The impact of variations in temperature on early *Plasmodium falciparum* development in *Anopheles stephensi*

B. H. NODEN, M. D. KENT *and* J. C. BEIER*

Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, The Johns Hopkins University, 615 N. Wolfe St, Baltimore, MD 21205, USA

SUMMARY

The effect of temperature on early *Plasmodium falciparum* development was examined in *Anopheles stephensi*. The rates of both ookinete development and bloodmeal digestion were lengthened as temperatures decreased from 27 to 21 °C. However, low temperatures (21–27 °C) did not significantly influence infection rates or densities of either ookinetes or oocysts. In contrast, high temperatures (30 and 32 °C) significantly impacted parasite densities and infection rates by interfering with developmental processes occurring between parasite fertilization and ookinete formation, especially during zygote and early ookinete maturation. This study demonstrates clearly that temperature affects the sporogonic development of *P. falciparum* in anophelines by altering the kinetics of ookinete maturation. These studies not only confirm the ookinete as the key development stage affecting the probability of vector infectivity, they provide new insights into the epidemiology of *P. falciparum* infections.

Key words: *Anopheles stephensi*, *Plasmodium falciparum*, sporogony, temperature.

INTRODUCTION

No single environmental factor influences the epidemiology of *Plasmodium falciparum* more than temperature (Boyd, 1949; Macdonald, 1957). While playing a significant role in the rate of bloodmeal digestion (Briegel & Lea, 1975) and the survival of anopheline vectors (Boyd, 1949), external temperature also affects the internal temperature of the poikilothermic mosquito thereby directly influencing the extrinsic incubation period of the malaria parasite (Boyd, 1949). Together, these factors contribute to the intensity of malaria parasite transmission. However, surprisingly little is known about the specific effect of temperature on early sporogonic development.

Early studies defined the thermal limits of *P. falciparum* development (18–32 °C) and reported the effect of temperature on exflagellation, oocyst infection rates, sporozoite presence in the salivary glands and sporozoite infectiousness (reviewed by Boyd, 1949). However, the development stage of *P. falciparum* most sensitive to temperature was not identified. Grassi in 1901 and Janesco in 1904 focused attention on the effect of cold temperature on early sporogony, but reported conflicting results

(reviewed by Mitzmain, 1917). While both observed that temperatures below 16 °C reduced oocyst densities in *P. falciparum*, Grassi claimed cold temperature influenced the fertilization process while Janesco concluded the ookinete–oocyst transition was most affected (Mitzmain, 1917). Subsequent studies using *P. vivax* (Stratman-Thomas, 1940), *P. relictum* (Chao & Ball, 1962; Ball & Chao, 1964) and *P. berghei* (Vanderberg & Yoeli, 1966) provided evidence that both high and low temperature affect early sporogony but no studies clarified the effects of temperature on discrete developmental processes.

The objective of this study was to evaluate the effect of temperature on *P. falciparum* within an anopheline vector, *A. stephensi*, define specific early sporogonic processes most affected by temperature, and highlight the impact of high temperature on early stages of parasite development.

MATERIALS AND METHODS

Anopheles stephensi (Dutch) mosquitoes were infected with gametocytes from *in vitro P. falciparum* cultures. *Plasmodium falciparum* NF54 strain, obtained from the Institute of Medical Parasitology in Nijmegen, The Netherlands, was cultured using modifications (Noden *et al.* 1994) of the method described by Ponnudurai *et al.* (1982).

* Present address: Department of Tropical Medicine, School of Public Health and Tropical Medicine, 1501 Canal Street, Tulane University Medical Center, New Orleans, LA 70112–2824, USA.

