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Responses of adult mosquitoes of two sibling species, *Anopheles arabiensis* and *A. gambiae* s.s. (Diptera: Culicidae), to high temperatures

M.J. Kirby and S.W. Lindsay*

School of Biological and Biomedical Sciences, University of Durham, South Road, Durham, DH1 3LE, UK

Abstract

It is well known that amongst the sibling species of the *Anopheles gambiae* complex, *A. arabiensis* Patton predominates over *A. gambiae* sensu stricto Giles in hotter, drier parts of Africa. Here it was investigated whether *A. arabiensis* is better adapted to higher temperatures than *A. gambiae* s.s. at the microclimatic level. Bioassays were used to assess behavioural avoidance activity of adult mosquitoes in the presence of increasing temperature. Female mosquitoes were introduced into a holding tube from which they could escape into a cage through a one-way funnel. From a starting temperature of 28°C they were exposed to a 2°C rise in temperature every 30 min until all mosquitoes had escaped or been knocked down. As temperature increased, *A. arabiensis* left the holding tube at higher temperatures than *A. gambiae* s.s. (*A. arabiensis* mean activation temperature = 35.7°C, 95% CIs = 35.4–36.1°C; *A. gambiae* s.s. = 33.0°C, 32.5–33.5°C). To determine the relative ability of both species to survive at extremely high temperatures, batches of insects were exposed to 40°C for different periods. It took considerably longer to kill 50% of *A. arabiensis* at 40°C than it did *A. gambiae* s.s. (112 min vs. 67 min). These data show that adult *A. arabiensis* are better adapted to hotter conditions than *A. gambiae* s.s., a characteristic that is reflected in their spatial and temporal distribution in Africa.

Introduction

A rapid change in environmental temperature is an important factor governing the behaviour of insects, not least because many find it difficult to maintain a constant internal temperature through physiological mechanisms alone (Uvarov, 1931; Denlinger & Yocum, 1988). Instead many insects, like mosquitoes, take advantage of small local variations in temperature and maintain an optimal body temperature by seeking out favourable microclimates in which to rest.

*Author for correspondence
Fax: +44 (0)191 334 1179
E-mail: S.W.Lindsay@durham.ac.uk

In mosquitoes (Diptera: Culicidae), temperature is important in a number of behaviours and physiological processes including host seeking (Petric *et al.*, 1995), blood feeding (Samish *et al.*, 1995; Crans *et al.*, 1996) and development (Lyimo *et al.*, 1992; Lanciani & Le, 1995), and is one of a number of climatic factors that affect their geographic distribution (Lindsay *et al.*, 1998; Bayoh *et al.*, 2001).

The influence of temperature on mosquito flight activity is clearly important for quantifying the risk of mosquito-borne diseases, particularly at high temperatures, since in the absence of flight pathogen transmission is unlikely to occur. Generally, catches of mosquitoes are greater on warmer nights than colder ones (Read & Adams, 1980; Petric *et al.*, 1995). The upper temperature threshold where mortality increases sharply appears to be between 38 and

45°C for most tropical mosquitoes, depending on the species and life-stage involved (Muirhead-Thompson, 1951; Benedict *et al*., 1991). For *Anopheles gambiae* s.s. Giles few adults survived longer than one day above 40°C, at 40% relative humidity (Bayoh, 2001).

Behavioural avoidance of extreme heat might be one of several putative survival strategies used by adult mosquitoes encountering such conditions in the field. For though they have a limited flight range of a few kilometres, they are regularly exposed to a range of conditions from which they must select suitable feeding, resting and breeding sites. Thus they are capable of responding to temperature fluctuations that can be quite large. For example, in Tigray, Ethiopia, air temperature can fluctuate daily from 11 to 27°C (Yohannes, 2002), whilst in Kaduna, Nigeria, it can range from less than 15 to nearly 35°C (Rishikesh *et al*., 1985) and in northern Sudan diurnal fluctuations from 24 to 49°C occur in the dry season (Dukeen & Omer, 1986).

