Medical and Veterinary Entomology

Medical and Veterinary Entomology (2012) **26**, 263–270 doi: 10.1111/j.1365-2915.2011.00987.x

Feeding frequency and survival of *Anopheles gambiae* in a rice-growing area in Ghana

J. D. CHARLWOOD[1], E. V. TOMÁS[1], A. EGYIR-YAWSON[2], A. A. KAMPANGO[1] and R. J. PITTS[3]

[1]MOZDAN (Mozambican-Danish Rural Malaria Initiative), Morrumbene, Mozambique, [2]Biotechnology and Nuclear Agriculture Research Institute, Accra, Ghana and [3]Department of Biological Sciences, Vanderbilt University, Nashville, TN, U.S.A.

Abstract. Mortality rates, determined by dissection, of predominantly M form female *Anopheles gambiae* (Diptera: Culicidae) were estimated. Mosquitoes were collected in tent traps and light traps in an irrigation project village in Ghana in June and July 2010, when much of the area was flooded. Both M and S form larvae were collected from rice fields (74 of 80 specimens were M form). Adults were collected in equal proportions from the two traps (90 of 107 specimens from the light trap and 106 of 116 specimens from the tent trap were M form). During the study, collection numbers rose from 105 to 972 per night. A total of 1787 of the 15 431 *An. gambiae* collected were dissected. Of these, 953 (53%) were found to have taken their first bloodmeal, either as virgins or following mating. The age profiles of mosquitoes collected alive and dead, respectively, were similar. Eighteen of 2933 ($0.61 \pm 0.49\%$) specimens were found to be positive for sporozoites in an enzyme-linked immunosorbent assay (ELISA). Lagged cross correlations among the different age groups implied that the mosquitoes fed on days 2 and 4 following emergence prior to oviposition and every 2.65 ± 0.17 days thereafter. The best model to describe the observed population patterns implied a daily mortality of 84%. The results are discussed in relation to possible mosquito control measures for the village.

Key words. *Anopheles gambiae*, cross correlation, feeding frequency, Furvela tent trap, survival, Okyereko.

Introduction

Mosquito blood-feeding frequency and survival are two important factors influencing the transmission of malaria. The feeding behaviour of mosquitoes from emergence to first oviposition may vary according to a number of factors, giving rise to irregular feeding cycles in which initial feeds are not necessarily associated with egg maturation. *Anopheles gambiae* Giles, the principal vector of malaria in much of sub-Saharan Africa, may take a 'pre-gravid' bloodmeal shortly after emergence (Gillies, 1953, 1956; Charlwood *et al.*, 1997, 2003). Factors affecting pre-gravid feeding remain poorly understood, although juvenile hormone titre at the time of feeding (Noriega, 2004) and mating status may be important (Klowden, 1999). In the laboratory, nutritional status on emergence may determine whether such a feed is required (Takken *et al.*, 1998) and in some field populations a pre-gravid meal appears to be obligatory (Gillies & Wilkes, 1965; Charlwood *et al.*, 1997). In these populations nutritional status on emergence may, however, be important in determining whether such a feed is taken before or after mating (Charlwood *et al.*, 2003).

In the laboratory, post-teneral *An. gambiae* may continue to take pre-gravid feeds (Briegel & Hörler, 1993), but in the field they are generally gonotrophically concordant and each bloodmeal gives rise to a complete batch of eggs (Gillies & De Meillon, 1968). Feeding frequency in these mosquitoes depends on the time required to mature eggs (which is temperature-dependent) and the time taken by the female to

Correspondence: Jacques D. Charlwood, PO Box 8, MOZDAN, Morrumbene 1412, Mozambique. Tel.: + 258 825 149 984; E-mail: jdcharlwood@gmail.com

return to feed following oviposition. Factors affecting post-oviposition behaviour in malaria vectors, however, remain poorly studied or understood. Different species may have inherently different cycles and a delay after oviposition may be obligatory in some species but may occur rarely in others [e.g. *Anopheles triannulatus* and *Chagasia bonnea* (Diptera: Culicidae) in Brazil (Wilkes & Charlwood, 1979; Charlwood & Wilkes, 1981)]. In practice, populations are likely to consist of some individuals that blood feed immediately after ovipositing and others that delay blood feeding, and environmental factors may determine post-oviposition behaviour as much as, or more than, inherent behavioural or physiological factors. For example, both *Anopheles farauti* from Papua New Guinea and *Anopheles funestus* from Mozambique return to feed more rapidly in the presence of moonlight (Charlwood *et al.*, 1986; Birley & Charlwood, 1989; Kampango *et al*., 2010).

