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Influence of indoor microclimate and diet on survival of *Anopheles gambiae* s.s. (Diptera: Culicidae) in village house conditions in western Kenya

Bernard A. Okech[1,2,3,*], Louis C. Gouagna[1], Bart G.J. Knols[1], Ephantus W. Kabiru[3], Gerry F. Killeen[1], John C. Beier[5], Guiyun Yan[4] and John I. Githure[1]

[1]International Centre of Insect Physiology and Ecology (ICIPE), PO Box 30772, Nairobi, Kenya: [2]Kenya Medical Research Institute, PO Box 54840-00100, Nairobi, Kenya: [3]Department of Zoology, Kenyatta University, PO Box 43844, Nairobi, Kenya: [4]Department of Biological Sciences, State University of New York, Buffalo, NY 14260, USA: [5]Department of Epidemiology and Public Health, University of Miami School of Medicine, Miami, FL 33136, USA

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Abstract. The survival of female *Anopheles gambiae* s.s. mosquitoes inside two village house types (grass-thatched and iron-roofed) was studied in relation to diet and ambient indoor microclimatic conditions. Two batches of 20–30, 1-day-old laboratory-bred mosquitoes were maintained inside cages in the grass-thatched ($n = 2$) and iron-roofed ($n = 2$) houses and fed daily, one group on 10% glucose and the other on human blood. Throughout the experiments, indoor temperature and relative humidity of the houses were recorded, and mortality of mosquitoes monitored daily until all had died. The experiments were replicated thrice. There was no significant variation in the overall mean temperature ($P = 0.93$) or relative humidity profiles ($P = 0.099$) between the two house types, although the iron-roofed houses recorded higher temperature peaks. A Kaplan–Meier survival analysis showed that the mean survival times of mosquitoes were 8 and 10 days in the two grass-thatched huts and 7 and 10 days in the two iron-roof houses for mosquitoes feeding on blood and sugar meals, respectively. The mean survival times of mosquitoes maintained inside similar house types differed only due to diet. In the proportionality of hazards model (Cox regression), the dietary regimes significantly influenced the probability of survival ($P = 0.0001$), with mosquitoes surviving longer on sugar meals than on blood. Microclimatic factors inside houses also significantly influenced mosquito survival. Although higher peak temperatures were recorded in corrugated iron-roofed houses, the survival of the mosquitoes resting in them did not differ significantly from that in grass-thatched houses. However, the impact of these temperatures on the development of malaria parasites inside the vector needs to be investigated.

Key words: mosquito, indoor survival, house construction material, *Anopheles gambiae*, indoor microclimate, western Kenya

Résumé. La survie des femelles d'*Anopheles gambiae* s.s à l'intérieur de deux types de maisons villageoises (toiture en chaume ou en tôles) a été étudiée en relation avec l'alimentation et les conditions microclimatiques rencontrées à l'intérieur. Deux groupes

*Email: bokech@nairobi.mimcom.net

de 20–30 femelles obtenues en conditions de laboratoire, âgées d'1 jour, ont été maintenus en cages dans les maisons à toiture en chaumes ($n = 2$) et dans des maisons à toiture en tôles ($n = 2$); ils ont été nourris chaque jour, un groupe avec du glucose à 10% et l'autre avec du sang humain. Pendant toute la durée de l'expérience, les températures et les humidités relatives intérieures sont enregistrées et les moustiques sont observés chaque jour jusqu'à leur mort. L'expérience est répétée trois fois. Il n'y a pas de différence significative entre la température moyenne ($P = 0{,}93$) et l'humidité relative moyenne ($P = 0{,}099$) des deux types de maisons, bien que les maisons avec la toiture en tôles enregistrent des pics de températures plus élevées. Une analyse de survie selon la méthode de Kaplan-Meier montre que la durée moyenne de survie des moustiques est de 8 et 10 jours dans les deux maisons à toiture en chaume et de 7 et 10 jours dans les deux maisons à toiture en tôles pour les moustiques se nourrissant de sang et d'eau sucrée respectivement. La différence de durée moyenne de survie des moustiques maintenues dans des maisons identiques est due uniquement au régime alimentaire. L'analyse par régression de Cox montre que le régime alimentaire influence significativement la probabilité de survie ($P = 0{,}0001$), avec des moustiques survivants plus longtemps avec des repas de sucre qu'avec des repas de sang. Les facteurs microclimatiques à l'intérieur des maisons influencent également significativement la survie des moustiques. Bien que des températures plus élevées aient été enregistrées dans les maisons à toiture en tôles, la survie des moustiques n'est pas significativement différentes de celle observée dans les maisons à toiture en chaume. Cependant, l'influence de ces températures sur le développement du parasite de la malaria dans le vecteur doit être étudiée.