Many previous behavioural studies of the *A. gambiae* complex, especially in the older literature, are difficult to interpret because the mosquitoes reported are a combination of different species (Muirhead-Thomson, 1951). *Anopheles gambiae* s.s. and *Anopheles arabiensis* Patton are the two most efficient malaria vectors of the complex, and though they are morphologically identical, they express distinct behavioural characteristics (e.g. preferred host, feeding and resting sites) which may relate to their differential use of habitat (Coluzzi, 1984). *Anopheles arabiensis* dominates in hot and dry conditions while *A. gambiae* s.s. thrives in cooler, wetter conditions (Lindsay *et al*., 1998). *Anopheles arabiensis* has a number of strategies allowing persistence in arid areas which include laying eggs on damp surfaces (Coluzzi, 1965), delayed egg hatching and reproductive quiescence, in which a single gonotrophic cycle is experienced over the whole period (Omer, 1970; White, 1974; Taylor *et al*., 1993). However these processes operate over periods of several days, and are not concerned with shorter-term responses to changing climate. This present study set out to test whether there are short-term differences in (i) tolerance to increasing temperature and (ii) survival of adults at high temperatures, between the two closely related species.

Materials and methods

Mosquitoes

The KWA and 16CSS strains of *A. gambiae* s.s. and KGB and Dondotha strains of *A. arabiensis* were supplied by the London School of Hygiene and Tropical Medicine. The London colony of *A. gambiae* s.s. KWA was established in 1975 from eggs collected in the field at Kwale, 35 km north of Tanga, Tanzania and 16CSS was derived in 1974 from Lagos, Nigeria. *Anopheles arabiensis* KGB was colonized in London in 1998. That population was raised from a colony held at the Medical Research Council's laboratory, Cape Town, South Africa, and was originally derived from wild material from the Kanyema Zambesi Valley and maintained in Harare, Zimbabwe. The Dondotha strain came from Kwa Zulu-Natal in South Africa. The Durham colonies of both species were established between 1998 and 2001.

Mosquitoes were raised in a standard manner (Bayoh & Lindsay, 2003). Larvae were reared at 25.0 ± 1.0°C and adults maintained in 30 cm^3 holding cages at 40–50% relative humidity and 27.5 ± 1.0°C with 10% glucose solution supplied *ad libitum*.

Adults of *A. gambiae* s.s. (KWA strain) and *A. arabiensis* (KGB strain) were evaluated at different ages and feeding conditions. To assess the effects of age, mosquitoes were tested within 24 h of emergence, at 3–6 days old and at 14–17 days old. In the oldest group, in order to simulate conditions experienced in nature, mosquitoes were blood-fed every 3 days on one of us (MK), with the final feed 2 days before the assay. Cohorts of both species aged 3–6 days were allowed to blood-feed to repletion to examine the responses of newly-fed females to rising temperature. Fully engorged females were immediately transferred to the holding tubes. The 16CSS *A. gambiae* s.s. and Dondotha *A. arabiensis* strains were used to test whether differences observed were consistent between species.

Temperature avoidance behaviour

Bioassay

The experimental system consisted of a plastic holding tube, heated by warm water, connected to an escape cage (fig. 1). The plastic tubes used were WHO insecticide resistance testing-kit tubes measuring 125 mm length × 44 mm internal diameter (WHO, Geneva). In the holding tube, the mesh screen at the end of the tube was replaced with a solid clear plastic disc, 46 mm in diameter, glued into place. The disc prevented the inflow of air at room temperature (28.0 ± 1.0°C) affecting internal tube conditions. A 3 mm diameter hole in the disc allowed a thermometer (probe type T, Digitron Instruments, UK) to be inserted into the tube. Humidity within the holding tube was monitored using a pen-type digital humidity meter (RS Components Ltd, Northamptonshire, UK). At the other end of the tube there was a sliding gate that allowed the introduction of mosquitoes into the holding tube through a 20 mm diameter hole. A water bath fitted with an immersion thermostat with integral pump unit (Grant Instruments Ltd, Cambridge, UK) for external circulation was used to drive heated water around the holding tube, at a flow rate of 350 ml min^{-1}. The water was circulated through translucent silicone tubing (4 mm internal diameter, 1 mm thick) coiled around the holding tube and taped into place in order to minimize internal thermal gradients.

A plastic funnel (70 mm and 15 mm diameter) was secured to the outside of a 15 cm^3 mesh cage. The wide end was fitted flush to the cage surface so that the small end protruded into the cage. This allowed easy entrance to the cage but prevented highly active mosquitoes returning into the escape tube, which linked the holding tube to the escape cage. The escape tube prevented mosquitoes returning to the holding tube if they did not enter the escape cage immediately.

