© 2014. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2014) 217, 3823-3833 doi:10.1242/jeb.104638

RESEARCH ARTICLE

Desiccation tolerance as a function of age, sex, humidity and temperature in adults of the African malaria vectors *Anopheles arabiensis* and *Anopheles funestus*

Candice L. Lyons[1,2,*], Maureen Coetzee[2], John S. Terblanche[3] and Steven L. Chown[4]

ABSTRACT

Adult mosquito survival is strongly temperature and moisture dependent. Few studies have investigated the interacting effects of these variables on adult survival and how this differs among the sexes and with age, despite the importance of such information for population dynamic models. For these reasons, the desiccation tolerance of *Anopheles arabiensis* Patton and *Anopheles funestus* Giles males and females of three different ages was assessed under three combinations of temperature and humidity. Females were more desiccation tolerant than males, surviving for longer periods than males under all experimental conditions. In addition, younger adults were more tolerant of desiccation than older groups. Both species showed reduced water loss rate (WLR) as the primary mechanism by which they tolerate desiccation. Although *A. arabiensis* is often considered to be the more arid-adapted of the two species, it showed lower survival times and higher WLR than *A. funestus*. The current information could improve population dynamic models of these vectors, given that adult survival information for such models is relatively sparse.

KEY WORDS: Age-related variation, Mosquito, Cross-tolerance, Population dynamics, Ecophysiology, Water loss

INTRODUCTION

Persistence of any natural biological population not being continually rescued by immigration is dependent on survival and reproduction. For insects, ambient temperature and water availability are the two key extrinsic factors influencing survival, with the latter being significant especially for smaller species (Benoit and Denlinger, 2010; Chown et al., 2011; Harrison et al., 2012). How survival is influenced by their interactions is not always clear (e.g. Hayward et al., 2001; Chown and Nicolson, 2004). 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 desiccation resistance or tolerance of a given species or population (Hoffmann, 1990; Gibbs and Markow, 2001; Gray and Bradley, 2005). By contrast, investigations of the effects of temperature × water interactions are uncommon (How and Lee, 2010; Kleynhans and Terblanche, 2011), and understanding interactions of this kind is generally considered a significant challenge in physiology (Chown and Nicolson, 2004; Gaston et al., 2009; Hoffmann, 2010).

Interactions among environmental variables are also unlikely to be consistent among age groups and sexes, given that responses to both temperature and water availability vary with age and sex (Bowler and Terblanche, 2008; How and Lee, 2010; Weldon et al., 2013). In several insect species, as they age, they become less tolerant of temperature extremes (e.g. Bowler and Terblanche, 2008; Lyons et al., 2012; Colinet et al., 2013). Differences between sexes can also be pronounced and significant, e.g. in the mosquito vectors *Anopheles arabiensis* and *Anopheles funestus* (Lyons et al., 2012) and in males of the tropical butterfly *Bicyclus anynana* (Dierks et al., 2012), although this is not always the case (e.g. Mironidis and Savopoulou-Soultani, 2010). Similarly, age- and/or sex-related differences in the survival of desiccation have been found in mosquitoes, *Drosophila* spp. and tephritid flies (Gibbs and Markow, 2001; Gray and Bradley, 2005; Benoit and Denlinger, 2007; Fouet et al., 2012; Weldon et al., 2013), although much variation among species is typical, and investigations of their interactions is uncommon. Survival time under dry conditions is also influenced by several other characteristics such as body size, associated with initial body water content and lipid stores, and reduced water loss rate (WLR) (Chown and Klok, 2003; Gibbs et al., 2003; Gray and Bradley, 2005). In addition, desiccation may be affected by variation in tolerance to water loss (i.e. body water content when the insect succumbs to death), although for insects this is typically not as significant as variation in other traits (Edney, 1977; Chown and Nicolson, 2004). In a similar manner, starvation resistance can be influenced by sex, age, strain or variation among individuals in size or lipid content, though again no general conclusions across insects are yet possible (e.g. Gibbs et al., 1997; Hoffmann et al., 2005; Ballard et al., 2008).

For these reasons, the influence of sex, age, temperature, water availability and their interactions on the desiccation tolerance of two of the most significant vectors of *Plasmodium falciparum* malaria in south-eastern Africa (Gillies and Coetzee, 1987), *A. arabiensis* Patton and *A. funestus* Giles were investigated here. Although only the females are vectors of the *Plasmodium* spp. parasites, population persistence is dependent on male and female survival. While efforts to control malaria are ongoing and new methods of control are constantly being developed and tested (e.g. Farenhorst et al., 2009; Munhenga et al., 2011), the disease remains a major public health concern (WHO, 2013). As climates continue to change, how malaria, and other vector-borne diseases, will be affected remains uncertain (Rogers and Randolph, 2000; Hay et al., 2002; Pascual et al., 2006; Githeko, 2009). Providing detailed physiological information at the species level will therefore improve forecast models, so reducing this uncertainty (Thomson et al., 2010; Buckley and Kingsolver, 2012; Woodin et al., 2013).

[1]Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa. [2]Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2000, South Africa. [3]Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Stellenbosch University, Matieland 7602, South Africa. [4]School of Biological Sciences, Monash University, VIC 3800, Australia.

*Author for correspondence (candice.lyons@hotmail.com)

Received 26 February 2014; Accepted 3 September 2014

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104638

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, sex, RH, temperature and age for *Anopheles funestus* and *Anopheles arabiensis*

Species	Predictors	Estimate	s.e.m.	*t*-statistic	*P*-value
A. funestus	Intercept	2.4484	0.095	25.77	<0.0005
	Mass	0.5347	0.0938	5.69	<0.0005
	Sex (male)	–0.5472	0.1759	–3.11	0.002
	RH L	1.0679	0.0436	24.49	<0.0005
	RH Q	–0.0282	0.0399	–0.71	0.4804
	Temperature L	–0.3116	0.0438	–7.12	<0.0005
	Temperature Q	–0.1268	0.0394	–3.22	0.0013
	Age L	–0.2862	0.0424	–6.75	<0.0005
	Age Q	0.0464	0.0404	1.15	0.2513
	Mass × sex (male)	0.3319	0.2894	1.15	0.2517
	Sex (male) × RH L	0.0342	0.0749	0.46	0.6481
	Sex (male) × RH Q	–0.1587	0.0655	–2.42	0.0156
	Sex (male) × temperature L	0.0585	0.0739	0.79	0.4288
	Sex (male) × temperature Q	–0.0281	0.0661	–0.43	0.6709
	RH L × temperature L	–0.0483	0.0808	–0.59	0.5498
	RH Q × temperature L	0.2145	0.0705	3.04	0.0024
	RH L × temperature Q	0.4612	0.0693	6.65	<0.0005
	RH Q × temperature Q	–0.1698	0.0654	–2.59	0.0096
	RH L × age L	0.2674	0.0781	3.42	0.0006
	RH Q × age L	0.0469	0.0679	0.69	0.4899
	RH L × age Q	0.2196	0.0721	3.04	0.0024
	RH Q × age Q	–0.0806	0.0676	–1.19	0.2332
	Sex (male) × age L	–0.0602	0.0705	–0.85	0.3937
	Sex (male) × age Q	–0.0797	0.0668	–1.19	0.2332
	Temperature L × age L	0.1592	0.0739	2.15	0.0316
	Temperature Q × age L	–0.2167	0.0657	–3.3	0.001
	Temperature L × age Q	0.0337	0.071	0.48	0.6349
	Temperature Q × age Q	–0.0179	0.0648	–0.28	0.7825
	Sex (male) × RH L × temperature L	0.138	0.1366	1.01	0.3124
	Sex (male) × RH Q × temperature L	–0.4423	0.1155	–3.83	0.0001
	Sex (male) × RH L × temperature Q	–0.0122	0.1179	–0.1	0.9173
	Sex (male) × RH Q × temperature Q	0.0232	0.1074	0.22	0.829
	Sex (male) × RH L × age L	–0.2214	0.1302	–1.7	0.0893
	Sex (male) × RH Q × age L	–0.2087	0.112	–1.86	0.0627
	Sex (male) × RH L × age Q	–0.1529	0.1185	–1.29	0.1971
	Sex (male) × RH Q × age Q	0.0436	0.1091	0.4	0.6895
	RH L × temperature L × age L	0.0538	0.1225	0.44	0.6609
	RH Q × temperature L × age L	0.2363	0.1004	2.35	0.0187
	RH L × temperature Q × age L	–0.0627	0.1016	–0.62	0.5369
	RH Q × temperature Q × age L	–0.0208	0.0912	–0.23	0.8198
	RH L × temperature L × age Q	–0.1393	0.1098	–1.27	0.2047
	RH Q × temperature L × age Q	0.0733	0.0974	0.75	0.4514
	RH L × temperature Q × age Q	0.2741	0.0965	2.84	0.0046
	RH Q × temperature Q × age Q	–0.0781	0.0904	–0.86	0.3877
	Sex (male) × temperature L × age L	0.2428	0.1023	2.37	0.0178
	Sex (male) × temperature Q × age L	0.1289	0.1011	1.27	0.2028
	Sex (male) × temperature L × age Q	0.0037	0.1027	0.04	0.9716
	Sex (male) × temperature Q × age Q	0.0141	0.099	0.14	0.8872
A. arabiensis	Intercept	2.0181	0.0794	25.421	<0.0005
	Mass	0.3563	0.0395	9.028	<0.0005
	Sex (Male)	–0.5591	0.1642	–3.41	0.0007
	RH L	0.9629	0.0349	27.52	<0.0005
	RH Q	0.0671	0.0329	2.035	0.0421
	Temperature L	–0.2679	0.0353	–7.59	<0.0005
	Temperature Q	–0.1592	0.0342	–4.66	<0.0005
	Age L	–0.1423	0.0354	–4.017	<0.0005
	Age Q	–0.1169	0.0343	–3.41	0.0007
	Mass × sex (male)	0.3387	0.1471	2.302	0.0215
	Sex (male) × RH L	0.0795	0.059	1.348	0.178
	Sex (male) × RH Q	–1.1311	0.0552	–2.374	0.01778
	Sex (male) × temperature L	–0.0207	0.05947	–0.348	0.7283
	Sex (male) × temperature Q	–0.0539	0.05601	–0.963	0.3357
	RH L × temperature L	0.2673	0.0679	3.936	<0.0005
	RH Q × temperature L	–0.0183	0.0587	–0.312	0.755
	RH L × temperature Q	0.3494	0.0573	6.102	<0.0005
	RH Q × temperature Q	0.0446	0.05576	0.8	0.4238

