The effects of natural *Plasmodium falciparum* infection on the fecundity and mortality of *Anopheles gambiae s. l.* in north east Tanzania

J. C. HOGG† *and* H. HURD*

Centre for Applied Entomology and Parasitology, Department of Biological Sciences, Keele University, Keele, Staffs ST5 5BG, UK

(*Received 23 July 1996; revised 30 September 1996; accepted 1 October 1996*)

SUMMARY

Rodent and avian malaria parasites have been reported to have an adverse affect upon the reproductive fitness of mosquitoes. In order to determine whether fecundity reduction occurs in *Anopheles gambiae s. l.* infected with human malaria a study of wild-caught mosquitoes was undertaken in the Muheza district of north east Tanzania. Fully engorged, indoor resting females were collected daily for 4 months and maintained for 5 days. A sporozoite rate of 11·5% was detected for the whole collection and of those females alive on day 6 an additional 17·5% were infected with oocysts alone. Oocyst, but not sporozoite, infection resulted in a 17·5% reduction in egg production. Fecundity reduction was not caused by a reduction in bloodmeal size in infected females and no size difference was detected between oocyst-infected and uninfected females although sporozoite-positive females were significantly larger. Comparisons in parity between uninfected and infected groups indicate that infection does not affect survival beyond the first gonotrophic cycle as no changes in survivorship occurred as a result of sporozoite infection.

Key words: *Plasmodium*, *Anopheles gambiae*, egg production, mortality, oocysts.

INTRODUCTION

The few studies that have been made of mosquitoes infected with *Plasmodium* have demonstrated several changes in vector behaviour and physiology, some of which have adverse effects upon blood feeding, fecundity and longevity (reviewed by Maier, Becker-Feldman & Seitz, 1987; Hurd, Hogg & Renshaw, 1995). We have described the deleterious effects of malaria infection on the fecundity of anopheline mosquitoes using the *Plasmodium yoelii nigeriensis*/*Anopheles stephensi* association as a laboratory model (Hogg & Hurd, 1995*a*). Compared with uninfected mosquitoes infected individuals in this model produced between 18 and 26% fewer eggs at both high and low oocyst burdens over 3 consecutive gonotrophic cycles post-infection (e.g. Hogg & Hurd, 1995*b*). The low oocyst burdens were comparable to those found in wild mosquito infections, raising the possibility that natural *Plasmodium*/*Anopheles* associations may also reduce mosquito fecundity.

In order to determine whether fecundity reduction did occur as a result of *Plasmodium* infections of wild-caught *A. gambiae sensu lato* we undertook a study in Tanzania. The investigation was designed to replicate our previous laboratory studies as far as possible, in that confounding influences upon fecundity such as mosquito size and bloodmeal size were taken into account (discussed by Hurd *et al.* 1995).

* Corresponding author. Tel: 01782 583034. Fax: 01782 63007. email: h.hurd@keele.ac.uk
† Present address: Department of Biological Sciences, University College North Wales, Bangor, Gwynedd, N. Wales.

MATERIALS AND METHODS

Study area

Field work was conducted in the village of Tengeni (38° 46′ E, 5° 11′ S) located 4 km west of Muheza (40 km from the coast) in the Muheza district of north east Tanzania. This region experiences 2 rainy seasons per year, the long rains between April and June and the short rains which usually occur in November or December. In addition to the major rains there is sometimes an additional week of rain at the start of August that increases the number of temporary water bodies during this month.

In this area, *P. falciparum* is transmitted by members of the *A. gambiae* species complex. Peak mosquito numbers coincide with the rains and transmission, although continuous, is most intense at these times (Mnzava & Kilama, 1986). The *A. gambiae s. l.* complex in this area has been reported to consist of over 90% *A. gambiae s. s.*, the remainder being *A. arabiensis* (Mnzava & Kilama, 1986). However, the ratio of sibling species may vary seasonally (White, Magayuka & Boreman, 1972). *A.*

https://doi.org/10.1017/S0031182096008542 Published online by Cambridge University Press

funestus also acts as a vector for *P. falciparum* but was not included in this study of fecundity reduction. *P. falciparum* is the predominant human malaria in Tanzania although *P. malariae* is also transmitted (Kilimali & Mkufya, 1985).

