**: 375-381.
- Bayoh, M.N. and Lindsay, S.W. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Medical and Veterinary Entomology* **18**: 174-179.

- Boko, M., Niang, I., Nyong, A., Vogel, C., Githeko, A., Medany, M., Osman-Elasha, B., Tabo, R. and Yanda, P. 2007. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Edited by Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. Cambridge: Cambridge University Press, UK: 433-467.
- Buckley, L.B. and Kingsolver, J.G. 2012. Functional and phylogenetic approaches to forecasting species' responses to climate. *Annual Review of Ecology, Evolution and Systematics* **43**: 205-226.
- Charlwood, J.D., Viji, R. and Billingsley, P.F. 2000. Dry season refugia of malaria-transmitting mosquitoes in a dry savannah zone of East Africa. *American Journal of Tropical Medicine and Hygiene* **62**: 726-732.
- Cox, J., Craig, M., Le Sueur, D. and Sharp, B. 1999. Mapping malaria risk in the highlands of Africa. MARA/HIMAL Technical Report December.
- Davis, C.L. 2011. *Climate Risk and Vulnerability: A Handbook for Southern Africa*. Council for Scientific and Industrial Research, Pretoria, South Africa.
- de Villiers, M., Hattingh, V. and Kriticos, D.J. 2012. Combining field phonological observations with distribution data to model the potential distribution of the fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Bulletin of Entomological Research*: doi:10.1017/S0007485312000454.
- Elith, J. and Leathwick, J.R. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution and Systematics* **40**: 677-697.
- Elith, J., Kearney, M. and Phillips, S. 2010. The art of modelling range-shifting species. *Methods in Ecology and Evolution* **1**: 330-342.

- Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D. and Harper, C.W. 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. *Global Change Biology* **14**: 1600-1608.
- Gillies, M.T. and Coetzee, M. 1987. *A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region)*. Publications of the South African Institute of Medical Research, Johannesburg, South Africa.
- Githeko, A.K., Service, M.W., Mbogo, C.M., Atieli, F.K. and Juma, F.O. 1994. Origin of blood meals in indoor and outdoor resting malaria vectors in western Kenya. *Acta Tropica* **58**: 307-316.
- Gray, E.M. and Bradley, T.J. 2005. Physiology of desiccation resistance in *Anopheles gambiae* and *Anopheles arabiensis*. *American Journal of Tropical Medicine and Hygiene* **73**: 553-559.
- Hay, S.I., Cox, J., Rogers, D.J., Randolph, S.E., Stern, D.I., Shanks, G.D., Myers, M.F. and Snow, R.W. 2002. Climate change and the resurgence of malaria in the East African highlands. *Nature* **415**: 905-909.
- Himeidan, Y.E. and Kweka, E.J. 2012. Malaria in East African highlands during the past 30 years: impact of environmental changes. *Frontiers in Physiology* **3**: 315.
- Hoffmann, A.A. and Sgró, C.M. 2011. Climate change and evolutionary adaptation. *Nature* **470**: 479-485.
- International Fund for Agricultural Development (IFAD). 2011. Addressing Climate Change in West and Central Africa. www.ifad.org.
- Jiménez-Valverde, A., Lobo, J.M. and Hortal, J. 2008. Not as good as they seem: the importance of concepts in species distribution modelling. *Diversity and Distributions* **14**: 895-890.

- Kearney, M., Phillips, B.L., Tracy, C.R., Christian, K.A., Betts, G. and Porter, W.P. 2008. Modelling species distributions without using species distributions: the cane toad in Australia under current and future climates. *Ecography* **31**: 423-434.
- Kearney, M., Porter, W.P., Williams, C., Ritchie, S. and Hoffmann, A.A. 2009. Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: the dengue mosquito *Aedes aegypti* in Australia. *Functional Ecology* **23**: 528-538.
- Kearney, M. and Porter, W.P. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters* **12**: 334-350.
- Kriticos, D.J., Yonow, T. and McFadyen, R.E. 2005. The potential distribution of *Chromolaena odorata* (Siam weed) in relation to climate. *Weed Research* **45**: 246-254.
- Kriticos, D.J., Webber, B.L., Leriche, A., Ota, N., Macadam, I., Bathols, J. and Scott, J.K.. 2012. CliMond: global high-resolution historical and future scenario climate surfaces for bioclimatic modelling. *Methods in Ecology and Evolution* **3**: 53-64.
- Lafferty, K.D. 2009. Calling for an ecological approach to studying climate change and infectious diseases. *Ecology* **90**: 932-933.
- Lozier, J.D. and Mills, N.J. 2011. Predicting the potential invasive range of light brown apple moth (*Epiphyas postvittana*) using biologically informed and correlative species distribution models. *Biological Invasions* **13**: 2409-2421.
- Lyons, C.L., Coetzee, M., Terblanche, J.S. and Chown, S.L. 2012. Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*. *Malaria Journal* **11**: 226.
- Macfadyen, S. and Kriticos, D.J. 2012. Modelling the geographical range of a species with variable life history. *PLoS ONE* **7**: e40313. doi:10.1371/journal.pone.0040313.

- Maharaj, R., Mthembu, D.J. and Sharp, B.L. 2005. Impact of DDT re-introduction on malaria transmission in KwaZulu-Natal. *South African Medical Journal* **95**: 871-874.
- Martens, W.J.M., Jetten, T.H. and Focks, D.A. 1997. Sensitivity of malaria, schistosomiasis and dengue to global warming. *Climatic Change* **35**: 145-156.
- Mbogo, C.N., Kabiru, E.W., Muiruri, S.K., Nzovu, J.M., Ouma, J.H., Githure, J.I. and Beier, J.C. 1993. Bloodfeeding behaviour of *Anopheles gambiae s.l.* and *Anopheles funestus* in Kilifi District, Kenya. *Journal of the American Mosquito Control Association* **9**: 225-227.
- Midgley, G.F., Hannah, L., Millar, D., Rutherford, M.C. and Powrie, L.W. 2002. Assessing the vulnerability of species richness to anthropogenic climate change in a biodiversity hotspot. *Global Ecology and Biogeography* **11**: 445-451.
- Molyneux, D.H. 2009. Climate change and vector-borne diseases (VBDs): Redrawing the epidemiology map. *Public Health Journal* **20**: 8-18.
- Muriu, S.M., Muturi, E.J., Shililu, J.I., Mbogo, C.M., Mwangangi, J.M., Jacob, B.G., Irungu, L.W., Mukabana, R.W., Githure, J.I. and Novak, R.J. 2008. Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya. *Malaria Journal* **7**: 43.
- Omumbo, J.A., Lyon, B., Waweru, S.M., Connor, S.J. and Thomson, M.C. 2011. Raised temperatures over the Kericho tea estates: revisiting the climate in the East African highlands malaria debate. *Malaria Journal* **10**: 12.
- Paaijmans, K.P. and Thomas, M.B. 2011. The influence of mosquito resting behaviour and associated microclimate for malaria risk. *Malaria Journal* **10**: 183.
- Parnesan, C. and Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37-42.

- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* **37**: 637-669.
- Pascual, M., Ahumada, J.A., Chaves, L.F., Rodó, X. and Bouma, M. 2006. Malaria resurgence in the East African highlands: temperature trends revisited. *Proceedings of the National Academy of Sciences of the U.S.A.* **103**: 5829-5834.
- Pattison, R.R. and Mack, R.N. 2008. Potential distribution of the invasive tree *Triadica sebifera* (Euphorbiaceae) in the United States: evaluating CLIMEX predictions with field trials. *Global Change Biology* **14**: 813-826.
- Pearson, R.G. and Dawson, T.P. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography* **12**: 361-371.
- Phillips, S.J., Anderson, R.P. and Schapire, R.E. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* **190**: 231-259
- Poutsma, J., Loomans, A.J.M., Aukema, B. and Heijerman, T. 2008. Predicting the potential geographical distribution of the harlequin ladybird, *Harmonia axyridis*, using the CLIMEX model. *BioControl* **53**: 103-125
- Robertson, M.P., Peter, C.I., Villet, M.H. and Ripley, B.S. 2003. Comparing models for predicting species' potential distributions: a case study using correlative and mechanistic predictive modelling techniques. *Ecological Modelling* **164**: 153-167.
- Robinson, M.T. and Hoffmann, A.A. 2001. The pest status and distribution of three cryptic blue oat mite species (*Penthaleus* spp.) and redlegged earth mite (*Halotydeus destructor*) in southeastern Australia. *Experimental and Applied Acarology* **25**: 699-716.
- Roca-Feltrer, A., Schellenberg, J.R.M.A., Smith, L. and Carneiro, I. 2009. A simple method for defining malaria seasonality. *Malaria Journal* **8**: 276.

- Rocca, K.A.C., Gray, E.M., Costantini, C. and Besansky, N.J. 2009. 2La chromosomal inversion enhances thermal tolerance of *Anopheles gambiae* larvae. *Malaria Journal* **8**: 147.
- Rogers, D.J. and Randolph, S.E. 2000. The global spread of malaria in a future, warmer world. *Science* **289**: 1763-1766.
- Sinka, M.E., Bangs, M.J., Manguin, S., Coetzee, C., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Okara, R.M., van Boeckel, T., Godfray, C.J., Harbach, R.E. and Hay, S.I. 2010. The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites and Vectors* **3**: 117.
- Solomon, S., Plattner, G.K., Knutti, R. and Friedlingstein, P. 2009. Irreversible climate change due to carbon dioxide emissions. *Proceedings of the National Academy of Sciences of the U.S.A.* **106**: 1704-1709.
- Stern, D.I., Gething, P.W., Kabaria, C.W., Temperley, W.H., Noor, A.M., Okiro, E.A., Shanks, G.D., Snow, R.W. and Hay, S.I. 2011. Temperature and malaria trends in highland East Africa. *PLoS ONE* **6**: e24524. Doi: 10.1371/journal.pone.00244524.
- Sutherst, R.W. and Maywald, G.F. 1985. A computerized system for matching climates in ecology. *Agriculture, Ecosystems and Environment* **13**: 281-299.
- Sutherst, R.W., Floyd, R.B. and Maywald, G.F. 1996. The potential geographical distribution of the cane toad, *Bufo marinus* L. in Australia. *Conservation Biology* **10**: 294-299.
- Sutherst, R.W., Maywald, G.F. and Bourne, A.S. 2007. Including species interactions in risk assessments for global change. *Global Change Biology* **13**: 1843-1859.
- Sutherst, R.W. and Bourne, A.S. 2009. Modelling non-equilibrium distributions of invasive species: a tale of two modelling paradigms. *Biological Invasions* **11**: 1231-1237.

- Tanser, F.C., Sharp, B. and Le Sueur, D. 2003. Potential effect of climate change on malaria transmission in Africa. *Lancet* **362**: 1792-1798.
- Thomson, L.J., Macfadyen, S. and Hoffmann, A.A. 2010. Predicting the effects of climate change on natural enemies of agricultural pests. *Biological Control* **52**: 296-306.
- van Lieshout, M., Kovats, R.S., Livermore, M.T.J. and Martens, P. 2004. Climate change and malaria: analysis of the SRES climate and socio-economic scenarios. *Global Environmental Change* **14**: 87-99.
- Wanji, S., Tanke, T., Atanga, S.N., Ajonina, C., Nicholas, T. and Fontenille, D. 2003. *Anopheles* species of the mount Cameroon region: biting habits, feeding behavior and entomological inoculation rates. *Tropical Medicine and International Health* **8**: 643-649.
- World Health Organization: World Malaria Report 2010. Geneva, Switzerland.
[<http://www.who.int/malaria>].

Figure Legends

Figure 1. Thermal physiology data for *Anopheles arabiensis* (blue) and *Anopheles funestus* (red). Black dotted lines indicate the lower (LLT) and upper lethal temperature (ULT) data of adults for the two species, while the green dotted lines indicate the average LLT and ULT for larval and pupal stages (combined) for each species. The development rate-temperature curves for each species are also shown, lines are not fitted, and are an indication of the general trend for each species.

Figure 2. Ecoclimatic Index (EI) indicating habitat suitability of current *Anopheles arabiensis* habitat (A), suitability under a moderate climate change scenario (B), and suitability under a more extreme scenario (C).

Figure 3. Ecoclimatic Index (EI) of current habitat suitability of *Anopheles funestus* (A), habitat suitability under a moderate climate change scenario (B), and habitat suitability under a more extreme climate change scenario (C).