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104638

Table 1. Continued

Species	Predictors	Estimate	s.e.m.	*t*-statistic	*P*-value
A. arabiensis	Sex (male) × age L	–0.0711	0.0575	–1.236	0.2167
	Sex (male) × age Q	0.1494	0.058	2.575	0.0102
	RH L × age L	–2.7251	0.0595	–4.581	<0.0005
	RH Q × age L	–0.0303	0.0577	–0.525	0.5996
	RH L × age Q	–0.2424	0.0611	–3.97	<0.0005
	RH Q × age Q	0.10662	0.0571	1.869	0.0619
	Temperature L × age L	–0.0414	0.0618	–0.67	0.5032
	Temperature Q × age L	–0.1071	0.0555	–1.928	0.0541
	Temperature L × age Q	0.0505	0.061	0.828	0.4077
	Temperature Q × age Q	–0.192	0.0581	–3.303	0.001
	Sex (male) × RH L × temperature L	–0.0944	0.1101	–0.856	0.3919
	Sex (male) × RH Q × temperature L	0.0377	0.09886	0.381	0.703
	Sex (male) × RH L × temperature Q	–0.3803	0.0977	–3.892	0.0001
	Sex (male) × RH Q × temperature Q	–0.083	0.0919	–0.903	0.3666
	Sex (male) × RH L × age L	0.2342	0.1012	2.315	0.0208
	Sex (male) × RH Q × age L	0.1189	0.0957	1.242	0.2146
	Sex (male) × RH L × age Q	0.116	0.1037	1.119	0.2635
	Sex (male) × RH Q × age Q	–0.1433	0.0956	–1.499	0.1342
	Sex (male) × temperature L × age L	0.0594	0.1039	0.571	0.5679
	Sex (male) × temperature Q × age L	0.2777	0.0922	3.014	0.0026
	Sex (male) × temperature L × age Q	–0.2059	0.1024	–2.01	0.0447
	Sex (male) × temperature Q × age Q	0.0966	0.0992	0.973	0.3307
	RH L × temperature L × age L	–0.2096	0.1096	–1.913	0.0559
	RH Q × temperature L × age L	–0.0513	0.1049	–0.489	0.6252
	RH L × temperature Q × age L	–0.3681	0.0972	–3.787	0.0002
	RH Q × temperature Q × age L	–0.1494	0.09559	–1.563	0.1183
	RH L × temperature L × age Q	–0.1151	0.1101	–1.046	0.2958
	RH L × temperature Q × age Q	0.1943	0.1031	1.885	0.0597
	RH L × temperature Q × age Q	–0.3101	0.1014	–3.06	0.0023
	RH Q × temperature Q × age Q	0.0896	0.0975	0.92	0.3579
	Sex (male) × RH L × temperature L × age L	–0.0946	0.1853	–0.511	0.6096
	Sex (male) × RH Q × temperature L × age L	0.0525	0.1752	0.3	0.7645
	Sex (male) × RH L × temperature Q × age L	0.5669	0.1623	3.494	0.0005
	Sex (male) × RH Q × temperature Q × age L	0.1736	0.1563	1.111	0.2669
	Sex (male) × RH L × temperature L × age Q	0.0189	0.1832	–0.104	0.9176
	Sex (male) × RH Q × temperature L × age Q	–0.2016	0.171	–1.179	0.2387
	Sex (male) × RH L × temperature Q × age Q	0.5941	0.1762	3.373	0.0007
	Sex (male) × RH Q × temperature Q × age Q	–0.1946	0.1619	–1.202	0.2298

L, linear; Q, quadratic; RH, relative humidity. Mass is in mg, temperature is in °C, and age is in days.

RESULTS

Mass (mean ± s.d. mass: *A. arabiensis* males 0.99±0.17 mg, females 1.88±0.57 mg; *A. funestus* males 0.52±0.08 mg, females 0.96±0.22 mg) and sex both had significant effects on survival time in *A. funestus* (Table 1; for averages for all groups see supplementary material Table S1). On average, females survived longer than males, and larger individuals survived longer than smaller individuals. As expected, survival was significantly affected by temperature and humidity. Older individuals of both sexes died more quickly than younger ones under the specific stress, although this was more evident at high humidity compared with low humidity (Fig. 1). The significant sex × relative humidity (RH) interaction indicates that the response of each sex was different across humidities (Table 1). In general, males died faster than females at all humidities. The significant RH × age interaction showed that the relationship between survival time and RH differs between age groups at each RH. However, across all RH treatments, younger individuals survived longer than older ones under the specific stress. The RH × temperature interaction suggests that the slopes of survival against temperature differed between humidity treatments, with individuals at the lowest temperatures and highest humidities having the highest survival, evident even in the oldest male and female age groups for this species (Fig. 2).

Mass, sex, RH, temperature and age all significantly influenced desiccation resistance in *A. arabiensis* (Table 1). As well as significant main effects in the models, there were also significant two-way interactions, between sex × mass, RH × temperature, sex × age, temperature × age, sex × RH and RH × age for *A. arabiensis* (Table 1). The sex × mass interaction showed differing responses amongst 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% RH relative to 55% or 5% RH treatments and higher survival at lower temperatures (Fig. 3). Survival showed a steady decline with increase in temperature across all humidity treatments (Fig. 3). The RH × age interaction for *A. arabiensis* again indicated a different relationship between RH and survival time for different age groups, with survival of younger age groups being higher across all humidities, but in different ways. The temperature × age interaction showed that high temperatures consistently led to faster death, across all ages and both sexes, especially at low (5%) RH (Fig. 4). At high temperatures, older individuals of both sexes survive for less time than younger individuals, even at 100% RH (Fig. 4). This model also had significant three- and four-way interactions between variables, which were not easily interpretable.