Constant temperature study

The initial constant temperature study was done to characterize the effect of 5 different temperatures on ookinete and oocyst development. Five cages containing similar numbers of *A. stephensi* were infected with *P. falciparum* gametocytes by membrane feeder as described by Ponnudurai *et al.* (1982). Following the infection of the 5 cages and removal of unfed mosquitoes, each cohort of *A. stephensi* was placed randomly in one of 5 diurnal (i.e. 12 h light: 12 h dark) environmental chambers (Revco, Fisher, Pittsburgh, PA, USA) set at constant temperatures (21, 24, 27, 30 and 32 °C) with 70 % relative humidity. Mosquitoes were maintained with access to 3 % Karo (Best Foods, Englewood Cliffs, NJ, USA).

Mean macrogametocyte density per mosquito was estimated by multiplying mean blood volume and mean blood erythrocyte density (Vaughan, Noden & Beier, 1994) with the percentage of macrogametocytes in the bloodmeal. Using the protocol described by Vaughan, Noden & Beier (1992), estimates of macrogametocytaemia were obtained from Geimsa-stained thin smears taken directly from blood in the membrane-feeders from which the *A. stephensi* were infected. Ookinete densities were also obtained following the protocol described by Vaughan *et al.* (1992). Four separate time-course experiments monitored ookinete development over time at the 5 constant temperatures. For each group of mosquitoes, ookinetes were sampled every 6 h, beginning 18 h post-infection (p.i.) until 54 h p.i. Oocysts were examined (400 ×) after dissecting mosquito midguts 7–17 days p.i., depending on the temperature at which the mosquitoes were held, and staining with mercurochrome.

Eighteen-hour temperature study

An additional study focused on the effect of high temperature (30 °C) on the ookinete–oocyst transition. In 3 separate experiments, 1 cage of *A. stephensi* was infected with *P. falciparum* and placed at 27 °C. After 18 h at 27 °C, half the mosquitoes were chosen randomly and placed into another cage which was held at 30 °C until the mosquitoes were dissected for oocysts. The original cage from which the anophelines were chosen remained at 27 °C. Ookinete and oocyst sampling followed protocols described earlier.

Six-hour reciprocal temperature study

A reciprocal study monitored the effect of temperature on discrete processes occurring during the developmental transition between the gametocyte, zygote and ookinete stages. In 2 separate experiments, 3 cages of *A. stephensi* were infected with

P. falciparum using the same methods described above. One of the 3 cages of infected *A. stephensi* was randomly selected as the experimental control and was held at 27 °C for the entire experiment. The mosquitoes in the second and third cages were each divided into 2 cages of equal number (cage 2 = cohort A and B; cage 3 = cohort C and D). Cohort A was exposed to 30 °C for 6 h then moved to 27 °C for the remainder of the experiment in order to focus on the effect of temperature during fertilization and early zygote formation. Mosquitoes in Cohort B were placed at 27 °C for 6 h then moved to 30 °C for the rest of the experiment to ascertain the influence of temperature on zygote maturation and ookinete development. Cohorts C and D were treated likewise at 32 and 27 °C. Ookinete and oocyst sampling followed protocols described earlier.

Analysis

Cohort life-tables (Varley, Gradwell & Hassell, 1973; Southwood, 1978) were constructed to determine stage-specific mortality. The decrease in parasite density from one stage to the next (i.e. mortality) was expressed as a k value, which is the difference in population density, expressed as logarithms, between one lifestage and the next. Thus, $k-1 = \log_{10}$ macrogametocytes $- \log_{10}$ ookinete, $k-2 = \log_{10}$ ookinete $- \log_{10}$ oocyst and $K =$ total mortality from macrogametocyte to oocyst (i.e. $k-1+k-2$). In biological terms, $k-1$ represents the decrease in parasites due to macrogametocytes not being fertilized, effects of mosquito factors on zygote development and/or failing to differentiate to ookinetes and $k-2$ represents the decrease in parasite numbers due to ookinetes failing to penetrate the midgut and/or failing to mature oocysts (Vaughan *et al.* 1992, 1994).

Appropriate transformations (e.g. $\log_{10} x+1$) were performed for statistical comparison of the geometric means of macrogametocytes, ookinetes and oocysts (analysis of variance (ANOVA), SPSS, SPSS, Inc., Chicago, IL, USA). Chi-square (χ^2) analysis for statistical comparison of oocyst infection rates was performed using Epi-Info 6·0 (CDC, Atlanta, GA) and the reported values were Yates corrected.