Mots clés: moustique, survie à l'intérieur, matériel de construction, *Anopheles gambiae*, microclimat intérieur, Ouest du Kenya

Introduction

Malaria transmission in much of sub-Saharan Africa has been sustained exclusively by the vectorial system that comprises *Anopheles gambiae* sensu lato and *An. funestus* sensu lato. Throughout most of Africa *Anopheles gambiae*, *An. arabiensis* and *An. funestus* are the three most important malaria vectors (Coluzzi, 1984, White, 1974). With the exception of *An. arabiensis*, which is sometimes zoophagic, *An. funestus* and *An. gambiae* are mainly anthropophagic, and derive 90% of their blood meals from human hosts (Beier, 1996). These mosquitoes tend to live indoors, close to their human hosts. In fact, they have been observed indoors during extended periods of dryness for up to 7 months, probably in a state of aestivation (Omer and Cloudsley-Thompson, 1970), even though predation may considerably reduce their indoor survival (Service, 1973). Behavioural characteristics of anthropophily and endophily in and around household environments (Githeko *et al.*, 1994; Smith *et al.*, 1995; Ribeiro *et al.*, 1996) predispose occupants to mosquito bites and leads to high malaria infection rates.

In addition to anthropophily, the design and construction of human dwellings has been found to be an important risk factor for mosquito bites and malaria infection (Lindsay and Snow, 1988; Gamage-Mendis *et al.*, 1991). Houses in much of rural tropical Africa are constructed from locally available materials such as grass or palm leaves, soil and cow dung. They are designed to minimize heat retention, and therefore many have huge eaves for improved air-flow. This feature results in increased mosquito populations indoors (Lindsay and Snow, 1988).

In addition to house design, human activities may influence the indoor microclimates in the houses. However, it is unknown what impact indoor microclimate may have on survival rates of resting *An. gambiae* mosquitoes in houses, and its influence in malaria transmission. For instance, the low sporozoite rates reported in Suba district in western Kenya (Shililu *et al.*, 2003) may be related to the low survival rates of mosquitoes in that area. In this study, we evaluated the influence of microclimate in two different village house designs on adult *An. gambiae* survival.

Materials and methods

Study area

The study was conducted from July to September 2001 and January to February 2002 in Mbita Division, Suba District, southwestern Kenya in villages surrounding ICIPE Mbita Point Field Station. The area has stable, endemic malaria that is vectored by *An. gambiae*, *An. arabiensis* Patton and *An. funestus* Giles (Mutero *et al.*, 1998; Minakawa *et al.*, 1999, 2001). Mosquito density is relatively high, and fluctuates with the two rainy seasons, from March–July and October–December, and consists predominantly of *An. gambiae* s.s.

Mud-walled houses predominate, of which 60% are grass-thatched and 25% have corrugated iron sheet roofing. Other houses, accounting for 15%, are made from wood, corrugated sheets, or stone walls with iron sheet or tile roofing.

Indoor microclimate monitoring

Four houses with earthen walls, two grass-thatched and two with corrugated iron sheet roofs, were selected for the study. The heads of the households were consulted and the purpose of the study was explained to them to obtain consent before experiments were started. Their indoor microclimatic conditions (temperature and humidity) were monitored using microclimate data recorders (Hobo® data loggers, Onset Computer Corporation, Bourne, Massachusetts, USA) throughout the duration of the experiments.