Making estimates of survival when feeding cycles are irregular requires data on the proportion of the population that takes different routes to the initial oviposition and the proportion that returns to feed without delay subsequent to ovipositing. These proportions can be established by examining the ovaries of female mosquitoes. Daily collections for a minimum of 10 days can produce significant cross correlations among different age categories of mosquitoes at lags equivalent to feeding frequency, assuming that sampling is not biased in favour of a particular age group (Lord & Baylis, 1999). We applied the technique to obtain estimates of feeding frequency and parous rates in a rapidly increasing population of *An. gambiae* from a village adjacent to an irrigated area used for rice cultivation in Ghana and used these to determine survival rates according to the methods of Garrett-Jones & Grab (1964), who describe six alternative rhythms of feeding and first oviposition (five of which are irregular), any of which might be found in a natural mosquito population. Garrett-Jones & Grab (1964) provided a series of graphs from which the proportion of the population surviving 1 day can be determined. Our results are discussed in the context of potential vector control techniques for the village.

Materials and methods

Description of study site

The Okyereko Irrigation Project (05°24.87′ N, 00°36.25′ W), located some 70 km to the west of Accra, was constructed in 1974 for sustainable rice agriculture (Dzodzomenyo *et al*., 1999; Okoye *et al*., 2005). The village of Okyereko, which consists of 110 relatively run-down cement houses, about 5 km from the coast, is bordered on two sides by extensive irrigated rice fields. The village population numbers approximately 700 residents, who are served by a clinic that is responsible for 10 000 people and is situated 1.5 km from the village. In a survey conducted in 2011, 30 of 50 (60%) women and eight of 30 (27%) men said that they had used a bednet the previous night. All but one of the 80 adults questioned reported having attended the clinic in the previous 6 months. Numerous small (but loud) sheep, goats and chickens roam throughout the village by both day and night. *Anopheles gambiae* is a common mosquito in the area. Reported proportions of M and S forms have varied from close to 100% M form (Dzodzomenyo *et al*., 1999; Charlwood *et al*., 2011) to 65% M form (Yawson *et al*., 2007). The collection and dissection methods used in this study were identical to those described for a study conducted in the village in 2009 (Charlwood *et al*., 2011). Specifically, a Furvela tent trap containing two hosts (JDC and EVT) was run on a nightly basis in the middle of the village from 18 June to 11 July 2010. At the start of the study, a Centers for Disease Control (CDC) light trap was also run in a bedroom in which the host slept under a mosquito bednet. Following the initial light trap collections, samples of host-seeking insects were restricted to the sentinel tent trap. Swarms of males were observed at dusk on a daily basis throughout the village. Samples of the swarms were collected by sweep net, as were pairs in copula when seen.

Dissection

Mosquitoes were dissected according to the schedule described by Charlwood *et al*. (2011). Insects were divided into the following categories:

1 First feeding

- virgin (*V*) mosquitoes with ovarioles at Stage I, spermatheca empty;
- unfed mosquitoes with sperm in the spermatheca and a mating plug (*Plug-unfed*), and
- nulliparous Stage I (*N-I*) mosquitoes with ovarioles at Stage I, with sperm in the spermatheca, but without a mating plug.

2 Second feeding

- mosquitoes with a mating plug and evidence of a previous bloodmeal in the stomach (*Plug-blood*), and
- nulliparous Stage II (*N-II*) mosquitoes with sperm in the spermatheca and yolk in the terminal ovariole.