Figures

Fig. 1

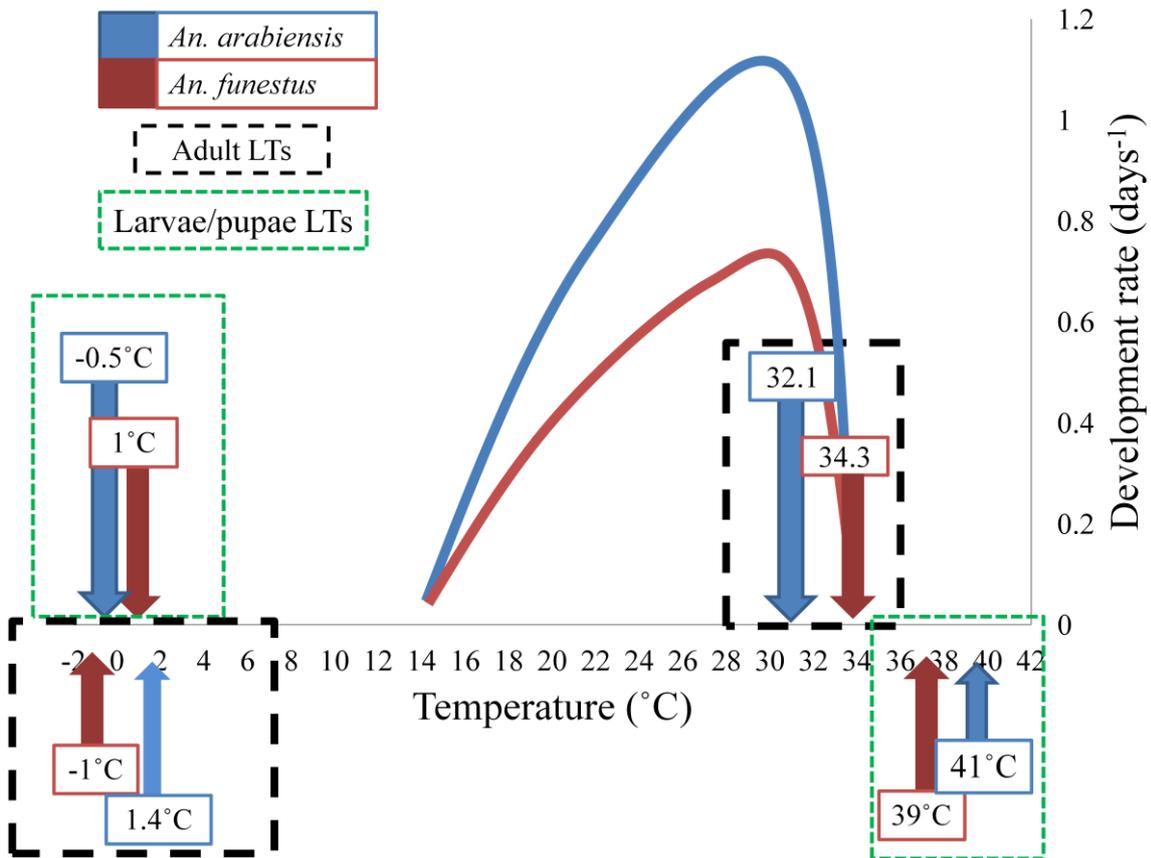


Fig. 2

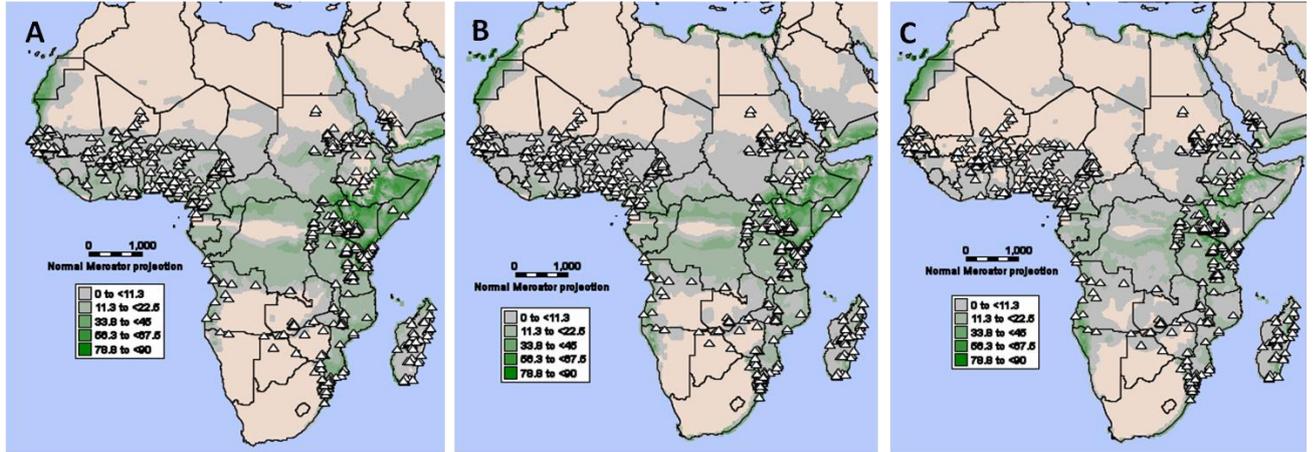
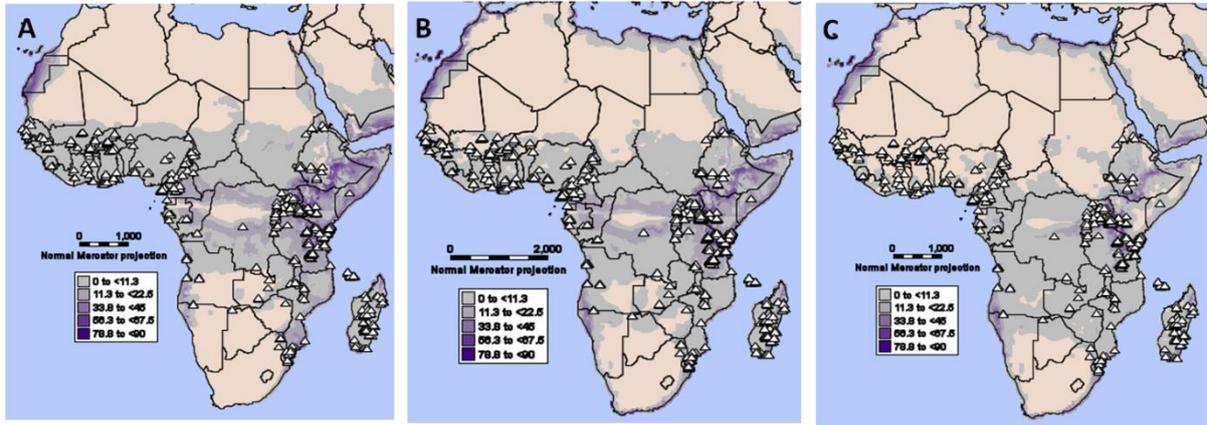