Mosquito collection and processing

Collections were performed daily for 4 months from 5 June to 16 September 1995. This period encompassed the end of the long rains and the beginning of the dry season. The same 24 houses, located throughout the village, were sampled routinely. Blood-fed anopheline mosquitoes were hand collected from the insides of villagers' sleeping quarters, between 08.00 and 10.00 h, using torches and aspirators. Captured mosquitoes were then transported to the Ubwari laboratory (Muheza) in muslin cages.

Fully engorged *A. gambia s. l.* were selected from the daily collections. These mosquitoes were kept individually in plastic vials (2·5 × 9 cm) covered with a layer of gauze and cotton wool soaked in 10% sucrose upon which they were allowed to feed *ad libitum*. Mosquitoes were maintained for 5 days in a humidified (80%) insectary on a 12:12 h light-dark cycle with the temperature ranging between 22 and 27 °C. On day 6 all survivors were killed and examined.

Mortality assessment

Mosquitoes were examined daily and all dead mosquitoes were removed. Wing length, sporozoite infection, the amount of haematin excreted (if any) and number of days survived were determined and recorded. Oocyst burden and parity could not be assessed for mosquitoes dying spontaneously before day 6.

Estimation of bloodmeal size

Anopheline mosquitoes concentrate their bloodmeal during feeding by expelling a plasma-rich prediuretic fluid. Estimations of bloodmeal size are therefore usually based upon the amount of haemoglobin ingested (discussed by Hurd *et al.* 1995) but these determinations must be made within 12 h of feeding. Bloodmeal size was thus expressed in terms of the digested product of haemoglobin, haematin. Bloodmeal digestion was usually completed in 2 days and haematin was then excreted into the vial and could be seen as a reddish-brown deposit. Once the mosquito had been removed from the vial the amount of haematin excreted was determined by the method of Briegel (1980). The haematin was dissolved in 1·0 ml of 1% lithium carbonate and the absorbance measured at an O.D. of 395 nm. The amount of haematin excreted by each female was calculated by reference to bovine haematin standards (Hogg & Hurd, 1995*a*; Hurd *et al.* 1995).

Identification of infected mosquitoes

The abdomen of each female was dissected and the midgut examined for the presence of oocysts; oocyst burdens were recorded. Sporozoite infections were detected by testing for the presence of circumsporozoite protein in the thorax by enzyme-linked immunoabsorbent assay (ELISA) using the 2A10 monoclonal antibody to *P. falciparum* circumsporozoite (CS) protein, (kindly supplied by R. Wirtz, Walter Reed Army Medical Centre). The protocol described by Beier *et al.* (1987) was followed (Hogg, Thomson & Hurd, 1996). Positive results were then re-tested to obtain confirmation.

Determination of mosquito fecundity, age-grading and size

To ensure that eggs were retained for 5 days post-blood feeding, mosquitoes were not provided with an oviposition site. Each surviving female was dissected and the development of the terminal follicles determined according to Christophers' stages (Clements & Boocock, 1984). Mature, stage V eggs were counted. Some attempt to age-grade the females was made by examination of the follicles for dilatations (Polovodova, 1949). Ovaries were classified as parous when caught (2 or more dilatations per follicle = 2 + parous) or nulliparous when caught (maximum of 1 dilatation per follicle = 1 parous). Mosquitoes that had blood fed but not produced eggs were classified as pre-gravid (nulliparous).

Mosquito size

Size was expressed in terms of wing length. One wing was randomly selected from each mosquito, mounted on a slide, and its length measured from the tip, excluding fringe, to the distal end of the allula (Lyimo & Koella, 1992) using an ocular micrometer.