Mosquito survival studies

A colony of *An. gambiae* s.s. mosquitoes, originally collected at Njage, 70 km from Ifakara, south-west Tanzania in April 1996, were reared in an insectary according to standard rearing procedures following a method similar to that described by Benedict (1997). In order to avoid mortality due to overcrowding, batches of 20 and 30 female mosquitoes (1–2-day-old) were aspirated into $15 \times 15 \times 15$ cm cotton-meshed experimental cages. Within each house, two cages were suspended from the ceiling using a steel wire, at a height of 1.5 m above the ground, close to one side of the wall facing the rising sun. The first cm of the wire was coated with Tanglefoot®, a castor oil-based odourless formulation for protecting against crawling insects and ants. Of the two mosquito batches, one was allowed access to a cotton pad soaked in a 10% (w/v) glucose solution, while the second batch was offered the same human forearm from which blood meal was drawn every day. Mosquitoes fed on blood meals were not provided oviposition substrates as we observed that they could oviposit on dry paper towels. Their ability to oviposit in dry conditions meant that their survival was not being physiologically compromised. In the evening of each day, dead mosquitoes were counted and removed from the cages and the remaining mosquitoes were provided with blood meals. The experiments were replicated three times and data pooled per household.

Statistical analysis

For each house type, the climatic data were downloaded from the individual Hobo® data logger and analysis was carried out using SPSS version 11 and Microsoft Excel 2000. Comparison of the mean temperature and relative humidity between house types was performed using an analysis of variance (ANOVA). The differences in survival times of mosquitoes in the different house types were estimated by a Kaplan–Meier survival analysis. The proportionality of hazards model (Cox regression) was used to estimate the specific survival functions for experimental groups on the different diet types or in relation to the microclimatic conditions of the house types where mosquitoes were held.

To evaluate the possible impact of diet and indoor microclimatic conditions on malaria transmission potential, we estimated the minimum sporogonic incubation period in the grass-thatched and iron-roofed house types; it was found to be approximately 17 and 18 days, respectively using mean indoor temperatures of the two house types in standard models (Craig *et al.*, 1999).

Results

Indoor microclimate by house type

The temperature and humidity profiles of the two house types are shown in Fig. 1 while the mean indoor temperature and humidity for each house type are summarized in Table 1. Overall temperature and humidity conditions did not vary significantly (ANOVA: $F = 1.45$, $P = 0.93$; $F = 1.56$, $P = 0.099$, respectively) between the house types (Table 1) although the temperature of iron-roofed houses reached higher peaks than that of grass-thatched ones (Fig. 1). The humidity of the grass-thatched houses was relatively higher than that of the iron-roofed houses.

Mosquito survival

In the grass-thatched houses, the mean survival times of mosquitoes fed on blood and sugar meals were 8 and 10 days, respectively. The survival times of mosquitoes fed on blood and those fed on sugar differed significantly when compared using a pair-wise log rank test in the K–M survival analysis (Table 2). In the iron-roofed houses, mean survival times were 7 and 10 days for mosquitoes fed on blood and sugar, respectively. Again, pair-wise log-rank test in K–M analysis showed significant differences in the mean survival times ($P < 0.0001$) of mosquitoes fed on the two diets. Further, intra-house comparisons of dietary influence (mosquitoes fed sugar meals or blood meals alone) on mosquito survival by the house type revealed no significant differences within the grass thatched houses ($P = 0.124$) or within the iron-roofed houses ($P = 0.095$). Because microclimatic