Fig. 3



Supplementary Materials

Fig. S1

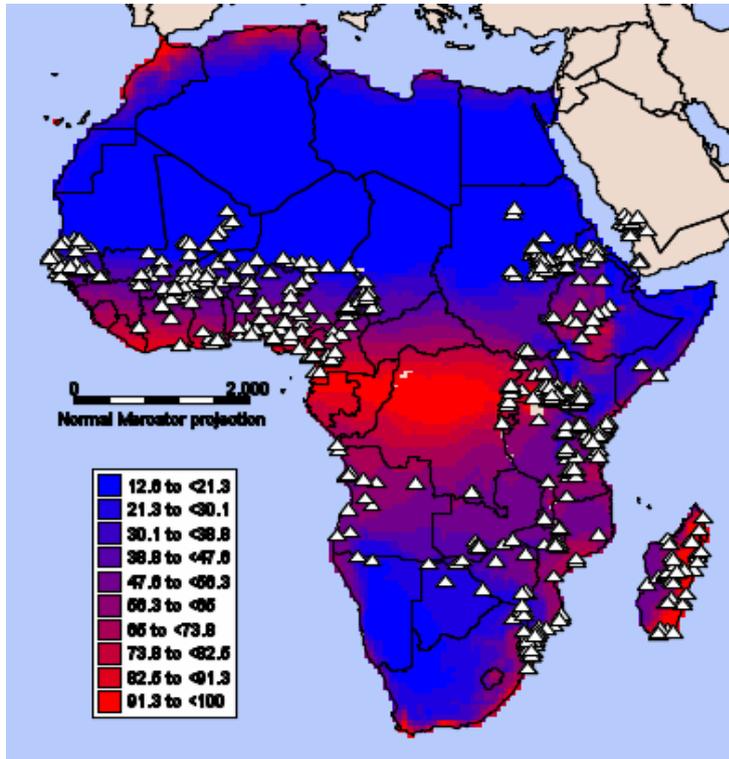


Figure S1. Moisture index for Africa and distribution points of *Anopheles arabiensis* (Δ) indicating moisture conditions associated with current distribution records.

Fig. S2

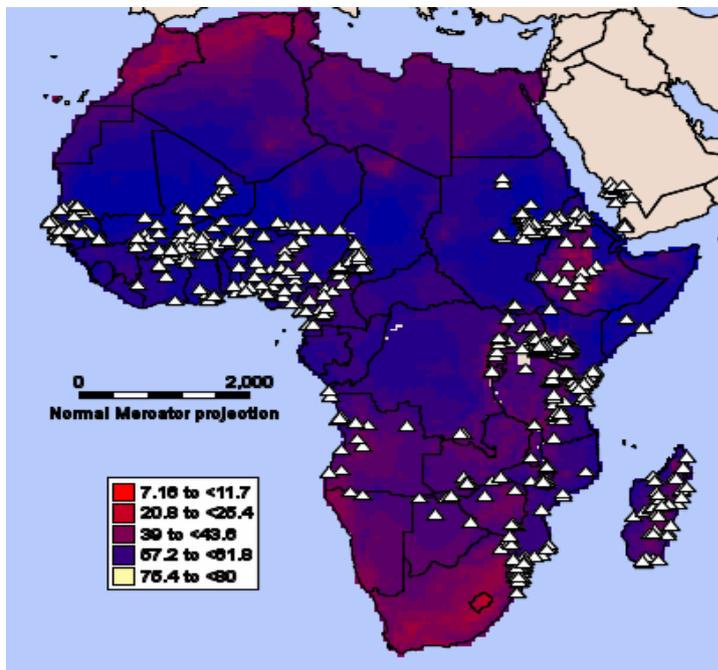


Figure S2. Temperature index for the African continent and distribution points of *Anopheles arabiensis* (Δ) indicating conditions associated with current distribution records.

Fig. S3

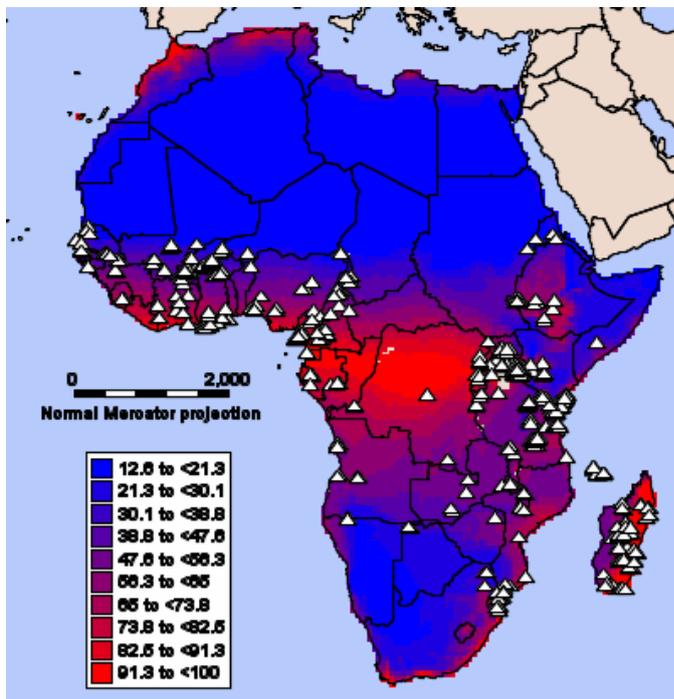


Figure S3. Moisture index for Africa and distribution points of *Anopheles funestus* (Δ) indicating moisture conditions associated with current distribution records of this species.

Fig. S4

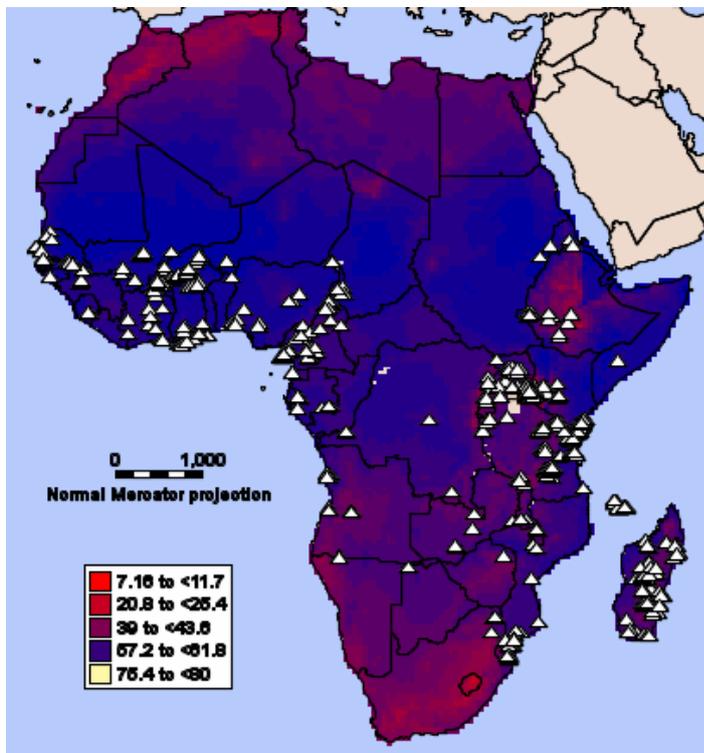


Figure S4. Temperature index for the African continent and distribution points of *Anopheles funestus* (Δ) indicating characteristics associated with current distribution records.

Table S1. Parameter estimates used in CLIMEX model fitting for *Anopheles arabiensis*.