Experimental procedure

Ten mosquitoes were introduced into the holding tube at the starting temperature of 28 ± 0.5°C. Mosquitoes were acclimated for 30 min, after which the gate was opened and the insects were free to move out of the holding tube. Any mosquitoes that left in the first 5 min were judged to have been disturbed by the gate opening and were not included in the final analysis. After 20 min, the numbers of mosquitoes in the holding tube and cage were recorded. In the next 10 min the water bath temperature was increased to create a holding tube temperature of 30 ± 0.5°C at a rate of 0.2°C min^{-1}. This procedure was repeated for all further

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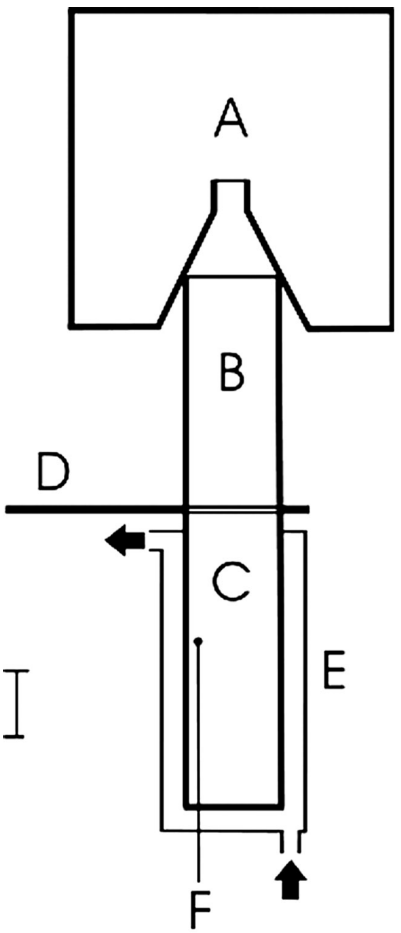

Fig. 1. Schematic representation of experimental apparatus. A, escape cage; B, escape tube; C, holding tube, where mosquitoes are introduced; D, sliding gate; E, silicone tubing 'water jacket'; F, temperature probe. Bar represents 3 cm.

temperature steps up to 42°C. Thus every 30 min the air temperature was increased by 2°C (fig. 2). Cage temperature was recorded continuously at each temperature step. Females remaining in the tube at the end of the experiment were removed to see if they were still responsive. Those unable to right themselves after probing with a pipette tip were recorded as 'knocked-down' and excluded from the final analysis. A paper facemask was worn by the observer when counting mosquitoes so as to minimize the impact of breathing carbon dioxide near the set-up. Each treatment group had ten runs, each with ten mosquitoes.

The experiment had two controls. Firstly, in order to demonstrate that mosquitoes responded to increasing temperature and not time, six runs each with ten mosquitoes for each treatment group were carried out at a constant temperature of 28 ± 0.5°C. The number of mosquitoes left in the holding tube at the end of 3 h was recorded. Secondly, it was necessary to show that the actual temperature values were important in dictating activity and not simply a 2°C change in ambient temperature. Therefore six runs each with ten mosquitoes for each treatment group were introduced into tubes at 24°C for 20 min and increased to 26°C at a rate of 0.2°C min^{-1}. This process was repeated up to 28°C.

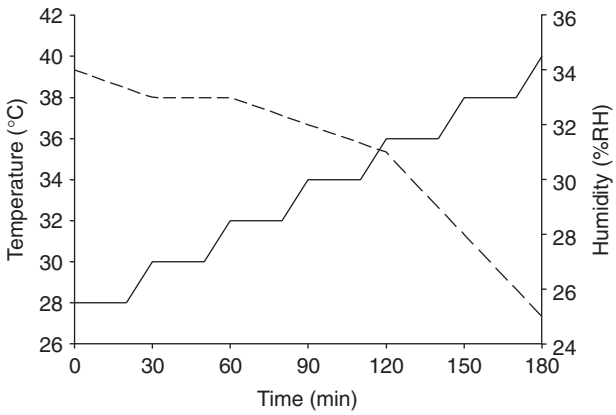

Fig. 2. Step-wise increase in experiment air temperature (—) and negative correlation with humidity (- - -).

Survival at 40°C

Ten 3- to 6-day-old unfed female mosquitoes were aspirated into 15 cm^3 wire-framed net-covered cages. These cages were placed into rectangular glass chambers 39 × 20 × 22 cm with fitted lids, which were put onto shelves within programmable incubators (S.H. Scientific, Kent, UK). Groups of ten mosquitoes were exposed to 40°C and 30% relative humidity (RH) for 30, 60, 90, 120, 240, 480 or 1440 min. At the conclusion of the experimental run, the cages were removed from the incubators and transferred to another glass chamber held at 27°C and > 80% RH to allow mosquito recovery. Mortality was determined 24 h later. Ten runs each with ten mosquitoes were completed for each species at all periods. LT_{50} values (time required to kill 50% of the population) were used to describe average survival.