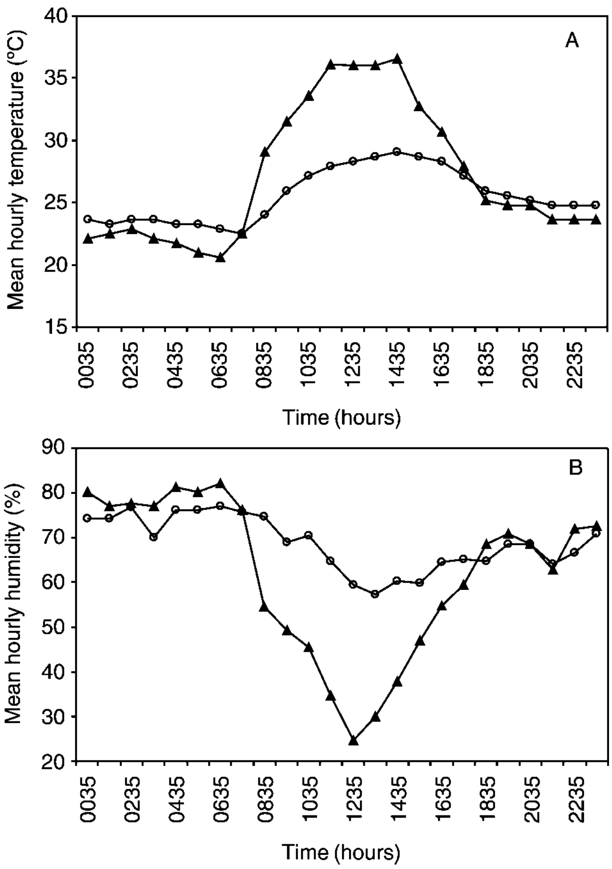

Fig. 1. Mean hourly temperature (A) and humidity (B) profiles in a grass-thatched (○) and corrugated iron-roofed (▲) houses measured over a 24-h period in Suba district, western Kenya

factors between the houses did not vary, further analysis was conducted with house ($n = 4$) as the main factor. Significant differences in the mean survival times of mosquitoes were observed in grass-thatched house no. 1 and iron-roofed house no. 2 between groups of mosquitoes fed on blood alone ($P = 0.019$) or those fed on sugar alone ($P = 0.0321$), suggesting variability in the indoor survival of mosquitoes between houses.

Because dietary factors may interact with microclimatic conditions to influence survival indoors, all these factors were included in a proportionality of hazards model (Cox regression) to identify the predictors of mosquito survival in the village houses. The diet (either sugar or blood) offered to the mosquitoes and prevailing temperature decreased the odds of mortality by 38 and 9.5%, respectively, while relative humidity increased the odds of mortality by 4.6% (Table 3).

Table 1. Mean temperature and relative humidity (± SEM, standard error of mean) of selected village huts in Suba district, western Kenya

Hut no.	Roof type	Temp. ± SEM	RH ± SEM
1	Thatch	23.80 ± 0.06	68.91 ± 0.45
2	Thatch	24.31 ± 0.04	66.10 ± 0.47
3	Iron	22.12 ± 0.57	68.31 ± 0.37
4	Iron	24.87 ± 0.08	64.10 ± 0.57

Discussion

This study has demonstrated that the type of diet mosquitoes feed on and house microclimatic conditions (temperature and humidity) are significant in determining the survival of mosquitoes in village houses in Suba district, western Kenya. The study has also shown that there are differences in survival of mosquitoes in houses constructed with different roofing materials. The variable nature of temperature and humidity conditions observed inside the different village houses in this study may correlate with the observed variable mosquito survival between these houses. These findings make an important contribution to our understanding of mosquito survival, distribution of populations and even indoor malaria transmission (Lindsay and Snow, 1988; Githeko *et al.*, 1994; Smith *et al.*, 1995; Ribeiro *et al.*, 1996).