Statistical analysis

As a result of mortality, lack of haematin excretion by some females and dissection errors complete sets of data were not collected for each female and thus sample sizes vary for the different parameters measured. All data were tested for normality by the Anderson Darling test. One-way ANOVA and '*t*'-tests were used to make comparisons between the infected and uninfected groups for haematin excretion, egg production and wing length. In the latter case, because of the large sample sizes involved, the use of ANOVA was valid even though one data

https://doi.org/10.1017/S0031182096008542 Published online by Cambridge University Press

set was not normally distributed (Zar, 1996). The effects of parity on these variables was determined by '*t*'-tests. Differences in the pre-gravid rate were tested by performing Chi-square tests using Yates' modification if any values were below 5 (Clarke, 1980). Comparisons in percentage mortality between uninfected and sporozoite-infected mosquitoes and parity of uninfected and infected females were assessed by Chi-square analysis. All statistical analysis was performed using Minitab software.

RESULTS

Prevalence and intensity of infection

A total of 967 *Anopheles gambiae s. l.* were maintained during this 4 month study and 11·5% of these tested positive for sporozoites. Of the 627 mosquitoes alive after 5 days 17·5% contained oocysts alone. The majority of infected females only harboured 1 or 2 oocysts. A breakdown of prevalence data showed that sporozoite and oocyst rates peaked in July (see Tables 1 and 2).

Effect of infection on survivorship

The effect of oocyst infection and parity on daily mortality could not be determined due to the rapid deterioration of mosquitoes after death. Comparisons were therefore only made between sporozoite-negative and sporozoite-positive mosquitoes. These females would have been infected during a blood feed previous to the one which initiated the gonotrophic cycle under investigation as the extrinsic period has been estimated as just over 3 gonotrophic cycles in this area (Lines, Wilkes & Lyimo, 1991). Mortality was high; a total of 40·9% of the sporozoite-negative and 44·6% of the sporozoite-positive females died during the 5 days of insectary maintenance. Presence of sporozoites did not exert an effect on the probability of survival during the 5 days of holding ($\chi^2 = 2{\cdot}114$, D.F. = 1, $P > 0{\cdot}05$). Thus, neither the presence of maturing oocysts that produced sporozoites during the maintenance period nor previous sporozoite infections in the salivary gland affected mortality.

Comparison of egg production by infected and uninfected mosquitoes

Egg production data were normally distributed between individuals of all groups. Infected mosquitoes (oocysts and/or sporozoites) produced significantly fewer eggs ($83{\cdot}3 \pm 1{\cdot}3$, $n = 129$) than uninfected mosquitoes ($93{\cdot}6 \pm 1{\cdot}3$, $n = 391$) (Student's '*t*' test, $P = 0{\cdot}0002$). However, partitioning of the infected category into those with oocysts and those with only sporozoites and testing of the data by one-way ANOVA ($F = 7{\cdot}34$, $P < 0{\cdot}0001$) and Tukey's test showed that fecundity was only significantly reduced in oocyst infected mosquitoes (mean egg production $= 79{\cdot}47 \pm 2{\cdot}8$; $n = 81$). Sporozoite-positive mosquitoes that had no visible oocysts did not produce significantly fewer eggs during this gonotrophic cycle than uninfected individuals ($94{\cdot}8 \pm 4{\cdot}9$, $n = 35$) (see Table 3).

Mosquito size and infection

Wing lengths were normally distributed in the infected group of mosquitoes but not the uninfected group. Infected mosquitoes were significantly larger than uninfected mosquitoes (one-way ANOVA, $F = 3{\cdot}28$, $P = 0{\cdot}21$). Although Tukey's test failed to distinguish between oocyst and sporozoite-infected groups, pairwise comparisons using Student's '*t*'-test demonstrated that sporozoite-infected females have significantly larger wing lengths than the uninfected mosquitoes of all parity categories but there was no significant difference between the oocyst-infected females and these uninfected mosquitoes (see Table 4).