Results from the generalized linear model of the effect of WLR and mass on the dependent variable, time, indicated that WLR and

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104638

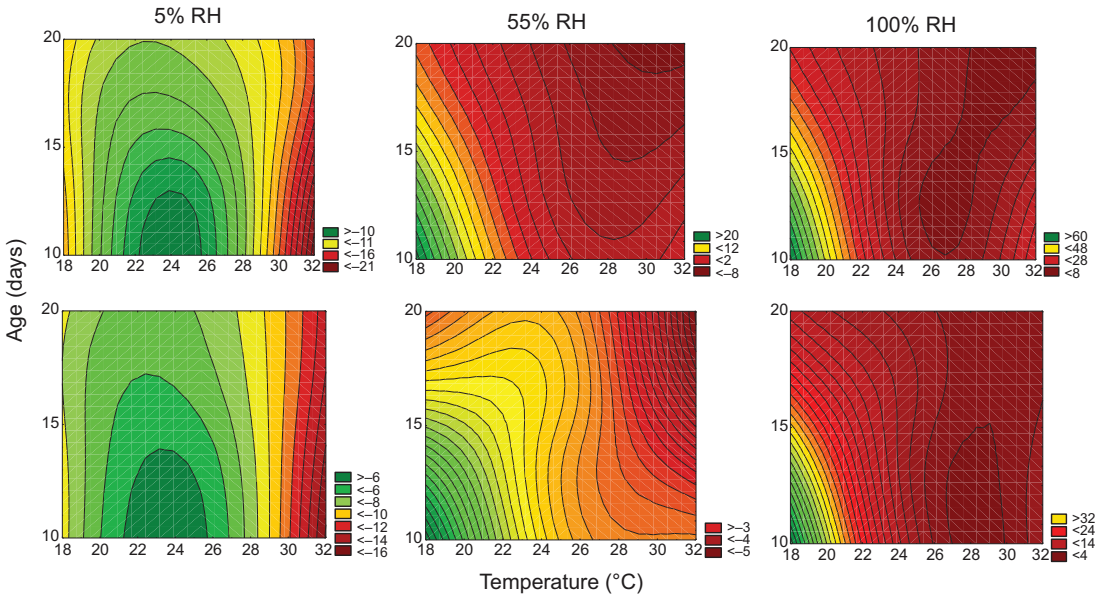

Fig. 1. Contour plots of the relationship between the residuals of a mass (mg) versus survival time (h) regression, and temperature (*x*-axis) and age (*y*-axis) for each of the three humidity treatments, for *Anopheles funestus* females (top row) and males (bottom row). Green indicates high survival while red indicates low survival. Note the substantially different scale values for each of the panels, indicating longer survival times at higher relative humidity (RH).

mass both significantly influenced survival time (desiccation tolerance) across all sexes, ages and temperatures for *A. funestus* at 5% RH (Table 2). WLR was most often more important in contributing to survival, given that it typically had the largest effect sizes, with lower WLR significantly increasing survival time. The positive estimate values for mass indicated that increased survival was associated with increased mass (Table 2).

For *A. arabiensis*, WLR again contributed most 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).

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 significant interactions and main effect involving the species term (Table 4). On average, *A. funestus* survived longer than *A. arabiensis* females across all treatments (Table 4; supplementary material Table S1). The RH × species interaction suggested that slopes of survival time versus RH differed between species, with a steeper slope in *A. 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.

When comparing survival times of males between species, no significant species main effect was observed (Table 4). However, mass, RH and temperature all significantly influenced survival (Table 4; supplementary material Table S1). 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 indicated that mass/time slopes were different between males of each species. The species × temperature and species × RH interactions showed that the species responded differently to RH and temperature in terms of their survival times, with *A. arabiensis* males dying faster than *A. funestus* males under high temperatures and low humidities.

Comparisons among the wild and laboratory strains of *A. arabiensis* revealed that under both sets of conditions (25°C/5% RH and 20°C/55% RH) the 10 day old adults showed no significant differences in water loss or lipid content at death (supplementary material Table S2). By contrast, time to death, the most significant variable from a fitness perspective, varied significantly among the strains, as did initial wet mass and final dry mass on death (supplementary material Table S3). When initial wet mass was included as a covariate, time to death did not vary among strains or the sexes (supplementary material Tables S2 and S3), indicating that

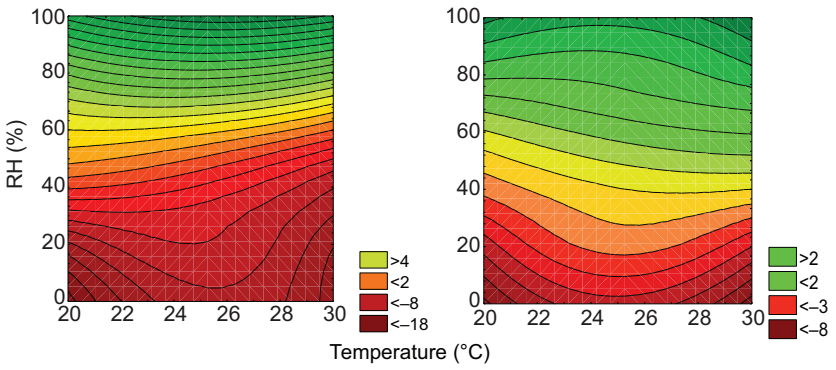

Fig. 2. Contour plots of the relationship between the residuals of a mass (mg) versus survival time (h) regression, and temperature and RH for each of the three humidity treatments, for 30 day old *Anopheles funestus* females (left) and males (right). Green indicates high survival while red indicates low survival.

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104638

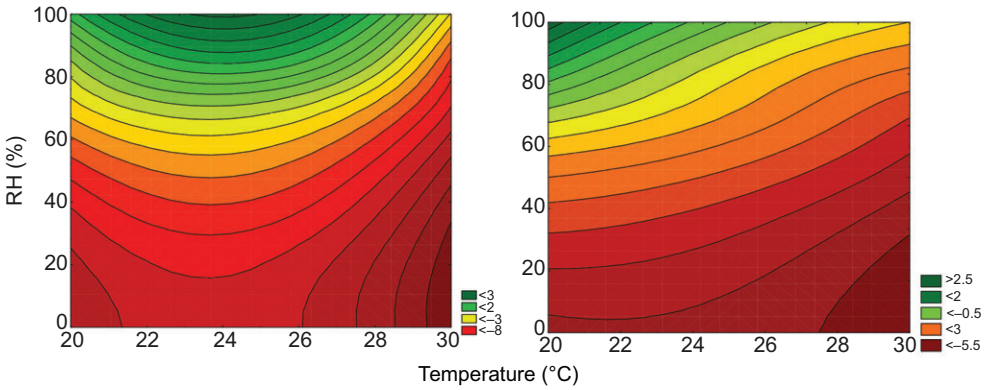

Fig. 3. Contour plots of the relationship between the residuals of a mass (mg) versus survival time (h) regression, and temperature and RH for each of the three humidity treatments, for 20 day old *Anopheles arabiensis* females (left) and males (right). Green indicates high survival while red indicates low survival.

variation in time to death among sexes and strains is a consequence of variation in mass.

DISCUSSION

Knowledge of the duration of survival of mosquitoes under different combinations of temperature and humidity, and among ages and sexes, is important for understanding population dynamics under various conditions, including those of the dry season. As might be expected (Chown and Nicolson, 2004), high humidity and lower temperature favour survival in both vector species across all ages and for both sexes, with the form of this response indicating survival times at low humidities typical of mesic/hygric insects this size, but with the interspecific variation characteristic of this trait (e.g. Hood and Tschinkel, 1990; Benoit and Denlinger, 2010).

One major concern with the current study might be that the findings reflect the situation only with the laboratory strains, given that laboratory adaptation has been recorded in several, though not all, species for several traits [see elsewhere for discussion (Chown and Terblanche, 2006; Parkash and Ranga, 2014) and for differing extents of laboratory adaptation in *Anopheles* species (Huho et al., 2007; Lyons et al., 2012)]. We investigated the extent of variation among laboratory and wild strains of *A. arabiensis* for the traits examined here. No differences were found in water loss tolerated and lipid content at death among the strains, although they differed in size (both initial wet mass and final dry mass), as did the sexes. This size difference accounted for the difference among the sexes and the strains in time to death, the key fitness trait (see Chown and Nicolson, 2004). Thus, the results presented here are considered generalizable to the field situation, once mass is taken into account as can readily be done, and indeed should be done given its key influence on survival time (see Tables 2 and 3 for relationships). Nonetheless, it is important to note the size differences among the laboratory and wild strains, and that for thermal tolerance traits of this species, laboratory strains showed significant differences from wild strains, but these differences were not large enough in effect size to render thermal tolerance work in the laboratory irrelevant to the field situation (Lyons et al., 2012). Other work has also found differences among laboratory and wild strains of *A. gambiae* in size and in internal nutrient resources (Huho et al., 2007). The size differences in *A. gambiae* males are similar to those found here for *A. arabiensis* males (larger individuals in the wild strain), but *A. arabiensis* females varied in the opposite direction [Huho et al. did not investigate females (Huho et al., 2007)]. Such trait variation among laboratory and field strains, although often small in effect, may nonetheless be important and should therefore be the subject of further investigation to improve the translation of laboratory to field