The kinetics of ookinete development from the constant temperature study were used to calculate the 'degree-hours' necessary for *P. falciparum* ookinete development. 'Degree-hours' represent 'the number of degrees by which the mean temperature of the [hour] exceeds the lower threshold temperature for the development of the organism of the given species, i.e. the temperature below which its development cannot occur' (Detinova, 1962). The calculation of 'degree-hours' was accomplished by, first, extrapolating the lowest developmental threshold (t) from the linear relationship between peak ookinete development (1/hours) and tempera-
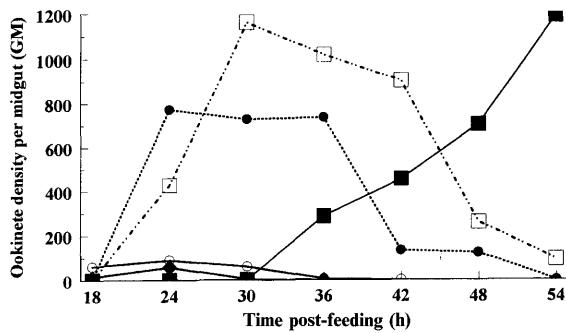
Fig. 1. The kinetics of *Plasmodium falciparum* ookinete development in *Anopheles stephensi* at 21 (■), 24 (□), 27 (●), 30 (○) and 32 °C (◆). The geometric means (GM) of ookinete densities per midgut were derived from 4 separate time-course experiments.

ture increasing from 21 to 27 °C. Next, the parasite development time in **days** (y) in the standard thermal constant degree-day accumulation formula [$K = y(d-t)$; y = developmental time in days, d = temperature, and t = developmental threshold] was replaced with the ookinete development time in **hours** (Davidson, 1944; Andrewartha & Birch, 1954). Only ookinete data obtained at 21, 24 and 27 °C were used to calculate 'degree-hours' because the ookinetes in mosquitoes at 30 and 32 °C experienced 'thermal death' and did not develop normally. Developmental data from these lethal temperatures, therefore, were spurious.

RESULTS

Constant temperature study

Ookinetes took longer to develop as temperatures decreased from 27 to 21 °C (Fig. 1). Ookinete densities in *A. stephensi* held at 27 and 24 °C peaked 24 and 30 h p.i., respectively, while ookinete densities appeared to peak 54 h p.i. in *A. stephensi* held at 21 °C, although no later observations were made. While ookinete densities appeared to peak 24 h p.i. in *A. stephensi* at 30 and 32 °C, the low number of ookinetes made it difficult to assess the kinetics of ookinete development. Additionally, oocyst development rates varied directly with temperature as oocysts were easily detected on days 6, 7, 9, 11, and 17 in *P. falciparum*-infected *A. stephensi* held at 32, 30, 27, 24 and 21 °C, respectively.

Ookinete development also corresponded to bloodmeal digestion. Regardless of temperature, the maximum number of ookinetes occurred during the peak of digestion when the peritrophic matrix was fully formed and waned as blood disappeared from the midgut. *Anopheles stephensi* held at 32, 30 and 27 °C completely digested their bloodmeals 30, 36, and 54 h p.i., respectively.

While temperature influenced the rates of development, neither ookinete (F = 1·4, D.F. = 2,9;

$P = 0·30$) nor oocyst (F = 1·3, D.F. = 2,9; $P = 0·32$) densities differed between *A. stephensi* held at 21, 24 and 27 °C (Table 1). Oocyst infection rates were also similar in *A. stephensi* at 24 °C ($\chi^2 = 0·3$, D.F. = 1, $P = 0·56$) and 21 °C ($\chi^2 = 0·9$, D.F. = 1, $P = 0·36$) when compared with *A. stephensi* held at 27 °C (Table 2). In contrast, high temperatures (30 and 32 °C) significantly altered ookinete (F = 15·2, D.F. = 4,14; $P = 0·0001$) and oocyst (F = 28·6, D.F. = 4,14; $P < 0·0001$) densities when compared with *A. stephensi* held between 21 and 27 °C (Table 1). Additionally, significant differences were observed in oocyst infection rates between *A. stephensi* held at 30 °C ($\chi^2 = 206·0$; D.F. = 1, $P < 0·0001$) and 32 °C ($\chi^2 = 193·0$; D.F. = 1; $P < 0·0001$) compared with those at constant 27 °C (Table 2).