Effect of infection on bloodmeal size

Haematin excretion was normally distributed for all groups. One-way ANOVA showed that there was no significant difference in the amounts of haematin excreted by uninfected ($15{\cdot}96 \pm 0{\cdot}32$ mg, $n = 301$), oocyst infected ($16{\cdot}80 \pm 0{\cdot}60$ mg, $n = 94$) and those infected with sporozoites ($16{\cdot}53 \pm 1{\cdot}08$ mg, $n = 26$) ($F = 0{\cdot}65$, $P = 0{\cdot}625$).

Parity, wing length, bloodmeal size and egg production

Nulliparous females caught in this study were not significantly different in size from females maturing eggs (Table 4). However, '*t*'-test comparisons of the mean haematin excretion showed that the nulliparous mosquitoes had taken a significantly smaller bloodmeal ($10{\cdot}77 \pm 0{\cdot}99$ mg haematin, $n = 25$) than the parous mosquitoes ($15{\cdot}9 \pm 0{\cdot}32$ mg haematin, $n = 330$).

The size of females that had undergone more than 1 gonotrophic cycle did not differ from those that were 1 parous in this study (see Table 4). Egg production was, however, significantly greater in 1 than in 2+ parous uninfected mosquitoes ($P = 0{\cdot}0008$) but not in one compared with 2+ parous oocyst-infected mosquitoes ($P > 0{\cdot}05$) (Table 3).

Parity and infection status

Of the 452 mosquitoes that survived for 5 days and on which parity was determined, 6·4% were nulliparous. As expected, none of these mosquitoes was found to be infected with oocysts or to be positive for

Table 1. Prevalence and intensity of infection of *Anopheles gambiae s. l.* with oocysts but no sporozoites

(These figures represent fully engorged females alive after 5 days maintenance.)

Month	Number of oocysts per mosquito						Mean number of oocysts/mosquito	Total infected	Percentage oocyst +ve
	0	1	2	3	4	5+			
June	108	12	7	5	0	2	2·31±0·48	26	19·4
July	136	16	6	5	2	4	2·85±0·65	33	19·5
Aug.	182	13	13	1	3	2	2·16±0·29	32	14·95
Sept.	91	12	3	0	2	2	2·07±0·33	19	17·27
Total	517	53	29	11	7	10	2·35±0·25	110	17·54

Table 2. Prevalence of infection with sporozoites alone or sporozoites and oocysts of fully engorged *Anopheles gambiae*

Month	Cs protein −ve	Cs protein +ve	Cs protein +ve and oocyst* infected	Sporozoite rate (%)†
June	240	15	2	5·9
July	286	49	1	14·62
August	196	30	6	13·27
September	134	17	4	11·25
Total	856	111	13	11·47

* Oocysts could only be detected in those mosquitoes that survived the 5 day maintenance period.

† Calculation of sporozoite rates may include mosquitoes that do not have salivary gland infections.

Table 3. Mean egg production during the gonotrophic cycle following capture

(Combined data from all ages of mosquitoes are given as all parities, this includes mosquitoes for which parity could not be determined.)

Gonotrophic status	Mean egg batch size ± S.E.	
	Uninfected females (*n*)	Infected females (*n*)
Nulliparous†	0	0
1 parous***	98·5±2·3 (113)	77·62±3·51 (47)
2+ parous**	88·4±1·9 (176)	72·96±5·14 (26)
2+ parous with sporozoites and no oocysts	—	94·8±4·9 (35)
All parities***	93·6±1·3 (391)	83·3±1·3 (129)

*** $P > 0{\cdot}0001$; ** $P > 0{\cdot}01$.

† Nulliparous females appeared fully engorged but did not mature an egg batch (pre-gravids).