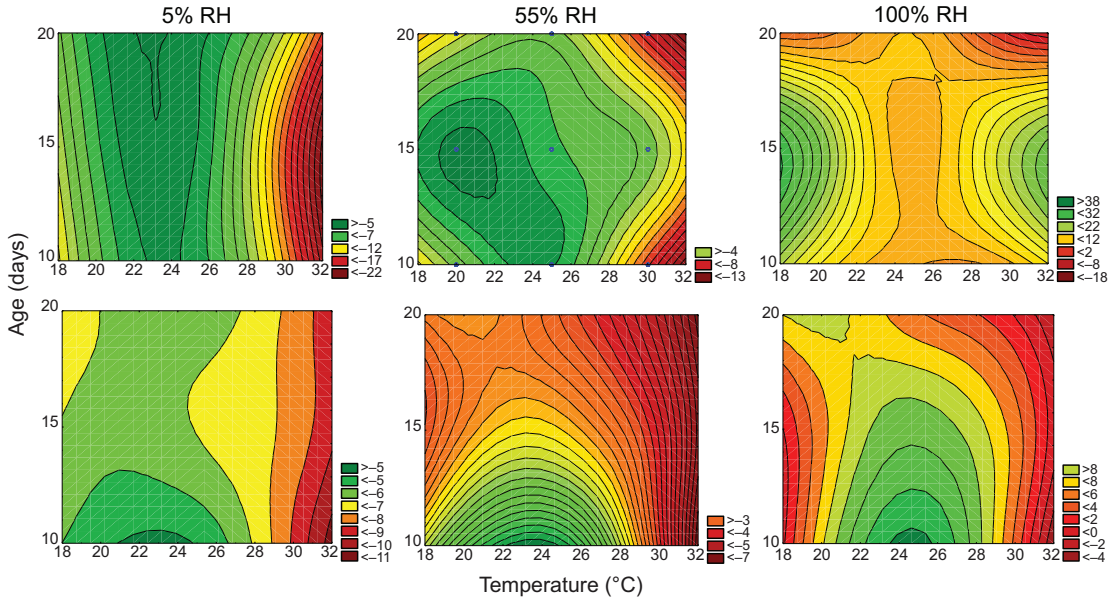

Fig. 4. Contour plots of the relationship between the residuals of a mass (mg) versus survival time (h) regression, and temperature and age for each of the three humidity treatments, for *Anopheles arabiensis* females (top row) and males (bottom row). Green indicates high survival while red indicates low survival. Note the substantially different scale values for each of the panels, indicating longer survival times at higher humidities.

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104638

Table 2. Results from a generalized linear model with quasipoisson distribution of errors and log link function, showing the relative contributions of WLR and initial mass to desiccation tolerance (time in h) of each group of *A. funestus* at each temperature and at the 5% RH treatment

Temp.	Group	Predictors	*N*	*F*	Estimate	s.e.m	*t*-statistic	*P*-value
20°C	10 d ♀	WLR	20	49.70	−16.01	6.48	−2.47	0.0063
	d.f.=17	Mass		5.13	1.15	0.51	2.26	0.0369
	10 d ♂	WLR	20	42.16	−22.68	4.33	−5.24	<0.0001
	d.f.=17	Mass		4.25	1.49	0.73	2.04	0.0549
	20 d ♀	WLR	40	35.92	−20.23	4.12	−4.91	<0.0001
	d.f.=37	Mass		22.34	0.86	0.18	4.7	<0.0001
	20 d ♂	WLR	40	102.13	−40.03	4.72	−8.48	<0.0001
	d.f.=37	Mass		21.32	2.27	0.49	4.56	<0.0001
	30 d ♀	WLR	20	124.36	−6.17	0.62	−10.02	<0.0001
	d.f.=17	Mass		5.16	1.05	0.47	2.23	0.0363
	30 d ♂	WLR	20	10.57	40.01	12.36	3.24	0.0050
	d.f.=17	Mass		20.42	14.93	3.32	4.50	0.0003
		Mass × WLR		14.90	−88.74	23.10	−3.84	0.0014
25°C	10 d ♀	WLR	20	20.15	−18.33	4.34	−4.23	0.0003
	d.f.=17	Mass		6.62	0.76	0.29	2.55	0.0198
	10 d ♂	WLR	20	10.79	−23.59	8.26	−2.86	0.0044
	d.f.=17	Mass		8.94	2.55	0.85	2.99	0.0082
	20 d ♀	WLR	20	12.42	−18.08	5.48	−3.3	0.0026
	d.f.=17	Mass		8.09	0.56	0.19	2.84	0.0112
	20 d ♂	WLR	20	9.18	−21.46	7.59	−2.83	0.0076
	d.f.=17	Mass		2.14	2.76	1.84	1.50	0.1622
	30 d ♀	WLR	20	47.81	−31.35	4.65	−6.74	<0.0001
	d.f.=17	Mass		14.91	1.01	0.27	3.80	0.0013
	30 d ♂	WLR	20	22.26	−24.97	6.12	−4.08	0.0002
	d.f.=17	Mass		2.33	1.32	0.88	1.49	0.1455
30°C	10 d ♀	WLR	20	260.32	−7.91	0.61	−13.00	<0.0001
	d.f.=17	Mass		21.28	1.24	0.27	4.56	0.0002
	10 d ♂	WLR	20	25.58	−6.85	1.41	−4.87	<0.0001
	d.f.=17	Mass		12.66	4.63	1.22	3.7	0.0024
	20 d ♀	WLR	20	56.33	−15.22	2.53	−6.01	<0.0001
	d.f.=17	Mass		13.53	1.73	0.47	3.68	0.0019
	20 d ♂	WLR	20	8.68	−4.70	1.68	−2.79	0.0090
	d.f.=17	Mass		0.09	−0.54	1.89	−0.29	0.7697
	30 d ♀	WLR	20	32.33	−4.54	0.93	−4.86	<0.0001
	d.f.=17	Mass		2.59	0.45	0.28	1.62	0.1258
	30 d ♂	WLR	20	22.26	−3.59	0.75	−4.78	0.0002
	d.f.=17	Mass		3.02	1.07	0.61	1.74	0.1001

Model degrees of freedom (d.f.) are shown under each group category. Temp., temperature; d, day (under Group); WLR, water loss rate (mg h^{-1}); Mass, initial mass (mg).

outcomes, given the many laboratory studies undertaken on these species.

Given the relatively small, and readily correctable, differences among laboratory and wild strains, the effects of age and sex, as well as the differing trial conditions, on water balance found can inform both fundamental understanding of water balance in this group and its application to field population dynamics. Differences between different age groups indicated a decrease in desiccation tolerance with an increase in age, similar to previous findings confined to females for *A. arabiensis* and *A. gambiae* (Gray and Bradley, 2005), and those of some other insect species, mostly species of *Drosophila* (Nghiem et al., 2000; Gibbs and Markow, 2001; Shahrestani et al., 2012). 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 *A. arabiensis* and *A. funestus* (Lyons et al., 2012) and in other insect species (Bowler and Terblanche, 2008). Such a response has not been documented across a wide variety of insect groups, except for several species of *Drosophila*, and even here variation is typically among early and late life and also among species and sex (e.g. Matzkin et al., 2007; Shahrestani et al., 2012; Parkash and Ranga, 2013; Aggarwal, 2014). Mechanisms that might underlie a decline in tolerance with age are thought to include changes in WLR, probably a function of variation in cuticular hydrocarbon content and composition, melanization, differences in initial body water content and tolerance of dehydration (Nghiem et al., 2000; Gibbs and Markow, 2001; Benoit and Denlinger, 2007; Weldon et al., 2013). Here, we found a pronounced difference in the influence of WLR on overall survival time among age groups, suggesting that a change in cuticular resistance with age lies at the heart of the differences. Work showing that cuticular hydrocarbon amount and composition can change substantially with circumstances in *Anopheles* mosquitoes (Caputo et al., 2005; Reidenbach et al., 2014; Wagoner et al., 2014) bears out this idea. Irrespective of its cause, including age-related variation in tolerance of abiotic conditions is important for improving mechanistic models of population dynamics in mosquitoes (Styer et al., 2007; Beck-Johnson et al., 2013).