Climate data

The World Meteorological Organization weather station data presented for the sites of origin of the mosquito strains was accessed from the International Centre of Insect Physiology and Ecology website *http://informatics.icipe.org/databank/wmo.htm*).

Analysis

Unfed female mosquitoes 3–6 days old were used as the standard to which all other treatments were compared. The one-sample Kolmogorov-Smirnov test was used to test the normal distribution of the data. Normally-distributed data were analysed by standard parametric methods, using SPSS for Windows v. 10.0. F tests were used to compare variances between groups, t-tests were used to test for equality of means between two groups and χ^2 was used to test for a trend in the data. Multivariate analysis of variance (general linear model) was used to identify the relative importance of species, adult age and feeding status as determinants of activation temperature. To assess the possibility that the activity of one mosquito stimulated the movement of others resulting in a non-independence of data points, Mann-Whitney U tests were employed to test for strain and species differences between the first mosquito to be activated in each run. Mantel-Haenzel χ^2 test was used to test for differences

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in survival at 40°C between the two species and probit analysis was used to generate respective LT_{50} values.

Results

Temperature avoidance behaviour

Of the mosquitoes observed, 5.9% (31 *A. gambiae* s.s. and 22 *A. arabiensis*) were either disturbed or trapped by the gate opening, and 4.6% were 'knocked down' at the end of the experiments (22 *A. gambiae* s.s. and 20 *A. arabiensis*). Thus the main analysis was based on observations of 458 *A. arabiensis* and 447 *A. gambiae* s.s.

The variances of all groups tested were similar in all comparisons (F-test, n.s.), and all data was normally distributed (Kolmorov-Smirnov Z, n.s.). *Anopheles arabiensis* consistently left the holding tube at higher temperatures than did *A. gambiae* s.s. (table 1). The greatest interspecific difference was observed between 3- to 6-day-old unfed mosquitoes (*A. arabiensis* KGB, mean activation temperature = 35.7°C, 95% CIs = 35.4–36.1; *A. gambiae* s.s. KWA = 33.0°C, 32.5–33.5, fig. 3). There was no difference between the mean activation temperatures for strains of the same species (*A. arabiensis*; KGB = 35.7, 95% CIs 35.4–36.1, Dondotha = 35.5, 35.2–36.0, t = 0.5, n.s., *A. gambiae* s.s.; KWA = 33.0, 32.5–33.5, 16CSS = 33.3, 32.8–33.8, t = −0.8, n.s.)

In all age treatment comparisons, *A. arabiensis* left the tubes at higher temperatures than *A. gambiae* s.s. (< 24 h, t = −2.06, $P = 0.04$; 3–6 days, t = −9.17, $P < 0.001$; ⩾ 14 days, t = −4.14, $P < 0.001$). The temperature at which mosquitoes were activated declined with age in *A. gambiae* s.s. (χ^2 for trend: *A. gambiae* s.s. at 36°C, $\chi^2 = 32.4$, $P < 0.001$), but not *A. arabiensis* (at 36°C, $\chi^2 = 0.2$, n.s., fig. 4). In both species newly emerged mosquitoes were activated at higher mean temperatures than adults ⩾ 14 days (*A. arabiensis* = 1.8°C higher, 95% CIs = 1.1–2.5°C, t = 5.4, $P < 0.001$; *A. gambiae* s.s. = 2.5°C, 1.9–3.2°C, t = 7.7, $P < 0.001$). Blood-fed mosquitoes were activated at higher temperatures than unfed mosquitoes for both *A. arabiensis* (1.1°C, 0.6–1.6°C, t = 4.2, $P < 0.001$) and *A. gambiae* s.s. (3.1°C, 2.4–3.7°C, t = 9.5, $P < 0.001$), though *A. arabiensis* was still activated at higher temperatures than *A. gambiae* s.s. (t = – 2.80, $P < 0.001$, fig. 5). Eighteen per cent of blood-fed *A. arabiensis* and 15% of blood-fed *A. gambiae* s.s. were 'knocked down' at high temperatures and did not leave the holding tube, even when the temperature exceeded 40°C. Forty four per cent of the knocked down *A. arabiensis* and 53% of the *A. gambiae* s.s. died less than 24 h after the bioassay.