Life-tables were constructed to indicate where mortality was most intense during the developmental transitions from macrogametocyte to oocyst (Table 1). In general, ookinete mortality rates ($k-2$) were higher than macrogametocyte mortality rates ($k-1$) in *A. stephensi* held at 21, 24 and 27 °C. The reverse was true in anophelines held at 30 and 32 °C. Overall, mortality rates (K) were almost 5-fold higher in anophelines held at 30 and 32 °C than in *A. stephensi* held at 21, 24 and 27 °C.

Under non-lethal conditions (21–27 °C), *P. falciparum* parasites required 258 ± 16 (S.D.) 'degree-hours' to develop to the ookinete stage. The lower developmental threshold temperature for *P. falciparum* ookinetes was estimated to be 16 °C.

Eighteen-hour temperature study

No differences were observed in ookinete numbers between *A. stephensi* held at constant 27 °C and those moved to 30 °C after 18 h at 27 °C (F = 1·6, D.F. = 1,28, $P = 0·22$) (Table 3). Additionally, oocyst infection rates did not differ between groups ($\chi^2 = 2·6$, D.F. = 1, $P = 0·11$). The mean oocyst infection rate in *A. stephensi* at constant 27 °C was 80 % (3 experiments, respectively: 75, 78 and 92 %) while mosquitoes moved to 30 °C after 18 h at 27 °C had a mean oocyst infection rate of 68 % (3 experiments, respectively: 69, 65 and 71 %). However, fewer oocysts developed on the midguts of anophelines moved to 30 °C after 18 h of development at 27 °C (F = 23·2, D.F. = 1,175, $P < 0·0001$) (Table 3).

Six-hour reciprocal temperature study

High temperatures significantly retarded *P. falciparum* development during the first 6 h of sporogonic development (Fig. 2 and Table 4). Both ookinete and oocyst densities were significantly lower in *A. stephensi* held at 30 and 32 °C for the first 6 h of *P. falciparum* development when compared with

Table 1. Stage-specific densities (95 % confidence limits) and mortalities (k-values) of *Plasmodium falciparum* developing in *Anopheles stephensi* at 21, 24, 27, 30 and 32 °C

(Data are derived from 4 experiments and expressed as geometric means of parasite densities/mosquito.)

Temperature (°C)	No. of infections	Macro-gametocytes	$k-1$*	Ookinetes	$k-2$†	Oocysts	K
21	4	49636 (18953–213105)	1·62 42-fold	1187 (429–3284)	2·30 200-fold	6·0 (2–13)	3·92 8318-fold
24	4	49636 (18953–213105)	1·63 43-fold	1160 (722–1864)	2·17 148-fold	7·8 (4–14)	3·80 6310-fold
27	4	49636 (18953–213105)	1·81 65-fold	772 (631–942)	2·24 174-fold	4·4 (1–10)	4·05 11220-fold
30	4	49636 (18953–213105)	2·75 562-fold	89 (15–504)	1·94 87-fold	0·03 (0–0·1)	4·69 48978-fold
32	3	42796 (18596–213105)	2·87 741-fold	58 (4–730)	1·76 58-fold	0·0	4·63 42658-fold

* $k-1 = \log_{10}$ (macrogametocytes) $- \log_{10}$ (ookinetes). Indicates macrogametocyte mortality.
† $k-2 = \log_{10}$ (ookinetes) $- \log_{10}$ (oocysts). Indicates ookinete mortality.