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 (e.g. Omer and Cloudsley-Thompson, 1970; Lehmann et al., 2010). The results from the present study clearly demonstrated a pronounced sex effect on desiccation tolerance in both *A. arabiensis* and *A. funestus*. In both

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104638

Table 3. Results from a generalized linear model with quasipoisson distribution of errors and log link function for *A. arabiensis* showing the influence of mass and WLR on desiccation tolerance (time in h) for each age and sex group at each temperature for the 5% RH treatment

Temp.	Group	Predictors	*N*	*F*	Estimate	s.e.m.	*t*-statistic	*P*-value
20°C	10 d ♀	WLR	20	45.94	−7.24	1.21	−6.0	<0.0001
	d.f.=17	Mass		8.49	0.35	0.11	3.06	0.0097
	10 d ♂	WLR	20	25.88	−6.81	1.67	−4.08	<0.0001
	d.f.=17	Mass		0.21	0.27	0.59	0.45	0.6538
	15 d ♀	WLR	20	82.75	−7.16	0.89	−7.96	<0.0001
	d.f.=17	Mass		5.90	0.30	0.12	2.56	0.0265
	15 d ♂	WLR	20	29.88	−6.62	1.4	−4.72	<0.0001
	d.f.=17	Mass		0.11	0.15	0.44	0.34	0.7487
	20 d ♀	WLR	20	8.87	−29.00	9.88	−2.94	0.0089
	d.f.=17	Mass		1.49	−0.54	0.45	−1.19	0.2401
		Mass × WLR		4.99	14.69	6.62	2.22	0.0401
	20 d ♂	WLR	20	41.47	−6.26	1.10	−5.71	<0.0001
	d.f.=17	Mass		13.05	2.61	0.73	3.59	0.0022
25°C	10 d ♀	WLR	19	0.27	−1.18	2.33	−0.51	0.6121
	d.f.=16	Mass		4.44	0.23	0.11	2.09	0.0510
	10 d ♂	WLR	21	5.20	−6.39	2.84	−2.25	0.0350
	d.f.=18	Mass		4.57	1.30	0.60	2.19	0.0464
	15 d ♀	WLR	20	17.68	−12.93	3.13	−4.13	0.0006
	d.f.=17	Mass		8.99	0.44	0.14	3.09	0.0081
	15 d ♂	WLR	20	30.22	−3.93	0.72	−5.46	<0.0001
	d.f.=17	Mass		0.78	0.61	0.67	0.90	0.3910
	20 d ♀	WLR	20	16.39	−7.77	1.97	−3.94	0.0008
	d.f.=17	Mass		17.47	0.43	0.10	4.21	0.0006
	20 d ♂	WLR	20	8.84	−14.16	5.19	−2.73	0.0085
	d.f.=17	Mass		0.67	1.06	1.34	0.79	0.4245
30°C	10 d ♀	WLR	20	22.10	−5.92	1.59	−3.73	0.0002
	d.f.=17	Mass		0.08	0.05	0.17	0.28	0.7829
	10 d ♂	WLR	20	8.19	−3.36	1.38	−2.43	0.0108
	d.f.=17	Mass		0.00	−0.03	0.95	−0.03	0.9750
	15 d ♀	WLR	20	49.78	−2.46	0.40	−6.21	<0.0001
	d.f.=17	Mass		2.95	0.37	0.23	1.65	0.1038
	15 d ♂	WLR	20	116.82	−3.81	0.34	−11.28	<0.0001
	d.f.=17	Mass		14.27	1.23	0.32	3.90	0.0015
	20 d ♀	WLR	20	9.34	−3.90	1.27	−3.08	0.0072
	d.f.=17	Mass		3.05	0.26	0.14	1.84	0.0987
	20 d ♂	WLR	20	13.15	−4.04	1.14	−3.54	0.0021
	d.f.=17	Mass		0.33	−0.32	0.55	−0.58	0.5725

Model degrees of freedom (d.f.) are shown under each group category. Temp., temperature; d, day (under Group); WLR, water loss rate (mg h^{-1}); Mass, initial mass (mg).

cases, females survived significantly longer than males under all combinations of temperature and humidity, consistent with the expectation that the females, which overwinter, should have higher innate desiccation resistance. Our data revealed that both mass and WLR have significant influences on survival time. In consequence, two mechanisms might explain sex-related differences. First, cuticular WLR, which generally dominates loss (Chown et al., 2011), differs among the sexes, as confirmed by pronounced effect size differences between males and females for the influence of WLR on survival. In turn, these differences are likely to be a consequence of variation in cuticle lipid content and/or composition, or cuticle thickness (Parkash and Ranga, 2013; Parkash and Ranga, 2014). Although some evidence exists for thicker cuticles in female mosquitoes (Wood et al., 2010), it seems likely that variation in cuticular lipid amount and composition is more likely to account for variation in WLR (Reidenbach et al., 2014). Second, larger mass in females means higher water content, which would further contribute to enhanced survival time in females (see also Reidenbach et al., 2014). Although higher body mass should also mean higher metabolic rates, and hence greater respiratory water loss (Chown et al., 2011), no evidence is available to indicate to what extent this mechanism may offset the two others, resulting in greater tolerance in females.

Overwintering females, by contrast to those examined in this study, are likely nulliparous (Omer and Cloudsley-Thompson, 1970), and probably have a reduction in blood feeding, associated with the reduction in metabolic demand in this season, thus increasing survival (Huestis et al., 2011). Along with the tendency of unfed mosquito adults to seek cool, humid refugia (Kessler and Guerin, 2008), this would probably explain their survival in the field during the dry season for several months (Omer and Cloudsley-Thompson, 1970; see also Lehmann et al., 2010), substantially longer than survival of any individual in the present study. Furthermore, overwintering females could be under strong selection for low WLR or high water storage [both known mechanisms of desiccation tolerance (Chown and Nicolson, 2004)]. The individuals used in this trial were provided constant access to sugar water and 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).

Although *A. arabiensis* is traditionally thought of as the more drought tolerant of the two vector species investigated (Gillies and Coetzee, 1987; Lindsay et al., 1998; Gray and Bradley, 2005), this species was shown consistently to survive for shorter periods than

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104638

Table 4. Generalized linear model results using a quasipoisson distribution of errors and log link function to determine the influence of mass, temperature, RH and species differences on desiccation tolerance (time in h) of *A. arabiensis* and *A. funestus* females and males separately

Sex	Predictors	Estimate	s.e.m.	*t*-statistic	*P*-value
Females	Intercept	2.31	0.14	16.30	<0.0005
	Species *Arabiensis*	–0.42	0.21	–1.99	0.0467
	Mass	0.49	0.14	3.55	0.0004
	RH L	1.34	0.07	18.76	<0.0005
	RH Q	–0.02	0.06	–0.35	0.7281
	Temperature L	–0.19	0.07	–2.67	0.0081
	Temperature Q	–0.28	0.06	–4.78	<0.0005
	Species *Arabiensis* × Mass	–0.14	0.16	–0.89	0.3703
	Species *Arabiensis* × RH L	–0.66	0.09	–6.73	<0.0005
	Species *Arabiensis* × RH Q	0.11	0.09	1.18	0.2364
	Species *Arabiensis* × temperature L	–0.08	0.10	–0.77	0.4444
	Species *Arabiensis* × temperature Q	–0.03	0.09	–0.29	0.7737
	RH L × temperature L	–0.04	0.13	–0.32	0.7513
	RH Q × temperature L	0.46	0.11	4.09	<0.0005
	RH L × temperature Q	0.50	0.11	4.63	<0.0005
	RH Q × temperature Q	–0.19	0.09	–1.89	0.0588
	Species *Arabiensis* × RH L × temperature L	0.11	0.18	0.61	0.5444
	Species *Arabiensis* × RH Q × temperature L	–0.44	0.16	–2.64	0.0087
	Species *Arabiensis* × RH L × temperature Q	–0.54	0.16	–3.45	0.0006
	Species *Arabiensis* × RH Q × temperature Q	0.16	0.15	1.08	0.2799
Males	Intercept	1.16	0.23	5.04	<0.0005
	Species *Arabiensis*	0.07	0.28	0.26	0.7964
	Mass	1.79	0.44	4.09	<0.0005
	RH L	1.18	0.07	17.10	<0.0005
	RH Q	–0.31	0.06	–5.49	<0.0005
	Temperature L	0.07	0.07	1.10	0.2719
	Temperature Q	–0.22	0.06	–3.78	0.0002
	Species *Arabiensis* × Mass	–1.00	0.47	–2.16	0.0316
	Species *Arabiensis* × RH L	–0.23	0.09	–2.40	0.0169
	Species *Arabiensis* × RH Q	0.29	0.08	3.55	0.0004
	Species *Arabiensis* × temperature L	–0.41	0.9	–4.45	<0.0005
	Species *Arabiensis* × temperature Q	0.09	0.08	1.08	0.2776
	RH L × temperature L	0.04	0.13	0.28	0.7809
	RH Q × temperature L	–0.14	0.10	–1.33	0.1852
	RH L × temperature Q	0.55	0.11	5.14	<0.0005
	RH Q × temperature Q	–0.22	0.09	–2.50	0.0128
	Species *Arabiensis* × RH L × temperature L	–0.13	0.17	–0.73	0.4654
	Species *Arabiensis* × RH Q × temperature L	0.15	0.15	1.06	0.2912
	Species *Arabiensis* × RH L × temperature Q	–0.32	0.15	–2.16	0.0313
	Species *Arabiensis* × RH Q × temperature Q	0.16	0.13	1.24	0.2154

Significant interactions between factors are also shown. L, linear; Q, quadratic. Mass is in mg, temperature is in °C.