Moisture Index			
SM0	SM1	SM2	SM3
0.1	0.3	0.6	0.8
Temperature Index			
DV0	DV1	DV2	DV3
14	20	32	36
Cold Stress			
TTCS	THCS	DTCS	DHCS
14	-0.002	14	-0.01
Wet Stress			
SMWS	HWS		
2	0.003		
Day-degree accumulation above			
DV0			
DV0	DV3	MTS	
14	36	7	
Day-degree accumulation above			
DVCS			
DVCS	DV4	MTS	
15	100	7	
Day-degree accumulation above			
DVHS			
DVHS	DV4	MTS	

36

100

7

Degree-days per generation

PDD

140

Table S2. Parameter estimates used in fitting CLIMEX models for *Anopheles funestus*.

Moisture Index			
SM0	SM1	SM2	SM3
0.2	0.4	0.6	0.8
Temperature Index			
DV0	DV1	DV2	DV3
14	20	30	35
Cold Stress			
TTCS	THCS	DTCS	DHCS
13	-0.002	14	-0.01
Day-degree accumulation above DV0			
DV0	DV3	MTS	
14	35	7	
Day-degree accumulation above DVCS			
DVCS	DV4	MTS	
14	100	7	
Day-degree accumulation above DVHS			
DVHS	DV4	MTS	
35	100	7	
Degree-days per generation			
PDD	167		

Table S3. Explanations of parameters used in CLIMEX model fitting (from Sutherst *et al.* 2007).

Parameter	Description
SM0	Lower soil moisture threshold
SM1	Lower optimal soil moisture
SM2	Upper optimal soil moisture
SM3	Upper soil moisture threshold
DV0	Lower temperature threshold
DV1	Lower optimum temperature
DV2	Upper optimum temperature
DV3	Upper developmental threshold
TTCS	Cold stress temperature threshold
THCS	Cold stress temperature accumulation rate
DTCS	Cold stress degree-day threshold
DHCS	Cold stress accumulation if weekly degree-days are not reached
DVCS	Threshold number of degree-days above developmental temperature threshold
DVHS	Heat stress degree-day threshold
PDD	Number of degree-days above DV0 to complete one generation
SMWS	Wet stress threshold
HWS	Wet stress accumulation rate

Chapter 6

General Conclusion

Conclusion

In the last ten years, significant progress has been made in reducing the global impact of malaria. Since 2000, the number of deaths resulting from the disease has declined from over one million to the current World Health Organisation's estimate of 800 000 (WHO 2010). The reductions are largely a result of control measures in the form of indoor residual spraying of insecticides and the use of insecticide treated bednets (ITNs) (Mabaso *et al.* 2004; Kleinschmidt *et al.* 2009). Novel approaches to control the spread of malaria include the development of a new vaccine against the *Plasmodium* parasites (Bejon *et al.* 2008), the introduction of a fungal biological control agent (Scholte *et al.* 2005; Farenhorst *et al.* 2009) and the proposed use of the sterile insect technique to control wild populations of the *Anopheles* vectors (Benedict and Robinson 2003; Munhenga *et al.* 2011). The application of larvicides to known breeding sites has also been proposed as an alternative and efficient control measure (Fillinger and Lindsay 2006).

Despite all these interventions and approaches to control the spread and extent of malaria, it still remains a major public health concern. This is largely because of the short generation times of the mosquitoes, and hence their ability to build up resistance to the insecticides currently in use (Hemingway and Ranson 2000). Furthermore, the lack of appropriate education on symptom recognition, disease prevention and cure, and the fact that malaria affects large parts of the under-developed African and Asian continents, means that medicine is not readily available in these regions and, often, the use of mechanical means to avoid mosquito bites (such as ITNs) is not well-enforced or properly adhered to (Schellenberg *et al.* 1999; Minakawa *et al.* 2008). Malaria is therefore, likely to remain a concern for many parts of these regions for decades to come (van Lieshout *et al.* 2004).

Understanding how malaria as a disease is likely to be impacted by climate change may provide an indication of where control efforts should be focused in the face of

potentially range-shifting vector populations. Although there is heated debate as to whether or not malaria will indeed be affected by climate change (Martens *et al.* 1997; Rogers and Randolph 2000; Hay *et al.* 2002; Tanser *et al.* 2003), this debate serves to highlight the uncertainty associated with this disease. This uncertainty is probably a result of the complex nature of malaria. *Anopheles* mosquitoes are the vectors of the *Plasmodium* parasite, and both of these organisms display strong associations with temperature (Pascual *et al.* 2009; Paaijmans *et al.* 2009). Increases in temperature are likely to affect not only the mosquito populations, but also the parasite (Pascual *et al.* 2009; Paaijmans *et al.* 2009; Paaijmans *et al.* 2010; Mordecai *et al.* 2012) with increases above a certain temperature threshold detrimental to survival of both the vector and the parasite (Pascual *et al.* 2009; Mordecai *et al.* 2012). Furthermore, the mosquito is highly dependent on moisture or water availability, not only for breeding but also for survival. The changes in temperature and rainfall predicted to occur with climate change (Eeley *et al.* 1999; Davis 2011; Hansen *et al.* 2012) may therefore significantly influence mosquito populations and through their effects on the entomological inoculation rate, malaria as well. Given the current extent of malaria and the environmental changes predicted to occur with climate change, the aim of this thesis was to provide a comprehensive set of physiological tolerances of two of the most important Afrotropical malaria vectors – *Anopheles arabiensis* and *An. funestus*, information which was previously lacking in the literature. In light of studies that have shown range changes in other insect species in response to climate change (Buckley and Kingsolver 2012; de Villiers *et al.* 2012), I also addressed the extent that climate change might influence malaria through its effect on the vectors.

In Chapter two, I investigated the thermal tolerance of these two vector species given their strong association and reliance on temperature for survival (Gillies and Coetzee 1987; Clements 2000; Mordecai *et al.* 2012). I determined the extent of phenotypic plasticity

(Scheiner 1993) within each species by investigating the influence of thermal acclimation, age and sex on critical thermal limits (CT) of both vector species. Significant phenotypic plasticity was evident across thermal acclimation treatments, suggesting that these species have some scope for thermal adaptation in the face of increasing temperatures. In accordance with other studies on insects (Bowler and Terblanche 2008; Dawes *et al.* 2009) a clear senescence response was evident in both vectors, with older age groups showing lower tolerance to increasing temperatures than younger age groups. The rate of temperature change used in this study is arguably not fully representative of what the rate of change in natural systems may be (Terblanche *et al.* 2007; Chown *et al.* 2008) and future studies should focus their efforts on determining the potential influence of different rates of change on the thermal tolerance of these vectors, especially given the important findings of other insect studies (Allen *et al.* 2012) which indicate that rate of change can significantly influence these critical thermal limits. Importantly, comparisons between early generations of the wild strains of both species show a high degree of similarity in CT limits, suggesting that the use of these colonies in assessing physiological traits is useful when inferring wild population responses. Chapter two also investigated the lethal temperature limits of different aged adults and of fourth instar larvae and pupae of both *An. arabiensis* and *An. funestus*. The immature stages were significantly more tolerant of high temperature than the adult groups, unsurprising given their habitat constraints and relatively sedentary lifestyle when compared to adults (Clements 2000; Chown and Nicolson 2004). The thermal tolerance data collected in chapter two provides an important indication of thermal limits to survival and behaviour for these species. Taken alone, it suggests that both species would be able to tolerate a certain degree of warming associated with climate change. However, to understand the full extent of climate change on these vectors, further physiological traits were examined.