CS protein. Of the parous, uninfected mosquito category collected during the period of the study, 38·5% were 1 parous and 61·5% were 2+ parous thus, a large proportion of the population survived to produce more than 1 egg batch. Chi-squared comparisons showed no significant difference between the percentage of non-infected and infected females out of the total examined that were 1 or more than 1 parous.

DISCUSSION

Parasite prevalence is usually measured in terms of sporozoite rates in field studies of mosquitoes infected with *Plasmodium* spp. as this information contributes to assessment of entomological inoculation rate. Our observed sporozoite rates of 11·5% during this study period is considerably higher than those that have been reported previously for this

https://doi.org/10.1017/S0031182096008542 Published online by Cambridge University Press

Table 4. Mean wing length of infected and uninfected fully engorged females

Gonotrophic status	Wing length, uninfected mm ± S.E. (*n*)	Wing length, infected mm ± S.E. (*n*)
Nulliparous	3·013 ± 0·035 (29)	—
1 parous	2·978 ± 0·012 (116)	2·968 ± 0·027 (48)
2+ parous	2·980 ± 0·012 (185)	3·012 ± 0·030 (27)
2+ parous with sporozoites and no oocysts	—	3·043 ± 0·022 (47)*
All parities	2·979 ± 0·008 (458)*	2·978 ± 0·013 (188)

* Significantly different (Student's *t*-test, $P < 0{\cdot}05$).

area: *A. gambiae s. l.* 3·1 % (Gillies & Wilkes, 1965); *A. gambiae s. s.* 4·23 % and *A. arabiensis* 0·32 % (White *et al.* 1972) and *A. gambiae s. l.* 6·1 % (Lines *et al.* 1991). These figures support the observation of a trend towards increasing parasite infectivity in this area, which has been attributed to widespread chloroquine resistance by Lines *et al.* (1991). However, it is difficult to make any firm conclusions from so short a study period at the time of highest transmission and some caution must be applied to the interpretation of the ELISA; they may suggest higher sporozoite rates than salivary gland examination as CS protein present in the thorax will also be detected (Beier & Koros, 1991).

Sporozoite rate is a measure of the number of mosquitoes capable of infecting humans and gives an underestimate of the number of malaria-infected mosquitoes in the population, as we found a further 17·54 % infected with oocysts alone. Clearly many females do not survive long enough for the parasite to mature but infection could exert pathological effects on a considerable proportion of the population during the extrinsic period.

Factors which influence mosquito longevity, (measured in terms of number of gonotrophic cycles completed (parity)) will affect vectorial capacity. Some authors have suggested that mosquito size may, in turn, affect survival (e.g. Nasci, 1986). However, we found no difference in the size of mosquitoes of different parities, with the exception of sporozoite-infected mosquitoes which were significantly larger than all other groups and must have undergone at least 3 gonotrophic cycles. This observation is in contrast to data produced by Lyimo & Koella (1992) who found that sporozoite prevalence decreased in the largest mosquitoes. They speculated that large mosquitoes harboured larger oocyst burdens which, in turn, induced higher mortality.

We were not able to detect a difference in mortality between sporozoite positive and negative mosquitoes during the course of the experiment. However, as we were unable to determine whether dead mosquitoes harboured oocysts, it is possible that mosquitoes infected during the bloodmeal immediately prior to collection (classed as sporozoite negative) did suffer higher mortality. In our laboratory model most mortalities within infected groups with a high oocyst burden occurred during the first 48 h p.i. (Hogg & Hurd, 1995*a*) and *P. berghei* has also been shown to increase mortality of *A. stephensi* immediately after infection (Gad, Maier & Piekarski, 1979). However, these unnatural associations often result in very large parasite burdens. Comparisons of the proportion of mosquitoes in our collection that were 1 or 2+ parous suggested that, if both oocyst and sporozoite-infected females are included, there is no drop in the proportion of mosquitoes surviving more than 1 gonotrophic cycle.