A. funestus under different combinations of temperature and humidity. *Anopheles funestus* is typically a behaviourally 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 (e.g. 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 *A. arabiensis* and *A. funestus*. Additionally, *A. funestus* adults have also been shown to be more tolerant of high temperatures than *A. arabiensis* adults (Lyons et al., 2012). In the present study, *A. funestus* survived for longer under desiccation trials than *A. arabiensis*, largely because of its reduced WLR, though, once corrected for size, the differences between the species (especially in survival time) are much less pronounced. Indeed, our analyses show (Table 3) that the interspecific difference disappears for males and is only marginally significant for females with relatively small effect sizes. 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 (Huestis et al., 2011). In some regions, *A. funestus* is thought to be more important in the persistence of malaria throughout a dry season than *A. arabiensis* or *A. gambiae* (Charlwood et al., 2000).

A further possible reason for these species differences might be the influence of laboratory adaptation on desiccation tolerance. Some physiological traits are known to be more affected by laboratory conditions than others (Hoffmann et al., 2001; Huho et al., 2007; but see Terblanche et al., 2006; Parkash and Ranga, 2014). Our data suggest that the maintenance of *A. arabiensis* under colony conditions over several decades has had an effect on body size, which in turn affects survival time, but that other traits such as water loss tolerated are not affected. Moreover, the 12.5 h survival time of 10 day old females at 25°C was indistinguishable statistically ($P>0.05$ by *t*-test) from the 13.4 h found by Gray and Bradley (Gray and Bradley, 2005) for the same strain investigated at similar temperatures (28°C) but maintained under different conditions for 37 generations. Alternatively, among-population variation in the two species could account for the unexpected finding of lower desiccation resistance in *A. arabiensis* than expected, given that population-level variation in physiological traits is commonly found

The Journal of Experimental Biology (2014) doi:10.1242/jeb.104638

(e.g. Hoffmann and Harshman, 1999; Terblanche et al., 2006; Rocca et al., 2009; Simard et al., 2009). *Anopheles gambiae* s.s., the sister taxon to *A. arabiensis*, exhibits chromosomal polymorphic inversions, one of which confers an advantage on the species in arid environments (Coluzzi et al., 1979; Gray et al., 2009; Rocca et al., 2009). *Anopheles funestus* has also recently been shown to consist of different chromosomal polymorphic inversion forms occurring in different regions of the African continent (Guelbeogo et al., 2009; Sinka et al., 2010). Furthermore, *A. arabiensis* in Sudan has been shown to survive during the dry season where temperatures spike to over 50°C (Omer and Cloudsley-Thompson, 1970), in contrast to lethal temperature estimates of only ~34°C over a 4 h period for the southern African strain of this species (Lyons et al., 2012). In addition, the *A. arabiensis* colony was originally collected from a population occurring in the Zambezi River Valley close to a permanent tributary of the Zambezi River (R. Hunt, personal communication). This original population is unlikely to be as desiccation tolerant as populations from more arid habitats based solely on their proximity to this humid refuge. The existence of different phenotypes of *A. arabiensis* probably accounts for the seasonality of malaria in certain regions (Hay et al., 1998; Tanser et al., 2003) and the overwintering of females in some populations (Taylor et al., 1993; Huestis et al., 2011). In consequence, further investigations should focus on among-population variation in desiccation resistance in both species. Importantly, though, the direction of evolution cannot be inferred from our investigation of the two species (see Garland and Adolph, 1994). Thus, for any evolutionary conclusions to be reached, differences among the species and among populations will have to be examined in a broader comparative context.

To survive throughout the dry season, it is clear that both species must seek out refuges, and that additional downregulation of water loss 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 (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 (e.g. Patz et al., 1998; Charlwood et al., 2000; Tanser et al., 2003; Ndiath et al., 2012).

Nonetheless, the current work provides information on the way in which different intrinsic and extrinsic factors interact to determine the survival of adults of *A. arabiensis* and *A. funestus*. Such information can considerably improve population dynamic models of these vectors and of the likelihood of malaria under a range of conditions (e.g. Mordecai et al., 2013; Beck-Johnson et al., 2013), especially given that even recent models consider adult mortality parameters uncertain (e.g. Tompkins and Ermert, 2013). Thus, in conjunction with information on thermal effects on development in the immatures and survival in adults (Lyons et al., 2012; Lyons et al., 2013), this information will improve current malaria forecasting abilities, especially in southern Africa.

MATERIALS AND METHODS

Study populations

Anopheles arabiensis mosquitoes from the KGB-strain, originally established from individuals caught in the Zambezi River Valley in 1975, and *A. funestus* mosquitoes from the FUMOZ-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±2°C, ±80% RH, 12 h:12 h light:dark cycle), checked with a Masons Thermohygrometer (Brannan, Cleator Moor, Cumbria, UK). During this time, all mosquitoes were provided with a 10% sugar water solution and females were offered a blood meal three times weekly. Three age groups for each species were used during desiccation resistance experiments. Age groups for *A. arabiensis* were 10, 15 and 20 day old adults, while ages for *A. funestus* were 10, 20 and 30 days old. Ages differed between the species because *A. funestus* adults are typically longer lived than those of *A. arabiensis* (Hunt et al., 2005; Munhenga et al., 2011).

Because laboratory populations may show adaptation to this situation (reviewed in Chown and Terblanche, 2006) [for *Anopheles* see Huho et al. and Lyons et al. (Huho et al., 2007; Lyons et al., 2012)], we sought to explore the extent of differences between the laboratory populations and a wild population in *A. arabiensis*, to which we had ready access in the field. The field population was collected from Malahapanga in the Kruger National Park, South Africa (22°53.23S, 31°02.22E), and experiments were undertaken (as described below) on the F1 generation [for further details of field collections and animal maintenance see above and Lyons et al. (Lyons et al., 2012)].

Desiccation tolerance and starvation resistance trials

Individual males and females from each of these age groups were exposed to different combinations of three RH treatments and three different experimental temperatures. The lowest humidity, ~5%, was maintained through the use of silica gel, the ~55% treatment through saturated $Mg(NO_3)_2$ solution (Winston and Bates, 1960), and the ~100% humidity treatment (i.e. a starvation assessment) through the use of double-distilled water. Temperature was controlled using PTC-1 cabinets (Sable Systems, Las Vegas, NV, USA) or a SANYO incubator (MIR-154, SANYO Electric Co. Ltd, Osaka, Japan). Temperature and humidity were checked using hygrochron i-buttons (DS 1923-F5, Maxim/Dallas Semiconductor, Sunnyvale, CA, USA) accurate to 5% RH and 0.5°C.

At the start of each trial, each individual mosquito was anaesthetized by brief CO_2 exposure (<10 s) so that initial mass could be obtained to the nearest 0.0001 mg (using a Mettler Toledo UMX2 microbalance, Greinfensee, Switzerland). Each individual was then placed into a clear, double open-ended 10 ml vial, closed on either end with 1 mm gauze mesh. Each of the 20 vials containing an individual mosquito was placed into one of four replicate clear containers (230×160×100 mm) containing the silica gel, $Mg(NO_3)_2$ solution or distilled water (four replicate containers of each at each temperature). Each replicate container was then sealed and placed at one of three temperatures (20, 25, 30°C) with a 12 h:12 h light:dark cycle. Mosquitoes were checked every 2–3 h for the first 24 h and then every 6 h until death or visible knockdown without any sign of recovery occurred. Following each experiment at each temperature, containers were opened to remove mosquitoes that were visibly knocked down and showed signs of desiccation stress. Knocked down or dead mosquitoes were weighed immediately after removal from the experimental conditions 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, which was attributed to desiccation, acknowledging that some mass was lost via metabolism [see Chown and Nicolson (Chown and Nicolson, 2004) for a discussion of various estimates of water loss]. Dividing this difference by the time each mosquito took to die provided an indication of WLR (in mg h^{-1}). In total, 1120 and 1060 *A. funestus* and *A. arabiensis* individuals, respectively, were used in these trials.

Influence of mass, age, sex, RH and temperature on desiccation resistance

To determine the influence of sex, mass (mg), RH (%), age (days) and temperature (°C), on desiccation resistance (response), measured as survival time (h), 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. Because data were zero-bounded on the left and showed positively skewed distributions, the quassipoisson error distribution was chosen (Crawley, 2007). Temperature, age and RH were used as ordered factors. 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 graphically present the influence of age and temperature on desiccation resistance (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 of the interactions among the factors.