The study has also shown that mosquitoes survive longer on sugar meals (glucose) than on blood meals. Glucose solution is absorbed and utilized more quickly, thereby contributing to the energy requirements of the mosquito at a faster rate than would blood meals. Although some studies have shown that mosquitoes may take multiple blood meals to make up for the energetic shortfall in the absence of sugar feeding (Briegel and Horler, 1993), our recent studies have suggested that mosquitoes would senesce faster upon feeding on blood meals (Okech *et al.*, 2003). However, the interpretation of our mosquito survival estimates should be done with caution because they may not be representative of the situation in nature, since the mosquitoes were fed within the safety of a cage, away from the natural hazards associated with the search for nutritional resources. Furthermore, in nature, mosquitoes feed on a variety of sugar sources, some of which may have large oligosaccharides, which are difficult to break down, and other plant components that may affect survival. Other studies have also shown that wild-caught *An. gambiae* mosquitoes may exhibit restrictive sugar feeding, instead, feeding frequently on blood (Beier, 1996). However, the contribution of sugar meals or blood meals to the survival probability and distribution of the vector populations is still unclear.

The results of this study also suggest that mosquito population densities indoors may vary

Table 2. Median survival times of mosquitoes fed on two diets and held in two house types in Suba district, western Kenya. Log rank statistics show the differences in survival times between the mosquito groups fed on blood or glucose

	Mean survival time (days)			
House type (house no.)	Blood meal	Glucose	Log rank statistic	*P*-value
Grass-thatched 1 (1)	7 (5–9)	10 (9–11)	11.31	0.0008
Grass-thatched 2 (2)	8 (7–8)	10 (9–11)	14.88	0.0001
Iron-roofed 1 (3)	7 (6–8)	9 (8–9)	23.03	<0.0001
Iron-roofed 2 (4)	7 (7–8)	10 (9–11)	15.32	0.0001

Confidence intervals (C.I.) in parentheses.

between house types as a function of the prevailing indoor microclimates. The uneven terrain in the study area, and even differences in the household activities (e.g. cooking indoors, number of occupants) may have contributed to differences in microclimates within the houses, although these were not measured in our study. However, we cannot make broad generalizations about the influence of heterogeneities of indoor microclimates on mosquito survival, because this study focused on a small sample of houses representative of the predominant house types in the rural areas of Suba district distributed in an area of approximately 10 km^2. However, some authors have reported on the physiological temperature thresholds for *Anopheles* mosquito survival (Clements, 1963), and the range of temperatures observed in our study falls within these limits. Other studies have indicated that mosquito densities and infection status may vary between and among house types (Copeland, 1994) and may be influenced by the topography of the study area. More recent data collected in houses within Mbita division (E.M. Mathenge, personal communication) show a consistently high number of host-seeking *An. gambiae* mosquitoes in both iron-roofed and grass-thatched houses. Although the proximity to mosquito breeding sites may be a more important factor influencing indoor resting densities (Minakawa *et al.*, 2002), no correlations have been made between the indoor mosquito densities and microclimates. More detailed analysis is needed into how prevailing environmental climatic conditions, design and type of material used for house construction, as well as other factors such as indoor human activities or impediments to air flow, together determine indoor microclimates.

In conclusion, our results have demonstrated that the indoor survivorship of mosquitoes is largely influenced by the availability of food resources and that microclimatic factors may also contribute. However, it is not known whether these factors also affect mosquito distributions or govern resting site selection, and there is much scope for further study.

Table 3. Cox regression analysis of the combined influence of indoor environmental conditions and diets upon survival of mosquitoes

Variable	β	SE	DF	Prob.	Exp(β)
Diet[1]					
Blood[2]	0^2	0^2	n/a	n/a	n/a
Glucose	−0.470	0.087	1	$<0.0001^3$	0.625
Temperature	−0.100	0.020	1	<0.0001	0.905
Relative humidity	0.045	0.008	1	<0.0001	1.045

n/a, not applicable; β, estimable regression coefficient (a negative β value indicates increased survival compared to reference group); SE, standard error (Odds of mortality increase if Exp(β) is >1 while it decrease if <1).
[1] Categorical variables.
[2] Reference group.
[3] Significance in relation to the reference group as specified during contrast selection of the Cox regression model fitting.

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