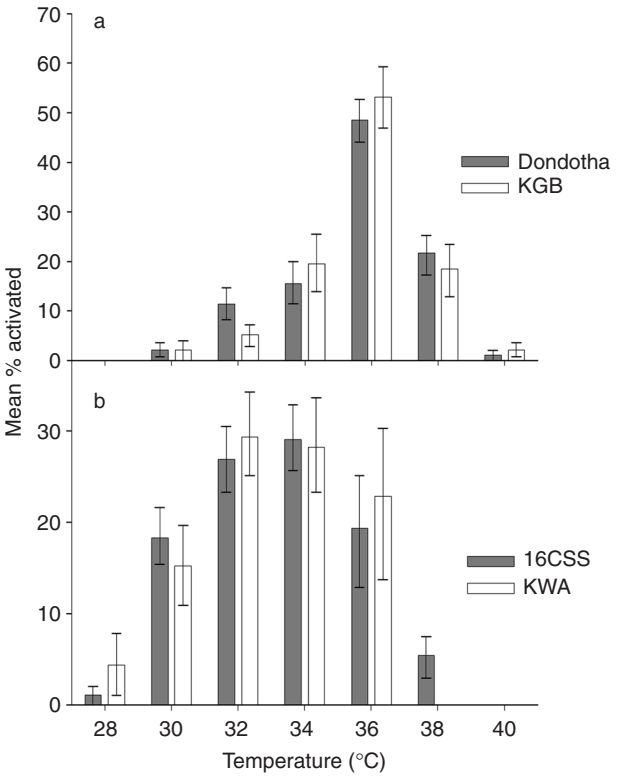

Fig. 3. Temperature activation profiles for 25°C-reared, 3–6 day unfed adults of *Anopheles arabiensis* strains (a) *A. gambiae* s.s. strains (b). Error bars represent 1 standard error.

Multivariate analysis revealed that all the treatment factors (feeding status, age and species) were significant predictors of the activation response ($P < 0.001$).

In the control trials only 3.6% (17/466) of mosquitoes left the holding tube after 3 h at constant 28°C, and only 5.4% (25/459) were activated by either of the step-wise temperature changes (24–26°C and 26–28°C). These findings are clearly different to the results under bioassay conditions (step-wise temperature changes between 28 and 40°C),

Table 1. Comparisons of mean activation temperatures for *Anopheles gambiae* s.s. KWA and *A. arabiensis* KGB for all treatments.

Adult status	*A. gambiae* s.s.			*A. arabiensis*			*P*
	n	Mean temperature (°C)	95% CI	*n*	Mean temperature (°C)	95% CI	
Age							
< 24 h	91	34.9	34.4–35.3	94	35.5	35.0–36.0	0.04
3–6 days*	92	33.0	32.5–33.5	92	35.7	35.4–36.1	<0.001
⩾ 14 days	86	32.4	31.9–32.8	89	33.7	33.3–34.9	<0.001
Feeding status							
Unfed*	92	33.0	32.5–33.5	92	35.7	35.4–36.1	<0.001
Blood-fed	85	36.1	35.6–36.5	80	36.8	36.5–37.2	<0.001

*Drawn from identical data. 25°C-reared, 3- to 6-day-old unfed female mosquitoes were the standard to which all other conditions were compared.

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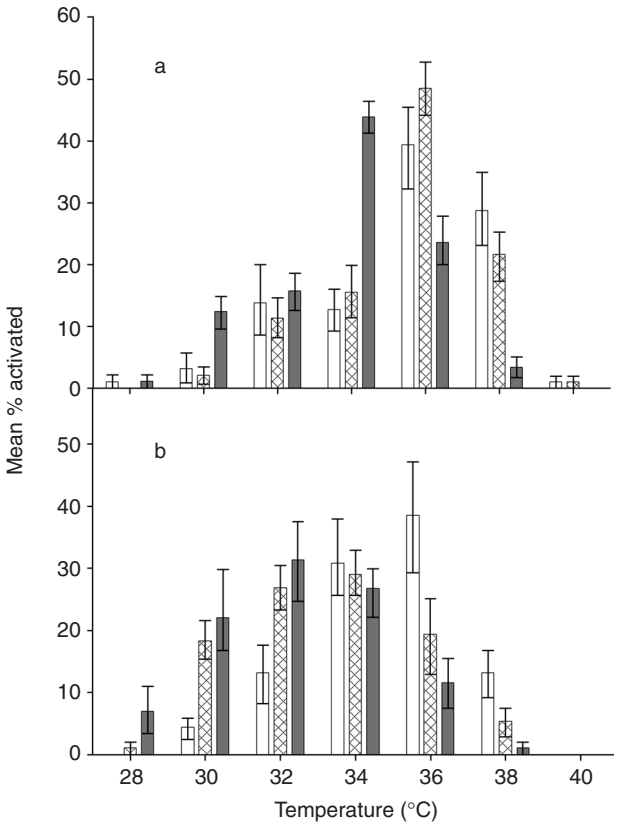

Fig. 4. Temperature activation profiles at three adult ages (□, ≤ 24 h; ⊠, 3–6 days; ■, 14–17 days) for *Anopheles arabiensis* KGB (a) and *A. gambiae* s.s. KWA (b). Error bars represent 1 standard error.