Table 2. Effect of 21, 24, 27, 30 and 32 °C on *Plasmodium falciparum* oocyst development in *Anopheles stephensi*

Temperature (°C)		Replicates				Total mean
		1	2	3	4	
21	Oocyst infection rate (%)	94	70	78	87	79
	Geometric mean (range)*	11·3 (0–91)	3·0 (0–21)	3·8 (0–49)	5·8 (0–49)	6·0
	N†	16	46	36	30	128
24	Oocyst infection rate (%)	96	83	74	84	86
	Geometric mean (range)	11·1 (0–82)	4·0 (0–30)	7·7 (0–69)	8·4 (0–44)	7·8
	N	26	69	52	31	178
27	Oocyst infection rate (%)	82	76	89	90	84
	Geometric mean (range)	3·0 (0–20)	1·7 (0–17)	7·2 (0–56)	5·7 (0–41)	4·4
	N	34	41	44	19	138
30	Oocyst infection rate (%)	6	0	2	0	2
	Geometric mean (range)	0·10 (0–3)	0·0	0·01 (0–1)	0·0	0·03
	N	48	27	49	41	165
32	Oocyst infection rate (%)	N.D.‡	0	0	0	0
	Geometric mean (range)	N.D.	0·0	0·0	0·0	0·0
	N	N.D.	72	49	13	134

* Range, range of oocysts/mosquito.
† N, number of midguts dissected.
‡ N.D., not determined.

Table 3. Effect of temperature on *Plasmodium falciparum* development in *Anopheles stephensi* after 18 h at 27 °C

(Data are derived from 3 experiments and expressed as geometric means of parasite densities/mosquito.)

Temperature first 18 h (°C)	Temperature after 18 h (°C)	Ookinetes (95 % CL)	$k-2$*	Oocysts (95 % CL)
27	27	1376 (1093–1732)	2·48 (302-fold)	4·5 (3·4–6·0)
27	30	1036 (674–1590)	2·81 (646-fold)	1·6 (1·1–2·1)

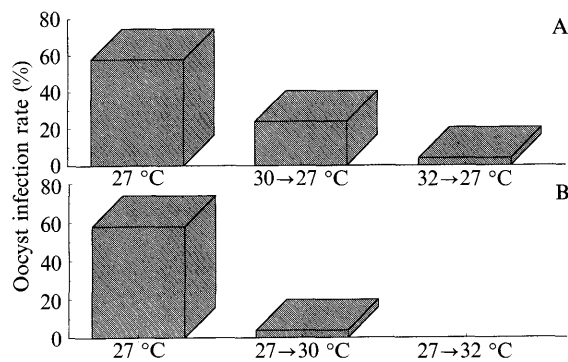* $k-2 = \log_{10}$ (ookinetes) $- \log_{10}$ (oocysts). Indicates ookinete mortality.

Fig. 2. The effect of 30 and 32 °C temperatures on the first 6 h of *Plasmodium falciparum* development in *Anopheles stephensi*. Oocyst infection rates are derived from 2 separate experiments. The experimental control were *P. falciparum*-infected *A. stephensi* held at constant 27 °C. (A) Oocyst infection rates from cages of infected anophelines held at 30 and 32 °C for 6 h p.i. then transferred to 27 °C for the remainder of the experiment. (B) Oocyst infection rates from cages of anophelines were held at 27 °C for the first 6 h p.i. then transferred to 30 and 32 °C for the remainder of the experiment.

those at constant 27 °C (Table 4). Similarly, oocyst infection rates (%) were lower in *A. stephensi* held at 30 °C (24%) ($\chi^2 = 13.2$, D.F. = 1, $P = 0.0003$) and 32 °C (4%) ($\chi^2 = 37.0$, D.F. = 1, $P < 0.0001$) for the first 6 h of *P. falciparum* development compared with those at constant 27 °C (56%) (Fig. 2).