In Chapter three, I investigated the development rate-temperature relationships of *An. arabiensis* and *An. funestus* under a range of biologically significant constant and fluctuating temperatures. The inclusion of fluctuating temperatures is especially significant, given the climate change predictions of increases in extreme weather events and changes in diurnal temperature variability (Hansen *et al.* 2012) and evidence that fluctuating temperatures could significantly influence transmission potential (Paaijmans *et al.* 2010; Mordecai *et al.* 2012). *Anopheles arabiensis* and *An. funestus* showed development rates in accordance with their breeding biology – faster development for the puddle-breeding *An. arabiensis* when compared to the pond-breeder, *An. funestus*. Higher survival of *An. arabiensis* when compared with *An. funestus* was also evident under all temperature treatments. Fluctuating temperatures led to significantly different development rates and survival for both species, in accordance with many other insect species (Hagstrum and Hagstrum 1970; Hagstrum and Leach 1972; Hagstrum and Milliken 1991; Worner 1992). Under warming conditions, *An. arabiensis* populations may be advantaged, by experiencing not only a faster development rate, but also high survival of adults, leading to possible increases in the populations of this species. In areas where *An. arabiensis* is the main vector, we might expect to see increased cases of malaria due to changes in the entomological inoculation rate, which is directly influenced by the number of mosquitoes in a population. In contrast, areas where *An. funestus* or *An. gambiae* (the third important African malaria vector) are the main vectors, may experience reductions in malaria cases as a result of lowered mosquito population output. The thermal range for development of *An. arabiensis*, *An. funestus* and *An. gambiae* (Lindsay and Bayoh 2004), the three main vectors in sub-Saharan Africa, lies between ~14°C and 35°C and is significantly narrower than the temperatures adults or fourth instar larvae and pupae are able to tolerate (Chapter two). Hence, it seems plausible that the distribution of these vectors may be limited to sites where they are able to develop into adults.

In Chapter four, I investigated the influence of temperature and humidity combinations on the survival of *An. arabiensis* and *An. funestus* adults. Given the importance of moisture availability and temperature for these vectors (Clements 1963; Clements 2000) and their large surface area to volume ratio relative to larger organisms (Gullan and Cranston 1994; Chown and Nicolson 2004) their ability to tolerate these conditions significantly impacts their survival. In areas where malaria displays seasonal transmission, understanding the ability of these vectors to tolerate conditions associated with the dry season, could provide an important clue to their overwintering ability (Lehmann *et al.* 2010; Huestis *et al.* 2011). Both species showed differences in the survival times of males and females under different temperature and humidity conditions, with females surviving on average, longer than males. Reduced desiccation tolerance was also observed with an increase in age, similar to desiccation trials done on other insect species (Gibbs and Markow 2001) including mosquitoes (Gray and Bradley 2005). Contrary to the accepted notion that *An. arabiensis* is the more arid-adapted species of the Afrotropical malaria vectors (Gillies and Coetzee 1987; Coetzee *et al.* 2000), it showed lower desiccation tolerance when compared to *An. funestus*. These species differences are probably a result of differences in water loss rates between the species, with *An. funestus* showing reduced water loss rates when compared with *An. arabiensis*. This therefore suggests that different phenotypes of each of these species probably exist (e.g. Gray and Bradley 2005; Rocca *et al.* 2009; Sinka *et al.* 2010) in order for them to survive the dry season in seasonal transmission areas. Different phenotypes, each associated with differences in desiccation tolerance are evident in *An. gambiae s.s.* (Rocca *et al.* 2009).

In Chapter five, I used the developmental data (lower developmental thresholds, upper temperature threshold, and degree-days) to provide an initial indication of which areas will be conducive to vector development under two different climate change scenarios

(Sutherst *et al.* 2007). Results suggest that areas on the east coast of southern Africa, predicted to experience increases in rainfall (Boko *et al.* 2007; Davis 2011), may become more suitable for vector development. In addition, areas in the species current northern range margins are predicted to become less suitable, with the result that both vectors will experience a southerly range shift, similar to those observed for several other plant and animal taxa (Parmesan and Yohe 2003; Parmesan 2006). Areas that currently experience seasonal transmission may become more suitable for year-round transmission. In the absence of appropriate control measures, these areas may therefore experience a surge in malaria cases as temperatures warm and rainfall intensity increases. However, these projections are only preliminary and based on only one set of physiological tolerances. Furthermore, malaria is a complex disease with several avenues, reliant on both mosquito and parasite survival and influenced to differing degrees by climate and by the geopolitics of control interventions, and the more threatening politics of armed conflict. The *Plasmodium* parasite is influenced by temperature just as the mosquito vectors are (Mordecai *et al.* 2012), and hence, temperature increases may result in reductions in the number of infective mosquitoes, although the number of mosquitoes may increase. Therefore, this may actually lead to reductions in malaria cases rather than increases. In addition, the ongoing control methods and development of new drugs and pesticides, could all ultimately influence whether or not malaria remains a major public health concern. In much the same way, the high degree of adaptability of the mosquito vectors and their development of resistance against insecticides, could further complicate matters.

Future Work

Given the role of species distribution modelling in forecasting potential range changes of species (Kearney *et al.* 2008; Elith and Leathwick 2009; Kearney *et al.* 2009; Elith *et al.*

2010; de Villiers *et al.* 2012) and the many successes in the field to date (Kearney *et al.* 2008; Buckley and Kingsolver 2012), applying such an approach to malaria or to the mosquito vector specifically, is likely to provide a robust indication of potential species ranges. Forecasts currently suggest that malaria is likely to increase on the range margins of current distributions and increase its distribution into high altitude regions (Omumbo *et al.* 2011; Himeidan and Kweka 2012). Because different phenotypes and genotypes of each species are present in different regions of the continent (e.g. Gray and Bradley 2005; Rocca *et al.* 2009; Sinka *et al.* 2010), their tolerance traits are likely to differ to some degree. Hence, to understand the full range of environmental conditions tolerated by these vectors, an assessment of the relative differences and changes in thermal tolerance (for example) should be made in as many malaria-prone regions as possible. Therefore, the data collected in this thesis will go a long way in use in mechanistic species distribution models to provide a comprehensive projection of potential mosquito ranges under climate change scenarios. Incorporating information such as evaporation, rainfall, monthly temperature and humidity data, together with the physiological tolerance data of the vectors should provide a more accurate estimate of these species' fundamental niches (Kearney and Porter 2004; Soberón 2005; Kearney *et al.* 2009). If we are able to develop models specific to the vectors in a particular region, given the likely differences in physiological traits associated with geographic location (Hoffmann 2010; Overgaard *et al.* 2011), our ability to control the disease may increase substantially. The development of a model which incorporates both vector and parasite thresholds, could provide the most accurate assessment of climate change impacts on malaria to date. These projections can then be used to inform policy and management. Finally, understanding where the mosquitoes are likely to establish or increase in abundance will enable efficient control measures to be put in place and may therefore act as early-warning systems for potential epidemics.