To determine the relative contributions of WLR and initial mass (body size) to desiccation resistance of *A. arabiensis* and *A. funestus*, a generalized linear model with quasipoisson distribution of errors (to correct for overdispersion) and log link function, using survival time of each group as the dependent variable and WLR and initial mass as the independent variables was implemented in R (v. 2.15.1). This analysis was performed only for the 5% RH treatment per temperature, age and sex category. Only one humidity treatment was chosen to provide an indication of possible mechanisms underlying desiccation resistance in these species.

To graphically present the influence of temperature and humidity on survival time for both species, the residuals from a mass versus time regression of the oldest groups for both species (20 days old for *A. arabiensis* and 30 days old for *A. funestus*) were plotted on a distance-weighted least squares 3D contour plot against temperature and RH.

Species comparison

How desiccation resistance (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). 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 RH and temperature were input as ordered factors.

Laboratory versus wild strains of *A. arabiensis*

To understand potential differences between field and wild strains of *A. arabiensis*, the following comparisons were made. First, using the methods described above, initial mass, time to death, dry mass at death, water loss tolerated (measured as the difference between initial wet mass and wet mass at death) and residual lipid content (measured as dry mass at death minus lipid free dry mass at death, with the latter determined after breaking individuals up and maintaining them for 72 h in a 1:1 chloroform:methanol solution exchanged every 24 h and then drying to constant mass) were determined for wild and laboratory populations. This was not done for the full suite of interactions, owing to limited numbers of wild-caught individuals, but rather for 25°C and 5% RH, a relatively extreme temperature × humidity treatment, and for 20°C and 55% RH, a less extreme situation, for 10 day old adults. Much of the data for the laboratory strain were drawn from the original experiments.

Generalized linear models (as above, conducted in R v. 3.0.2) were then used to examine the effects of population and sex on initial mass (to assess size differences among the strains), water lost and residual lipid content (as a measure of desiccation tolerance), and time to death (an integrated fitness measure). The analyses were then repeated, where significant differences among strains were found, to investigate the impacts of initial mass on these differences (using initial wet mass as the covariate).

Acknowledgements

Thanks to staff and students of the Vector Control Reference Laboratory of the Centre for Opportunistic and Hospital Infections, National Health Laboratory Service (NHLS) in Johannesburg for colony maintenance and upkeep. Four anonymous reviewers made very helpful suggestions for improvements.

Competing interests

The authors declare no competing financial interests.

Author contributions

All authors conceptualized the design of the research. C.L.L. carried out all research and C.L.L. and S.L.C. analyzed the data. All authors interpreted the results and drafted and read the final manuscript.

Funding

C.L.L. and S.L.C. were funded by the Department of Science and Technology National Research Foundation (DST-NRF) Centre of Excellence for Invasion Biology at Stellenbosch University, through a HOPE project grant. M.C. is supported by a DST-NRF South African Research Chairs Initiative (SARChI) Grant. J.S.T. is supported by the National Research Foundation (NRF) Incentive Funding for Rated (IFR) researchers scheme.

Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.104638/-/DC1

References

Aggarwal, D. D. (2014). Physiological basis of starvation resistance in *Drosophila leontia*: analysis of sexual dimorphism. *J. Exp. Biol.* **217**, 1849-1859.
Ballard, J. W. O., Melvin, R. G. and Simpson, S. J. (2008). Starvation resistance is positively correlated with body lipid proportion in five wild caught *Drosophila simulans* populations. *J. Insect Physiol.* **54**, 1371-1376.
Beck-Johnson, L. M., Nelson, W. A., Paaijmans, K. P., Read, A. F., Thomas, M. B. and Bjørnstad, O. N. (2013). The effect of temperature on *Anopheles* mosquito population dynamics and the potential for malaria transmission. *PLoS ONE* **8**, e79276.
Benoit, J. B. and Denlinger, D. L. (2007). Suppression of water loss during adult diapause in the northern house mosquito, *Culex pipiens*. *J. Exp. Biol.* **210**, 217-226.
Benoit, J. B. and Denlinger, D. L. (2010). Meeting the challenges of on-host and off-host water balance in blood-feeding arthropods. *J. Insect Physiol.* **56**, 1366-1376.
Bowler, K. and Terblanche, J. S. (2008). Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biol. Rev. Camb. Philos. Soc.* **83**, 339-355.
Buckley, L. B. and Kingsolver, J. G. (2012). Functional and phylogenetic approaches to forecasting species' responses to climate. *Annu. Rev. Ecol. Evol. Syst.* **43**, 205-226.
Caputo, B., Dani, F. R., Horne, G. L., Petrarca, V., Turillazzi, S., Coluzzi, M., Priestman, A. A. and della Torre, A. (2005). Identification and composition of cuticular hydrocarbons of the major Afrotropical malaria vector *Anopheles gambiae* s.s. (Diptera: Culicidae): analysis of sexual dimorphism and age-related changes. *J. Mass Spectrom.* **40**, 1595-1604.
Charlwood, J. D., Vij, R. and Billingsley, P. F. (2000). Dry season refugia of malaria-transmitting mosquitoes in a dry savannah zone of east Africa. *Am. J. Trop. Med. Hyg.* **62**, 726-732.
Chown, S. L. and Klok, C. J. (2003). Water-balance characteristics respond to changes in body size in subantarctic weevils. *Physiol. Biochem. Zool.* **76**, 634-643.
Chown, S. L. and Nicolson, S. W. (2004). *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford: Oxford University Press.
Chown, S. L. and Terblanche, J. S. (2006). Physiological diversity in insects: ecological and evolutionary contexts. *Adv. In Insect Physiol.* **33**, 50-152.
Chown, S. L., Sørensen, J. G. and Terblanche, J. S. (2011). Water loss in insects: an environmental change perspective. *J. Insect Physiol.* **57**, 1070-1084.
Colinet, H., Siaussat, D., Bozzolan, F. and Bowler, K. (2013). Rapid decline of cold tolerance at young age is associated with expression of stress genes in *Drosophila melanogaster*. *J. Exp. Biol.* **216**, 253-259.
Coluzzi, M., Sabatini, A., Petrarca, V. and Di Deco, M. A. (1979). Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Trans. R. Soc. Trop. Med. Hyg.* **73**, 483-497.
Crawley, M. J. (2007). *The R Book*. Chichester: John Wiley & Sons Ltd.
Dierks, A., Hoffmann, B., Bauerfeind, S. S. and Fischer, K. (2012). Effects of inbreeding on life history and thermal performance in the tropical butterfly *Bicyclus anynana*. *Popul. Ecol.* **54**, 83-90.
Edney, E. B. (1977). *Water Balance in Land Arthropods*. Berlin: Springer.
Farenhorst, M., Mouatcho, J. C., Kikankie, C. K., Brooke, B. D., Hunt, R. H., Thomas, M. B., Koekemoer, L. L., Knols, B. G. J. and Coetzee, M. (2009). Fungal infection counters insecticide resistance in African malaria mosquitoes. *Proc. Natl. Acad. Sci. USA* **106**, 17443-17447.
Fouet, C., Gray, E., Besansky, N. J. and Costantini, C. (2012). Adaptation to aridity in the malaria mosquito *Anopheles gambiae*: chromosomal inversion polymorphism and body size influence resistance to desiccation. *PLoS ONE* **7**, e34841.
Garland, T. and Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* **67**, 797-828.
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. et al. (2009). Macrophysiology: a conceptual reunification. *Am. Nat.* **174**, 595-612.
Gibbs, A. G. and Markow, T. A. (2001). Effects of age on water balance in *Drosophila* species. *Physiol. Biochem. Zool.* **74**, 520-530.
Gibbs, A. G., Chippindale, A. K. and Rose, M. R. (1997). Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* **200**, 1821-1832.
Gibbs, A. G., Fukuzato, F. and Matzkin, L. M. (2003). Evolution of water conservation mechanisms in *Drosophila*. *J. Exp. Biol.* **206**, 1183-1192.