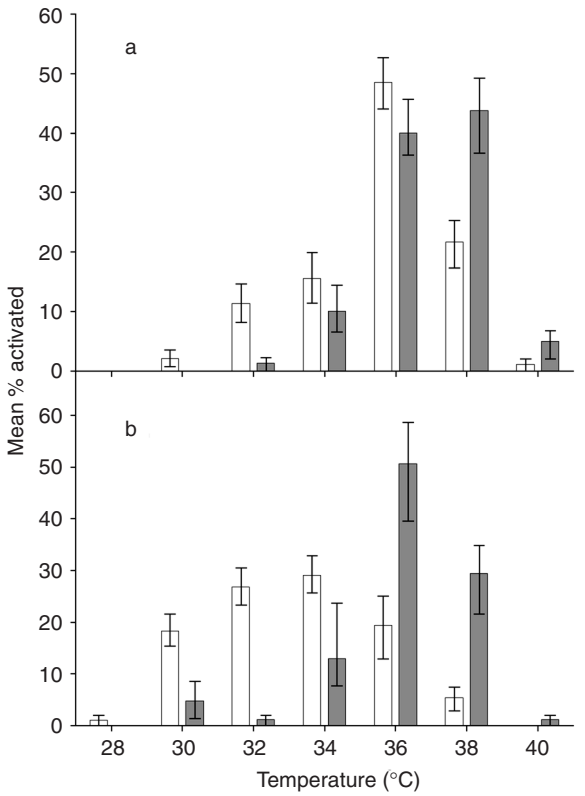

Fig. 5. Temperature activation profiles for unfed (□) and blood-fed (■) *Anopheles arabiensis* KGB (a) and *A. gambiae* s.s. KWA (b) Error bars represent 1 standard error.

where 100% of the mosquitoes capable of leaving the holding tube had done so within 3 h.

Observations of mosquitoes in the holding tube indicated that when one mosquito left the tube it did not cause others to leave at the same time. This is also reflected in the normal distributions seen in figs 3–5. If movement of the first mosquito had activated others, one would expect to see positively skewed distributions. Analysis of the movement of the first mosquito in each test is also consistent with our main findings. There was a significant interspecific difference between the temperatures at which the first mosquitoes were activated (*A. arabiensis* = 32°C, IQR = 32–34°C; *A. gambiae* s.s. = 30°C, IQR = 30–30°C, $Z = -4.3$, $P < 0.001$), but again there were no intraspecific differences (*A. gambiae* s.s. KWA median activation temperature = 30°C, interquartile range = 29.5–32°C; 16CSS = 30°C, IQR = 30–30°C, $Z = -0.9$, $P = 0.93$: *A. arabiensis* KGB = 33°C, IQR = 32–34°C; Dondotha = 32°C, IQR = 31.5–32.5°C, $Z = -1.3$, $P = 0.19$).

Survival at 40°C

Anopheles arabiensis exhibited a three-fold longer survival over *A. gambiae* s.s. at 40°C ($\chi^2_{M\text{-}H}$, adjusted for different time periods = 58.3, df = 5, $P < 0.001$, Mantel-Haenzel weighted odds ratio = 3.3, 95% C.I.s = 2.4–4.7, fig. 6). The equations for the lines describing the relation between probit survival (y) and time (t) were $y = -5.472 + 1.159 \times \ln t$ for *A. arabiensis* and $y = -6.441 + 1.534 \times \ln t$ for *A. gambiae*. The LT_{50} value derived from probit analysis was 112.2 (90.4–139.4) min for *A. arabiensis*, whilst for *A. gambiae* it was 66.7 (51.3–82.3) min.