References

- Allen, J.L., Clusella-Trullas, S. and Chown, S.L. 2012. The effects of acclimation and rates of temperature change on critical thermal limits in *Tenebrio mollitor* (Tenebrionidae) and *Cyrtobagous salviniae* (Curculionidae). *Journal of Insect Physiology* **58**: 669-678.
- Bejon, P., Lusingu, J., Olotu, A., Leach, A., Lievens, M., Vekemans, J., Mshamu, S., Lang, T., Gould, J., Dubois, M.C., Demoitié, M.A., Stallaert, J.F., Vansadia, P., Carter, T., Njuguna, P., Awuondo, K.O., Malabeja, A., Abdul, O., Gesase, S., Mturi, N., Drakeley, C.J., Savarese, B., Villafana, T., Ballou, W.R., Cohen, J., Riley, E.M., Lemnge, M.M., Marsh, K. and von Seidlein, L. 2008. Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. *New England Journal of Medicine* **359**: 2521-2532.
- Benedict, M.Q. and Robinson, A.S. 2003. The first release of transgenic mosquitoes: an argument for the sterile insect technique. *Trends in Parasitology* **19**: 349-355.
- Boko, M., Niang, I., Nyong, A., Vogel, C., Githeko, A., Medany, M., Osman-Elasha, B., Tabo, R. and Yanda, P. 2007. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Edited by Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. Cambridge: Cambridge University Press, UK: 433-467.
- Bowler, K. and Terblanche, J.S. 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biological Reviews* **83**: 339-355.
- Buckley, L.B. and Kingsolver, J.G. 2012. Functional and phylogenetic approaches to forecasting species' responses to climate. *Annual Review of Ecology, Evolution and Systematics* **43**: 205-226.

- Chown, S.L. and Nicolson, S.W. 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford: Oxford University Press, UK.
- Chown, S.L., Jumbam, K.R., Sørensen, J. G. and Terblanche, J.S. 2008. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology* **23**: 133-140.
- Clements, A.N. 1963. *The Physiology of Mosquitoes*. The MacMillan Company, New York, USA.
- Clements, A.N. 2000. *The Biology of Mosquitoes: Development, Nutrition and Reproduction*. CABI Publishing, Oxfordshire, UK.
- Coetzee, M., Craig, M. and le Sueur, D. 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today* **13**: 149-151.
- Davis, C.L. 2011. *Climate Risk and Vulnerability: A Handbook for Southern Africa*. Council for Scientific and Industrial Research, Pretoria, South Africa.
- Dawes, E.J., Churcher, T.S., Zhuang, S., Sinden, R.E. and Basáñez, M-G. 2009. *Anopheles* mortality is both age- and *Plasmodium*-density dependent: implications for malaria transmission. *Malaria Journal* **8**: 228.
- de Villiers, M., Hattingh, V. and Kriticos, D.J. 2012. Combining field phenological observations with distribution data to model the potential distribution of the fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Bulletin of Entomological Research*: doi:10.1017/S0007485312000454.
- Eeley, H.A.C., Lawes, M.J. and Piper, S.E. 1999. The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *Journal of Biogeography* **26**: 595-617.

- Elith, J. and Leathwick, J.R. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution and Systematics* **40**: 677-697.
- Elith, J., Kearney, M. and Phillips, S. 2010. The art of modelling range-shifting species. *Methods in Ecology and Evolution* **1**: 330-342.
- Farenhorst, M., Mouatcho, J.C., Kikanke, C.K., Brooke, B.D., Hunt, R.H., Koekemoer, L.L., Knols, B.G.J. and Coetzee, M. 2009. Fungal infection counters insecticide resistance in African malaria mosquitoes. *Proceedings of the National Academy of Sciences of the U.S.A.* **106**: 17442-17447.
- Fillinger, U. and Lindsay, S.W. 2006. Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in a rural Kenyan town. *Tropical Medicine and International Health* **11**: 1-14.
- Gibbs, A.G. and Markow, T.A. 2001. Effects of age on water balance in *Drosophila* species. *Physiological and Biochemical Zoology* **74**: 520-530.
- Gillies, M.T. and Coetzee, M. 1987. *A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region)*. Publications of the South African Institute of Medical Research, Johannesburg, South Africa.
- Gray, E.M. and Bradley, T.J. 2005. Physiology of desiccation resistance in *Anopheles gambiae* and *Anopheles arabiensis*. *American Journal of Tropical Medicine and Hygiene* **73**: 553-559.
- Gullan, P.J. and Cranston, P.S. 1994. *The Insects: An Outline of Entomology*. First Edition. Chapman and Hall, London, UK.
- Hagstrum, D.W. and Hagstrum, W.R. 1970. A simple device for producing fluctuating temperatures, with an evaluation of the ecological significance of fluctuating temperatures. *Annals of the Entomological Society of America* **63**: 1385-1389.

- Hagstrum, D.W. and Leach, C.E. 1972. Role of constant and fluctuating temperatures in determining development time and fecundity of three species of stored-products Coleoptera. *Annals of the Entomological Society of America* **66**: 407-410.
- Hagstrum, D.W. and Milliken, G.A. 1991. Modelling differences in insect developmental times between constant and fluctuating temperatures. *Annals of the Entomological Society of America* **84**: 369-379.
- Hansen, J., Sato, M. and Ruedy, R. 2012. Perception of climate change. Proceedings of the National Academy of Sciences of the U.S.A.: Early Edition: pnas.org/cgi/doi/10.1073/pnas.1205276109.
- Hay, S.I., Rogers, D.J., Randolph, S.E., Stern, D.I., Cox, J., Shanks, G.D. and Snow, R.W. 2002. Hot topic or hot air? Climate change and malaria resurgence in East African highlands. *Trends in Parasitology* **18**: 530-534.
- Hemingway, J. and Ranson, H. 2000. Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology* **45**: 371-391.
- Himeidan, Y.E. and Kweka, E.J. 2012. Malaria in East African highlands during the past 30 years: impact of environmental changes. *Frontiers in Physiology* **3**: 315.
- Hoffmann, A.A. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. *Journal of Experimental Biology* **213**: 870-880.
- Huestis, D.L., Yaro, A.S., Traoré, A.I., Adamou, A., Kassogué, Y., Diallo, M., Timbine, S., Dao, A. and Lehmann, T. 2011. Variation in metabolic rate of *Anopheles gambiae* and *A. arabiensis* in a Sahelian village. *Journal of Experimental Biology* **214**: 2345-2353.
- Kearney, M. and Porter, W.P. 2004. Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology* **85**: 3119-3131.