Gillies, M. T. and Coetzee, M. (1987). *A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region)*. Johannesburg: South African Institute of Medical Research.
Githeko, A. K. (2009). Malaria and climate change. In *Commonwealth Health Ministers Update*, pp. 40-43. London: The Commonwealth.
Gray, E. M. and Bradley, T. J. (2005). Physiology of desiccation resistance in *Anopheles gambiae* and *Anopheles arabiensis*. *Am. J. Trop. Med. Hyg.* **73**, 553-559.
Gray, E. M., Rocca, K. A. C., Costantini, C. and Besansky, N. J. (2009). Inversion 2La is associated with enhanced desiccation resistance in *Anopheles gambiae*. *Malar. J.* **8**, 215.
Guelbeogo, W. M., Sagnon, N., Grushko, O., Yameogo, M. A., Boccolini, D., Besansky, N. J. and Costantini, C. (2009). Seasonal distribution of *Anopheles funestus* chromosomal forms from Burkina Faso. *Malar. J.* **8**, 239.
Hahn, D. A. and Denlinger, D. L. (2011). Energetics of insect diapause. *Annu. Rev. Entomol.* **56**, 103-121.
Harrison, J. F., Woods, H. A. and Roberts, S. P. (2012). *Ecological and Environmental Physiology of Insects.* Oxford: Oxford University Press.
Hay, S. I., Snow, R. W. and Rogers, D. J. (1998). Predicting malaria seasons in Kenya using multitemporal meteorological satellite sensor data. *Trans. R Soc. Trop. Med. Hyg.* **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 Parasitol.* **18**, 530-534.
Hayward, S. A. L., Bale, J. S., Worland, M. R. and Convey, P. (2001). Influence of temperature on the hygropreference of the Collembolan, *Cryptopygus antarcticus*, and the mite, *Alaskozetes antarcticus* from the maritime Antarctic. *J. Insect Physiol.* **47**, 11-18.
Hoffmann, A. A. (1990). The influence of age and experience with conspecifics on territorial behaviour in *Drosophila melanogaster*. *J. Insect Behav.* **3**, 1-12.
Hoffmann, A. A. (2010). Physiological climatic limits in *Drosophila*: patterns and implications. *J. Exp. Biol.* **213**, 870-880.
Hoffmann, A. A. and Harshman, L. G. (1999). Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation 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., Hallas, R., Anderson, A. R. and Telonis-Scott, M. (2005). Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *J. Evol. Biol.* **18**, 804-810.
Hood, W. G. and Tschinkel, W. R. (1990). Desiccation resistance in arboreal and terrestrial ants. *Physiol. Entomol.* **15**, 23-35.
How, Y. F. and Lee, C. Y. (2010). Effects of temperature and humidity on the survival and water loss of *Cimex hemipterus* (Hemiptera: Cimicidae). *J. Med. Entomol.* **47**, 987-995.
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. *J. Exp. Biol.* **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. J. Exp. Biol.* **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*. *Med. Vet. Entomol.* **19**, 271-275.
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. *J. Vector Ecol.* **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. *Front. Physiol.* **2**, 74.
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. *Am. J. Trop. Med. Hyg.* **83**, 601-606.
Lindsay, S. W., Parson, L. and Thomas, C. J. (1998). Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae sensu stricto* and *An. arabiensis*, using climate data. *Proc. Biol. Sci.* **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*. *Malar. J.* **11**, 226.
Lyons, C. L., Coetzee, M. and Chown, S. L. (2013). Stable and fluctuating temperature effects on the development rate and survival of two malaria vectors, *Anopheles arabiensis* and *Anopheles funestus*. *Parasit. Vectors* **6**, 104.
Matzkin, L. M., Watts, T. D. and Markow, T. A. (2007). Desiccation resistance in four *Drosophila* species: sex and population effects. *Fly (Austin)* **1**, 268-273.
Mironidis, G. K. and Savopoulou-Soultani, M. (2010). Effects of heat shock on survival and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) adults. *J. Therm. Biol.* **35**, 59-69.
Mordecai, E. A., Paaijmans, K. P., Johnson, L. R., Balzer, C., Ben-Horin, T., de Moor, E., McNally, A., Pawar, S., Ryan, S. J., Smith, T. C. et al. (2013). Optimal temperature for malaria transmission is dramatically lower than previously predicted. *Ecol. Lett.* **16**, 22-30.
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. *Parasit. 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. *Malar. J.* **7**, 43.
Ndiath, M. O., Sarr, J. B., Gaayeb, L., Mazenot, C., Sougoufara, S., Konate, L., Remoue, F., Hermann, E., Trape, J. F., Riveau, G. et al. (2012). Low and seasonal malaria transmission in the middle Senegal River basin: identification and characteristics of *Anopheles* vectors. *Parasit. Vectors* **5**, 21.
Nghiem, D., Gibbs, A. G., Rose, M. R. and Bradley, T. J. (2000). Postponed aging and desiccation resistance in *Drosophila melanogaster*. *Exp. Gerontol.* **35**, 957-969.
Omer, S. M. and Cloudsley-Thompson, J. L. (1970). Survival of female *Anopheles gambiae* Giles through a 9-month dry season in Sudan. *Bull. World Health Organ.* **42**, 319-330.
Parkash, R. and Ranga, P. (2013). Sex-specific divergence for adaptations to dehydration stress in *Drosophila kikkawai*. *J. Exp. Biol.* **216**, 3301-3313.
Parkash, R. and Ranga, P. (2014). Seasonal changes in humidity impact drought resistance in tropical *Drosophila leontia*: testing developmental effects of thermal versus humidity changes. *Comp. Biochem. Physiol.* **169A**, 33-43.
Pascual, M., Ahumada, J. A., Chaves, L. F., Rodó, X. and Bouma, M. (2006). Malaria resurgence in the East African highlands: temperature trends revisited. *Proc. Natl. Acad. Sci. USA* **103**, 5829-5834.
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. *Trop. Med. Int. Health* **3**, 818-827.
Reidenbach, K. R., Cheng, C., Liu, F., Liu, C., Besandsky, N. J. and Syed, Z. (2014). Cuticular differences associated with aridity acclimation in African malaria mosquitoes carrying alternative arrangements of inversion 2La. *Parasit. Vectors* **7**, 176.
Rocca, K. A. C., Gray, E. M., Costantini, C. and Besansky, N. J. (2009). 2La chromosomal inversion enhances thermal tolerance of *Anopheles gambiae* larvae. *Malar. J.* **8**, 147.
Rogers, D. J. and Randolph, S. E. (2000). The global spread of malaria in a future, warmer world. *Science* **289**, 1763-1766.
Shahrestani, P., Quach, J., Mueller, L. D. and Rose, M. R. (2012). Paradoxical physiological transitions from aging to late life in *Drosophila*. *Rejuvenation Res.* **15**, 49-58.
Simard, F., Ayala, D., Kamdem, G. C., Pombi, M., Etouna, J., Ose, K., Fotsing, J. M., Fontenille, D., Besansky, N. J. and Costantini, C. (2009). Ecological niche partitioning between *Anopheles gambiae* molecular forms in Cameroon: the ecological side of speciation. *BMC Ecol.* **9**, 17.
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. et al. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasit. Vectors* **3**, 117.
Styer, L. M., Carey, J. R., Wang, J. L. and Scott, T. W. (2007). Mosquitoes do senesce: departure from the paradigm of constant mortality. *Am. J. Trop. Med. Hyg.* **76**, 111-117.
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. *Med. Vet. Entomol.* **7**, 351-357.
Terblanche, J. S., Klok, C. J., Krafsur, 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. *Am. J. Trop. Med. Hyg.* **74**, 786-794.
Thomson, L. J., Macfadyen, S. and Hoffmann, A. A. (2010). Predicting the effects of climate change on natural enemies of agricultural pests. *Biol. Control* **52**, 296-306.
Tompkins, A. M. and Ermert, V. (2013). A regional-scale, high resolution dynamical malaria model that accounts for population density, climate and surface hydrology. *Malar. J.* **12**, 65.
Wagoner, K. M., Lehmann, T., Huestis, D. L., Ehrmann, B. M., Cech, N. B. and Wasserberg, G. (2014). Identification of morphological and chemical markers of dry- and wet-season conditions in female *Anopheles gambiae* mosquitoes. *Parasit. Vectors* **7**, 294.
Weldon, C. W., Yap, S. and Taylor, P. W. (2013). Desiccation resistance of wild and mass-reared *Bactrocera tryoni* (Diptera: Tephritidae). *Bull. Entomol. Res.* **103**, 690-699.
Winston, P. W. and Bates, D. H. (1960). Saturated salt solutions for the control of humidity in biological research. *Ecology* **41**, 232-237.
Wood, O. R., Hanrahan, S., Coetzee, M., Koekemoer, L. L. and Brooke, B. D. (2010). Cuticle thickening associated with pyrethroid resistance in the major malaria vector *Anopheles funestus*. *Parasit. Vectors* **3**, 67.
Woodin, S. A., Hilbish, T. J., Helmuth, B., Jones, S. J. and Wethey, D. S. (2013). Climate change, species distribution models, and physiological performance metrics: predicting when biogeographic models are likely to fail. *Ecol. Evol.* **3**, 3334-3346.
World Health Organization (2013). *World Malaria Report.* Geneva: WHO. Available at: http://www.who.int/malaria.