Climate data

The KGB *A. arabiensis* strain studied was originally collected from the Kanyemba Zambesi Valley, Zimbabwe where *A. gambiae* s.s. is not present. At Kanyemba the mean daily maximum temperature is 32.3°C and temperatures in September to November regularly exceed 34°C (World Meteorological Organization, WMO, weather station 677670, data for September 1989 to October 1997). On the other hand, the *A. gambiae* s.s. strain KWA is derived from wild stock near Tanga, Tanzania. At Tanga, the mean daily maximum is more than 2°C lower (29°C) and maximum temperatures above 34°C have only been recorded on 18 days in 17 years (WMO weather station 638440, data for December 1978 to May 1995). World Meteorological Organization climate data is very limited for Dondotha, South Africa, origin of the Dondotha strain of *A. arabiensis*, and for Lagos, Nigeria, origin of the 16CSS strain of *A. gambiae* s.s., and was not evaluated.

Discussion

These findings show that adult *A. arabiensis* is better adapted to higher temperatures than *A. gambiae* s.s. *Anopheles arabiensis* tolerated higher temperatures before

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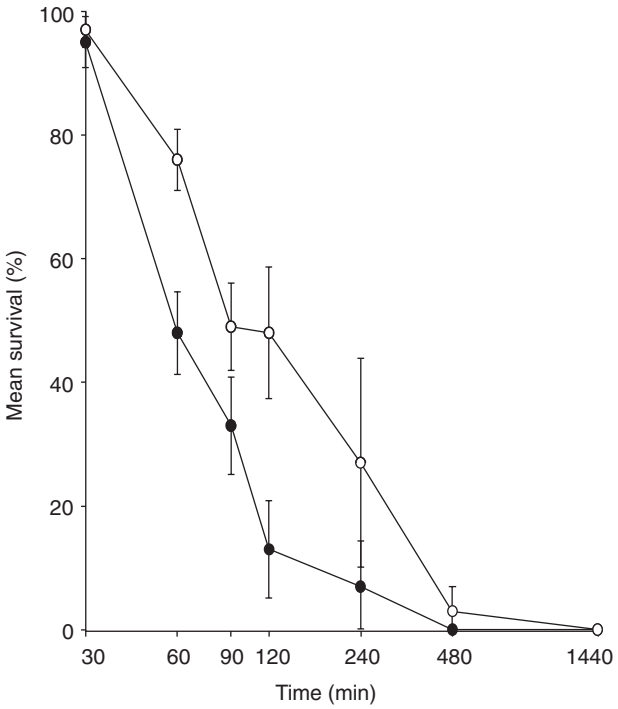

Fig. 6. Survival over time of *Anopheles arabiensis* (○) and *A. gambiae* s.s. (●) at 40°C and 30% relative humidity. Error bars represent 1 standard error.

moving and survived for longer at higher temperatures than did *A. gambiae* s.s. When holding tube temperature was progressively increased, unfed *A. arabiensis* consistently tolerated temperatures 2.7°C higher than *A. gambiae* s.s. Nearly all *A. arabiensis* treatment groups avoided temperatures at or above 36.0°C, in contrast to *A. gambiae* s.s. of which almost 60% were activated at or before 34.0°C. This difference in response between these closely-related species is impressive considering the relative homogenous nature under which these laboratory-adapted strains have been maintained for many years. The fact that this behaviour is the same for strains of the same species supports the notion that an innate difference in thermotolerance exists between the two species. This suggestion is supported by the evidence that *A. arabiensis* is more capable than *A. gambiae* s.s. of surviving periods of exposure to extreme temperature (40°C).

These findings reflect what is known of the geographic and temporal distribution of the two sibling species. *Anopheles arabiensis* is more common on the fringes of the complex's range, being endemic in the hotter and drier savanna areas of the African continent and steppes of the Arabian peninsula (Coluzzi *et al.*, 1979; Lindsay *et al.*, 1998; Coetzee *et al.*, 2000). In contrast, *A. gambiae* s.s. is more common in humid savanna and forest zones (White, 1974; Rishikesh *et al.*, 1985; Lindsay *et al.*, 1998; Rogers *et al.*, 2002). This situation is mirrored by the climate for the sites of origin of the studied strains; the KGB strain of *A. arabiensis* originates from a hotter region than that of the KWA strain of *A. gambiae* s.s. Where the two species are sympatric, they differ in their temporal distribution. Typically *A. arabiensis* is more common in the dry season, whilst *A. gambiae* s.s. predominates in the wet season (White *et al.*, 1972; Lindsay *et al.*, 1991).