High temperatures, also, impacted the development of *P. falciparum* after 6 h at 27 °C (Fig. 2 and Table 4). Significantly fewer ookinetes and oocysts were detected in *A. stephensi* held at 30 and 32 °C after 6 h at 27 °C when compared with anophelines at constant 27 °C (Table 4). Additionally, oocyst infection rates (%) were lower in *A. stephensi* held at 30 °C (3%) and 32 °C (0%) after 6 h at 27 °C when compared with those at constant 27 °C (56%) (Fig. 2).

DISCUSSION

This study demonstrates clearly the influence of temperature on early *P. falciparum* ookinete development in the anopheline midgut. The most dramatic effect was observed in *A. stephensi* held at 30 and 32 °C as significantly fewer parasites developed into mature ookinetes. Although results from the constant temperature study indicated initially that the macrogametocyte–zygote–ookinete transition ($k-1$) was most affected by high temperature, the number of parasites that survived the transition were so few that the actual impact of temperature on the ookinete-oocyst transition ($k-2$) was not obvious.

Subsequent studies, focusing on the effect of high temperature on the maturation of ookinetes during early formation, provided evidence that 30 °C

reduced the ability of ookinetes to develop into oocysts by more than 2-fold. This 2-fold reduction was considerably less than the effect of 30 °C on the macrogametocyte–zygote–ookinete transition observed initially (Table 1). Therefore, temperatures above 30 °C exerted the greatest influence on *P. falciparum* development during the first 18 h of development ($k-1$), thereby, confirming the observations of earlier studies using other *Plasmodium* species (Stratman-Thomas, 1940; Ball & Chao, 1964; Vanderberg & Yoeli, 1966).

In order to define the discrete developmental processes most affected by high temperature, the macrogametocyte–zygote–ookinete transition ($k-1$) was divided into 2 distinct time-periods. One part of the study monitored the effect of high temperature on the first 6 h of *P. falciparum* development, a period during which fertilization and genetic reorganization of early zygotes occurs (Sinden, 1984). Exflagellation, the process most sensitive to temperature (Ogwan'g *et al.* 1993), most likely occurred immediately after the infectious bloodmeal, while the unfed mosquitoes were being removed from the cages, and was not influenced by incubation temperatures. The reciprocal part of the 6 h temperature study monitored the effect of high temperature on zygote maturation and early ookinete formation (Sinden & Hartley, 1985). Even though results indicate that high temperature significantly impacts the entire macrogametocyte–zygote–ookinete transition, ookinete densities decreased 5 to 144-fold when parasites were exposed to 30 and 32 °C during the period of zygote maturation and early ookinete formation. High temperature, therefore, has the greatest impact on *P. falciparum* development between 6 and 18 h after the initiation of sporogonic development.

Six h after parasite development begins in the mosquito midgut, the developing zygotes undergo a complex series of genetic reorganization events as they begin to differentiate into retort-form ookinetes (Sinden & Hartley, 1985). At the same time, mosquitoes are synthesizing and beginning to secrete enzymes, such as trypsin (Briegel & Lea, 1975) and aminopeptidase (Billingsley & Hecker, 1991) which digest the bloodmeal. At 27 °C, the digestive enzymes do not appear to limit ookinete development as ookinete densities (Vaughan *et al.* 1992) and digestive enzyme levels (Billingsley & Hecker, 1991) peak at the same time. In fact, developing ookinetes may actually need trypsin to efficiently penetrate the peritrophic matrix (Shahabuddin & Kaslow, 1994; Shahabuddin, Criscio & Kaslow, 1995). However, these digestive enzymes have been reported to damage young retort-form ookinetes (Gass & Yeates, 1979).