Other field studies have attempted to discover whether there are any deleterious effects associated with natural *Plasmodium*/*Anopheles* associations. Studies of wild-caught *A. maculatus* infected with *P. falciparum* suggested an increased mortality of larger infected mosquitoes (Kittayapong *et al.* 1992). Lyimo & Koella (1992) suggested that high oocyst burdens in *A. gambiae s. l.* may increase the mortality of larger mosquitoes. However, field studies performed by Sinton & Shute (1938), Chege & Beier (1990), Robert, Verhave & Carnevale (1990) and Gamage-Mendis *et al.* (1993) all found no evidence of increased mortality of *P. falciparum*-infected anophelines in the wild.

To date, most attempts to assess pathology of *Plasmodium*-infected wild mosquito populations have been confined to investigating the effect of infection on mortality. However, studies of blood-feeding by naturally infected anophelines have shown that probing behaviour (Wekesa, Copeland & Mwangi, 1992), blood-feeding behaviour (Koella & Packer, 1996) and feeding time (Bockarie *et al.* 1996) are changed in sporozoite-infected females compared to non-infective individuals. In the laboratory, the effect of sporozoites in the salivary glands on probing behaviour and blood feeding time has been investigated in detail (e.g. Rossignol, Ribeiro & Spielman, 1984; Ribeiro, Rossignol & Spielman, 1985).

Although changes in probing behaviour may affect parasite transmission it is the quantity and quality of the bloodmeal that will have an impact upon

https://doi.org/10.1017/S0031182096008542 Published online by Cambridge University Press

fecundity. This field study has confirmed our laboratory findings (Hogg & Hurd 1995*a*, *b*) that malaria infection does not decrease the size of the bloodmeal available for development of an egg batch. In addition, it would appear that nulliparous females that became infected as a result of blood feeding just prior to collection, also ingested a bloodmeal of the same size as uninfected females undergoing their first gonotrophic cycle.

The experimental design used in this study did not enable us to assess the impact, if any, of infection on nulliparous females. Surprisingly, no mosquitoes that were fully engorged and remained pre-gravid were found to be infected. As the completion of a gonotrophic cycle is linked to bloodmeal size, the majority of pre-gravid females collected would not have been fully engorged and thus were not selected for study. Some females undergoing their first gonotrophic cycle may, however, have been infected during a previous, small, bloodmeal as many authors now accept that anophelines take varying numbers of bloodmeals per cycle in the wild (e.g. Briegel & Horler, 1993).

Our findings, that the presence of *Plasmodium* oocysts on the midguts of *A. gambiae s. l.* reduces the mean fecundity of these mosquitoes by 17·5%, were comparable to the degree of fecundity reduction observed in our laboratory, rodent malaria (Hogg & Hurd, 1995*b*). Here too, fecundity reduction cannot be attributed to small mosquito body size or bloodmeal size. It would appear that malaria-induced fecundity reduction is common to several species associations (reviewed by Hurd *et al*. 1995) and may be the result of complex physiological interactions between *Plasmodium* and the mechanisms controlling mosquito vitellogenesis (Hogg *et al*. 1996).

It is now clear that the reproductive fitness of individual mosquitoes is adversely affected by infection. This study also demonstrated that egg production declines with age for uninfected females although other workers have not found this to be the case (Gillies & Wilkes, 1965; Suleman, 1990, Clements, 1992).

It is uncertain whether this reduction in individual fitness would have an impact on vector population dynamics or select for refractoriness to the parasite in wild mosquito populations. The effect may fluctuate according to the season and have more impact at the end of the rains when the numbers of mosquitoes are low and the infected proportion is high. Lines *et al*. (1991) estimated that 21% of human bloodmeals are infectious in this area and suggested that the high sporozoite rate implies an absence of genetic refractoriness to infection. Information concerning the presence of inherent refractoriness in this wild population needs to be collected before we can assess the effect of *Plasmodium*-induced pathology on the vector population structure or speculate upon the possible imposition of selective pressure in favour of refractoriness.