Although both species exhibit a high degree of anthropophily throughout much of their distribution (Costantini *et al.*, 1999), *A. arabiensis* is more exophilic than *A. gambiae* s.s. (White, 1974; Coluzzi, 1984; Mnzava *et al.*, 1995; Githeko *et al.*, 1996). Because of this, *A. arabiensis* may experience higher temperatures more frequently than *A. gambiae* s.s., since mean maximum outdoor air temperatures are often 3–6°C greater than indoor temperatures in mud and thatch houses (De Meillon, 1934). Thus one would expect *A. arabiensis* to be better adapted to higher temperatures than *A. gambiae* s.s.. The endophilic nature of *A. gambiae* s.s. protects the mosquito from the highly variable and more extreme external climate. The high endophily of this species may reflect its evolutionary past since it is thought to have originally developed from a forest dweller (Coluzzi *et al.*, 1985), adapted for a more benign and less extreme climate . The proliferation of settled human communities in Africa over the last 7000 years (Roberts, 1998) created an extremely favourable environment for *A. gambiae* s.s., namely a reliable food source and a stable thermal environment inside houses that resembled the forest climate (Costantini *et al.*, 1999). *Anopheles arabiensis*, by contrast, is more catholic in its feeding habits, taking bloodmeals from a range of animals. This flexibility in feeding preference may promote adaptation to the more extreme temperatures encountered outdoors.

Anopheles arabiensis and *A. gambiae* s.s. are able to detect and respond to increasing temperature by moving away from extreme heat. The fact that both species move away from temperatures several degrees lower than 40°C may seem premature, given that they are capable of surviving for short periods of time at such an extreme. However, at temperatures over 38°C, 96% of *A. gambiae* s.s. and 74% of *A. arabiensis* are knocked down and do not escape. The behavioural avoidance experiment tested a behaviour that mosquitoes are only likely to perform as a last resort – it is an escape mechanism. In nature this probably results in short distance flights to seek cooler spots, typically the shaded resting sites under vegetation outdoors or cool dark corners indoors. For *A. arabiensis*, being activated by rapidly rising temperatures outdoors will most likely expose the insect to an increased risk of desiccation exhaustion and higher predation risks. A higher thermal tolerance may therefore minimize risky shade-seeking behaviour in this species. For *A. gambiae* s.s., becoming active at lower temperatures than *A. arabiensis* is less of a problem, provided the insect does not stray outdoors where it is liable to experience much hotter conditions.

The ability to respond to microclimatic changes depends on the age of the insect and its stage of the gonotrophic cycle. Much higher temperatures were required to initiate activation in recently-emerged mosquitoes than in older ones. Higher temperature tolerance in young adults is common in Diptera and has been demonstrated for blowflies (Davison, 1969) and *Drosophila* (Bowler & Hollingsworth, 1966) amongst others. In mosquitoes it probably also occurs because there is little flight activity in the first few days after eclosion (Gillett, 1971). Eclosion is an energy-expensive process, and rest after emergence is necessary to allow for the hardening of the cuticle (Gullan & Cranston, 2000).

Blood-fed mosquitoes were the least responsive to increasing temperature. Whilst only 1.6% of unfed

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mosquitoes were knocked down as temperatures rose, 15.5% of blood-fed mosquitoes failed to escape and were knocked down. The reluctance of blood-fed mosquitoes to fly, even when temperatures became lethal, reflects the behaviour of this feeding stage in the wild. A blood-fed mosquito is not only typically 80–200% heavier when fed (Clements, 1992), its energy is also largely invested in utilizing the blood to produce eggs. For *A. gambiae* s.s., remaining indoors after a bloodmeal may reduce the exposure to attack by predators, parasites and parasitoids. Blood-fed mosquitoes are more visible and slower flying, increasing the likelihood of being preyed upon or killed by their host. Muirhead-Thompson (1951) found similar trends in temperature sensitivity in *Anopheles minimus* Theobald. Female temperature avoidance was most pronounced in hungry females (which avoid temperatures above 25°C), less strong in blood-feds (above 30°C) and least strong in newly emerged females (above 32°C).

This simple laboratory study illustrates pronounced differences in the way two closely related species of mosquito respond to increasing temperature. It is clearly not temperature alone that affects the behaviour or survival of the mosquitoes in our experiments or indeed in nature. As temperature rises, relative humidity declines and the drying capacity of the air increases. Thus the responses of the mosquitoes to rising temperature should be seen as a response to the risk of desiccation, as well as temperature.

Although these species have been reared under similar laboratory conditions for many generations, their responses to increasing temperature reflect the different environments these insects have adapted to in the wild. Thus our study helps confirm that the geographical and temporal distribution of *A. arabiensis* and *A. gambiae* s.s. across Africa is characterized by species and stage-specific adaptations to climate at the microclimatic level.

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