- Kearney, M., Phillips, B.L., Tracy, C.R., Christian, K.A., Betts, G. and Porter, W.P. 2008. Modelling species distributions without using species distributions: the cane toad in Australia under current and future climates. *Ecography* **31**: 423-434.
- Kearney, M., Porter, W.P., Williams, C., Ritchie, S. and Hoffmann, A.A. 2009. Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: the dengue mosquito *Aedes aegypti* in Australia. *Functional Ecology* **23**: 528-538.
- Kleinschmidt, I., Schwabe, C., Shiva, M., Segura, J. L., Sima, V., Mabunda, S. J. A. and Coleman, M. 2009. Combining indoor residual spraying and insecticide treated net interventions. *American Journal of Tropical Medicine and Hygiene* **81**: 519– 524.
- Lehmann, T., Dao, A., Yaro, A.S., Adamou, A., Kassogue, Y., Diallo, M., Sékou, T. and Coscaron-Arias, C. 2010. Aestivation of the African malaria mosquito, *Anopheles gambiae* in the Sahel. *American Journal of Tropical Medicine and Hygiene* **83**: 601-606.
- Lindsay, S.W. and Bayoh, M.N. 2004. Mapping members of the *Anopheles gambiae* complex using climate data. *Physiological Entomology* **29**: 204-209.
- Mabaso, M.L.H., Sharp, B. and Lengeler, C. 2004. Historical review of malaria control in southern Africa with emphasis on the use of indoor residual house-spraying. *Tropical Medicine and International Health* **9**: 846-856.
- Martens, W.J.M., Jetten, T.H. and Focks, D.A. 1997. Sensitivity of malaria, schistosomiasis and dengue to global warming. *Climatic Change* **35**: 145-156.
- Minakawa, N., Dida, G.O., Sonye, G.O., Futami, K. and Kaneko, S. 2008. Unforeseen misuses of bed nets in fishing villages along Lake Victoria. *Malaria Journal* **7**: 165.
- Mordecai, E.A., Paaijmans, K.P., Johnson, L.R., Balzer, C., Ben-Horin, T., Moor, E., McNally, A., Pawar, S., Ryan, S.J., Smith, T.C. and Lafferty, K.D. 2012. Optimal

- temperature for malaria transmission is lower than previously predicted. *Ecology Letters*: doi: 10.1111/ele.12015.
- Munhenga, G., Brooke, B.D., Chirwa, T.F., Hunt, R.H., Coetzee, M., Govender, D. and Koekemoer, L.L. 2011. Evaluating the potential of the sterile insect technique for malaria control: relative fitness and mating compatibility between laboratory colonized and a wild population of *Anopheles arabiensis* from the Kruger National Park, South Africa. *Parasites and Vectors* **4**: 208.
- Omumbo, J.A., Lyon, B., Waweru, S.M., Connor, S.J. and Thomson, M.C. 2011. Raised temperatures over the Kericho tea estates: revisiting the climate in the East African highlands malaria debate. *Malaria Journal* **10**: 12.
- Overgaard, J., Kristensen, T.N., Mitchell, K.A. and Hoffmann, A.A. 2011. Thermal tolerance in widespread and tropical *Drosophila* species: does phenotypic plasticity increase with latitude? *American Naturalist* **178**: S80-96.
- Paaijmans, K.P., Read, A.F. and Thomas, M.B. 2009. Understanding the link between malaria risk and climate. *Proceedings of the National Academy of Sciences of the U.S.A.* **106**: 13844-13849.
- Paaijmans, K.P., Imbahale, S.S., Thomas, M.B. and Takken, W. 2010. Relevant microclimate for determining the development rate of malaria mosquitoes and possible implications of climate change. *Malaria Journal* **9**: 196.
- Parmesan, C. and Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37-42.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* **37**: 637-669.

- Pascual, M., Dobson, A.P. and Bouma, M.J. 2009. Underestimating malaria risk under variable temperatures. *Proceedings of the National Academy of Sciences USA* **106**: 13645-13646.
- Rocca, K.A.C., Gray, E.M., Constantini, C. and Besansky, N.J. 2009. 2La chromosomal inversion enhances thermal tolerance of *Anopheles gambiae* larvae. *Malaria Journal* **8**: 147.
- Rogers, D.J. and Randolph, S.E. 2000. The global spread of malaria in a future, warmer world. *Science* **289**: 1763-1766.
- Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **24**: 35-68.
- Schellenberg, J.R.M.A., Abdulla, S., Minja, H., Nathan, R., Mukasa, O., Marchant, T., Mponda, H., Kikumbih, N., Lyiom, E., Manchester, T., Tanner, M. and Lengeler, C. 1999. KINET: a social marketing programme of treated nets and net treatment for malaria control in Tanzania, with evaluation of child health and long-term survival. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**: 225-231.
- Scholte, E.J., Ng'habi, K., Kihonda, J., Takken, W., Paaijmans, K., Abdulla, S., Killeen, G.F. and Knols, B.G.J. 2005. An entomopathogenic fungus for control of African malaria mosquitoes. *Science* **308**: 1641-1642.
- Sinka, M.E., Bangs, M.J., Manguin, S., Coetzee, C., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Okara, R.M., van Boeckel, T., Godfray, C.J., Harbach, R.E. and Hay, S.I. 2010. The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites and Vectors* **3**: 117.
- Soberón, J. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics* **2**: 1-10.

- Sutherst, R.W., Maywald, G.F. and Bourne, A.S. 2007. Including species interactions in risk assessments for global change. *Global Change Biology* **13**: 1843-1859.
- Tanser, F.C., Sharp, B. and le Sueur, D. 2003. Potential effects of climate change on malaria transmission in Africa. *Lancet* **362**: 1792-1798.
- Terblanche, J.S., Deere, J.A., Clusella-Trullas, S., Janion, C. and Chown, S.L. 2007. Critical thermal limits depend on methodological context. *Proceedings of the Royal Society B* **274**: 2935-2943.
- van Lieshout, M., Kovats, R.S., Livermore, M.T.J. and Martens, P. 2004. Climate change and malaria: analysis of the SRES climate and socio-economic scenarios. *Global Environmental Change* **14**: 87-99.
- Worner, S.P. 1992. Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. *Environmental Entomology* **21**: 689-699.
- World Health Organization: World Malaria Report 2010. Geneva, Switzerland.
[<http://www.who.int/malaria>].