The authors wish to thank the Medical Research Council for financial support. Grateful thanks are also given to Professor C. F. Curtis and Mr T. J. Wilkes for helpful discussions and advice, to field workers at the Ubwari laboratory for their assistance and to Professor Kilama and the Tanzanian National Institute for Medical Research for provision of facilities.

REFERENCES

BEIER, J. C. & KOROS, J. K. (1991). Anatomical dissemination of circumsporozoite protein in wild Afrotropical *Anopheles* affects malaria sporozoite rate determination by ELISA. *Medical and Veterinary Entomology* **5**, 81–85.

BEIER, J. C., PERKINS, P. V., WIRTZ, R. A., WHITMERE, R. E., MUGAMBI, M. & HOCKMEYER, W. T. (1987). Field evaluation of an enzyme-linked immunoabsorbant assay (ELISA) for *Plasmodium falciparum* detection in anopheline mosquitoes from Kenya. *American Journal of Tropical Medicine and Hygiene* **36**, 459–468.

BOCKARIE, M. J., ALEXANDER, N., BOCKARIE, F., IBAM, E., BARNISH, G. & ALPERS, M. (1996). The late biting habit of parous *Anopheles* mosquitoes and pre-bedtime exposure of humans to infective female mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 23–25.

BRIEGEL, H. (1980). Determination of uric acid and hematin in a single sample of excretia from blood fed insects. *Experimentia* **36**, 142.

BRIEGEL, H. & HORLER, E. (1993). Multiple blood meals as a reproductive strategy in *Anopheles* (Diptera: Culicidae). *Journal of Medical Entomology* **30**, 975–985.

CHEGE, G. M. M. & BEIER, J. C. (1990). Effect of *Plasmodium falciparum* on the survival of naturally infected Afrotropical *Anopheles* (Diptera: Culicidae). *Journal of Medical Entomology* **27**, 454–458.

CLARKE, G. M. (1980). *Statistics and Experimental Design*. 2nd edn. Edward Arnold, London.

CLEMENTS, A. N. (1992). *The Biology of Mosquitoes*, Vol. 1. Chapman and Hall, London.

CLEMENTS, A. N. & BOOCOCK, M. R. (1984). Ovarian development in mosquitoes; stages of growth and arrest and follicular resorption. *Physiological Entomology* **9**, 1–8.

GAD, A. M., MAIER, A. & PIEKARSKI, G. (1979). Pathology of *Anopheles stephensi* after infection with *Plasmodium bergei bergei*. 1. Mortality rate. *Zeitschrift für Parasitenkunde* **60**, 249–261.

GILLIES, M. T. & WILKES, T. J. (1965). A study of the age composition of populations of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in North Eastern Tanzania. *Bulletin of Entomological Research* **56**, 237–262.

GAMAGE-MENDIS, A. C., RAJARUNA, J., WEERASINGHE, S., MENDIS, C. & CARTER, R. (1993). Infectivity of *Plasmodium vivax* and *P. falciparum* to *Anopheles*

tessellatus; relationship between oocyst and sporozoite development. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 3–6.

HOGG, J. C. & HURD, H. (1995*a*). Malaria-induced reduction of fecundity during the first gonotrophic cycle of *Anopheles stephensi* mosquitoes. *Medical and Veterinary Entomology* **9**, 176–180.

HOGG, J. C. & HURD, H. (1995*b*). Effects of *Plasmodium yoelii nigeriensis* upon the fecundity of *Anopheles stephensi* over three consecutive gonotrophic cycles. *Parasitology* **111**, 555–562.

HOGG, J. C., THOMSON, M. & HURD, H. (1996). Comparative fecundity and associated factors for two sibling species of the *Anopheles gambiae* complex occurring sympatrically in the Gambia. *Medical and Veterinary Entomology*, (in the Press).

HURD, H., HOGG, J. C. & RENSHAW, M. (1995). Interactions between blood-feeding, fecundity and infection in mosquitoes. *Parasitology Today* **11**, 411–416.