The increase of temperature appears to disrupt the relationship between digestive enzymes and ookinete development observed at 27 °C. While high

Table 4. *The effect of temperature on the first 6 h of* Plasmodium falciparum *development in* Anopheles stephensi

(Data are derived from 2 experiments and expressed as geometric means of parasite densities/mosquito.*)

Temperature first 6 h (°C)	Temperature after 6 h (°C)	$k-1$†	Ookinetes (95 % CL)	$k-2$‡	Oocysts (95 % CL)	K
27	27	1·98 95-fold	872 (693–1098)	2·76 575-fold	1·50 (1·0–2·1)	4·74 54954-fold
30	27	2·32 210-fold	395 (192–810)	2·51 324-fold	0·22 (0·1–0·4)	4·83 67608-fold
32	27	2·62 414-fold	200 (116–345)	2·29 195-fold	0·03 (0–0·1)	4·91 81283-fold
27	30	2·77 585-fold	141 (36–550)	2·15 141-fold	0·01 (0–0·1)	4·92 81283-fold
27	32	4·14 13804-fold	5 (0·1–33)	0·70 5-fold	0·0	4·92 81283-fold

* Macrogametocyte densities/mosquito for all groups were 83115 (18357–376313).
† $k-1 = \log_{10}$ (macrogametocytes) $- \log_{10}$ (ookinetes). Indicates macrogametocyte mortality.
‡ $k-2 = \log_{10}$ (ookinetes) $- \log_{10}$ (oocysts). Indicates ookinete mortality.

temperatures increase the rate of digestive enzyme synthesis and secretion (Briegel & Lea, 1975), *P. falciparum* ookinete densities do not appear to peak before 24 h p.i. at 30 and 32 °C. The temperature-related increase of digestive enzymes during the period of early zygote development could easily cause the large reduction in ookinete numbers (Gass & Yeates, 1979). Furthermore, the small number of ookinetes observed 18–30 h p.i. were probably damaged earlier by digestive enzymes and unable to efficiently continue development into oocysts. High temperatures, therefore, appear to reduce successful ookinete formation by accelerating the kinetics of protease secretion.

In contrast, temperatures between 21 and 27 °C do not seem to have any influence on efficient parasite development in anophelines. While the rates of ookinete development and blood digestion slowed as temperatures decreased, no differences between infection rates or densities were detected between *P. falciparum*-infected *A. stephensi* at 21, 24 and 27 °C. The lower rate of blood digestion was accompanied by a temperature-related decrease in the secretion of digestive enzymes (Briegel & Lea, 1975). Temperatures between 21 and 27 °C may not alter successful parasite development because the synchronous relationship between ookinete development and digestive enzymes at 27 °C may also exist as temperatures decrease.

While providing unique glimpses into mosquito–parasite interactions, these studies reinforce the central importance of the ookinete in the malaria parasite's life-cycle. Earlier studies, using oocyst and sporozoite data, identified 32 °C as the upper temperature limit of *P. falciparum* (reviewed by Boyd, 1949; Macdonald, 1957). This study shows clearly that 32 °C specifically limits ookinete development. Additionally, the lowest developmental threshold for *P. falciparum* development was calculated at 16 °C using the kinetics of ookinete development (in hours) while earlier studies calculated the same threshold temperature using extrinsic incubation periods (in days) (reviewed by Detinova, 1962). These results indicate that both the highest and lowest temperature ranges of *P. falciparum* development in anophelines is determined by the successful maturation of the ookinete. Therefore, *P. falciparum* ookinetes are not only the key transitional stage affecting the probability of vector infectivity in the lab (Vaughan *et al.* 1992) and in the field (Beier *et al.* 1992), they also define the thermal limits for parasite development throughout the world. Such conclusions provide valuable information when attempting to understand the effect of climatic change on the transmission of malaria parasites in ecological zones and latitudes where *P. falciparum* transmission is usually limited by temperature (Loevinsohn, 1994; Martens, Rotmans & Niessen, 1994; Stone, 1995).

In conclusion, this study indicates that temperature impacts the sporogonic development of *P. falciparum* in anophelines by altering the kinetics of ookinete maturation. While temperatures between 21 and 27 °C do not appear to retard successful ookinete development, the strong limiting role of high temperature is demonstrated clearly. These studies not only confirm the ookinete as the key developmental stage affecting the probability of vector infectivity, they provide new insights into the epidemiology of *P. falciparum* infections.

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