KILIMALI, V. A. E. B. & MKUFYA, A. R. (1985). *In vivo* assessment of the sensitivity of *Plasmodium falciparum* to sulphadoxine/pyrimethamine combination (Fansidar) in six localities in Tanzania where chloroquine resistant *P. falciparum* has been detected. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 482–483.

KITTAYAPONG, P., EDMAN, J. D., HARRISON, B. A. & DELORME, D. R. (1992). Female body size, parity and malaria infection of *Anopheles maculatus* (Diptera: Culicidae) in peninsular Malaya. *Journal of Medical Entomology* **29**, 379–383.

KOELLA, J. C. & PACKER, M. J. (1996). Malaria parasites enhance blood-feeding of their naturally infected vector *Anopheles punctulatus*. *Parasitology* **113**, 105–109.

LINES, J. D., WILKES, T. J. & LYIMO, E. O. (1991). Human malaria infectiousness measured by age-specific sporozoite rates in *Anopheles gambiae* in Tanzania. *Parasitology* **102**, 167–177.

LYIMO, E. O. & KOELLA, J. C. (1992). Relationship between body size of adult *Anopheles gambiae s. l.* and infection with the malaria parasite *Plasmodium falciparum*. *Parasitology* **104**, 233–237.

MAIER, W. A., BECKER-FELDMAN, H. & SEITZ, H. M. (1987). Pathology in malaria infected mosquitoes. *Parasitology Today* **3**, 216–218.

MNZAVA, A. E. P. & KILAMA, W. L. (1986). Observations on the distribution of the *Anopheles gambiae* complex in Tanzania. *Acta Tropica* **43**, 227–282.

NASCI, R. S. (1986). Relationship between adult mosquito (Diptera: Culicidae) body size and parity in field populations. *Environmental Entomology* **15**, 874–876.

POLOVODOVA, V. P. (1994). The determination of the physiological age of female *Anopheles*, by the number of gonotrophic cycles completed (in Russian). *Meditsinskaya Parazitologiya Bolezni* (Moscow) **18**, 352–355.

RIBEIRO, J. M. C., ROSSIGNOL, P. A. & SPIELDMAN, A. (1985). *Aedes aegypti*: Model for blood finding strategy and prediction of parasite manipulation. *Experimental Parasitology* **60**, 118–132.

ROBERT, V., VERHAVE, J. P. & CARNEVALE, P. (1990). *Plasmodium falciparum* infection does not increase the precocious mortality rate of *Anopheles gambiae*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 346–347.

ROSSIGNOL, P. A., RIBEIRO, J. M. & SPIELMAN, A. (1984). Increased intradermal probing time in sporozoite-infected mosquitoes. *American Journal of Tropical Medicine and Hygiene* **33**, 17–20.

SINTON, J. A. & SHUTE, P. G. (1938). A report on the longevity of mosquitoes in relation to the transmission of malaria in nature. *Ministry of Health Reports on Public Health and Medicine* **85**, 45.

SULEMAN, M. (1990). Intraspecific variation in the reproductive capacity of *Anopheles stephensi* (Diptera: Culicidae). *Journal of Medical Entomology* **27**, 819–828.

WHITE, G. B., MAGAYUKA, S. A. & BOREHAM, P. F. L. (1972). Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Dipt., Culicidae): bionomics and vectorial activity of species A and species B at Segera, Tanzania. *Bulletin of Entomological Research* **62**, 295–317.

WEKESA, J. W., COPELAND, R. & MWANGI, R. W. (1992). Effect of *Plasmodium falciparum* on blood feeding behaviour of naturally infected *Anopheles* mosquitoes in western Kenya. *American Journal of Tropical Medicine and Hygiene* **47**, 484–488.

ZAR, J. H. (1996). *Biostatistical Analysis*, 3rd edn. Prentice-Hall, New Jersey.