3 Subsequent feeding

- parous mosquitoes with sacs (*Sac*) with some distension still present; these mosquitoes were considered to have oviposited on the night they were collected and thus to have a 2-day feeding cycle, and
- parous mosquitoes without sacs (*No-sac*), in which the sac from the previous oviposition had contracted, thereby indicating a delay between oviposition and returning to feed.

It was assumed that gonotrophic development (from blood feeding to becoming gravid) takes 2 days in Okyereko; hence mosquitoes with sacs were considered to have a 2-day feeding cycle and those without to have added an extra day (i.e. to have a 3-day cycle). Estimates of the population mean duration

of the feeding cycle (μ) in parous insects were therefore determined according to the proportions of *Sac* and *No-sac* mosquitoes in the collection where μ is the mean feeding frequency of parous insects in days:

$$\mu = [(n\ Sac \times 2) + (n\ No\text{-}sac \times 3)]/(n\ Sac + n\ No\text{-}sac) \quad (1)$$

The *V* : *Plug* ratio provided an estimate of mating success in newly emerged females, and the number of first-feeding insects with undeveloped ovaries provided an estimate of the pre-gravid rate. Based on the assumption that survival was independent of age and that all age classes were sampled equally, the parous rate was used to estimate survival.

In order to determine whether survival in the trap was affected by age, on 4 days mosquitoes that arrived at the laboratory dead and those that arrived alive were dissected separately. The abdomens of dead mosquitoes were punctured with needles to allow saline solution to enter and rehydrate the internal organs. These specimens were then left for 10–15 min, after which they could, with care, be dissected in the usual manner.

Selected samples were preserved in a solution of RNA Later (Ambion, Inc., Austin, TX, U.S.A.) + Triton X-100 (0.1% v/v). These were stored at 4 °C for the duration of the field collection period and then frozen at −20 °C until removal for polymerase chain reaction (PCR) analysis. Single fly PCRs were performed as follows. Heads of selected individuals were removed. Carcasses were crushed in 25 µL of a buffered solution (10 mM Tris pH 8.2, 1 mM EDTA, 25 mM NaCl, 200 µg/mL proteinase K) and incubated at 37 °C for 20 min. Samples were heated to 95 °C for 2 min to inactivate proteinase K and centrifuged briefly at 4 °C. One microlitre of each sample was used as a PCR template to determine M or S molecular form using the S200 X6.1 method as described by Santolamazza *et al.* (2008). Individual samples displaying the S form PCR amplicon were subjected to subsequent PCR analysis to clarify *An. gambiae* complex species as described by Scott *et al*. (1993).

The presence of circumsporozoite antigens of *Plasmodium falciparum* in whole mosquitoes was determined using the sandwich enzyme-linked immunosorbent assay (ELISA) according to Wirtz *et al*. (1987).

Environmental monitoring

Air temperatures were measured with a digital logger (Tinytag™; Gemini Data Loggers Ltd., Chichester, U.K.) that recorded every 30 min. However, during a number of evenings temperatures were recorded every minute.

Data analysis

Feeding intervals (in days) between successive age groups were determined by cross correlations at different lags using the statistical software MINITAB (Minitab, Inc., State College, PA, U.S.A.) according to methods described earlier (Charlwood *et al*., 1985; Holmes & Birley, 1987; Mutero & Birley, 1989; Hii *et al*., 1990).

Following Holmes & Birley (1987), cross correlations (R) were considered to be significant if greater than R:

$$R = 2/\sqrt{(\mathrm{d})} \quad (2)$$

where d = the number of observations (in our case, 23); hence $R = 0.417$.

Estimates of survival were determined based on the relative feeding frequencies of different age groups, according to the method described by Garrett-Jones & Grab (1964).

The regularity by which numbers of the different age groups increased was assessed by determining the Pearson correlation coefficient for a comparison of numbers collected by day of collection and the rank order of the numbers collected. Completely regular increases in numbers would be the same as the rank order and thus produce coefficients approaching 1. Irregular increases would produce lower correlation coefficients.

Results

Anopheles gambiae s.l. was the most common mosquito collected; it accounted for 90% of the 14 518 mosquitoes collected over 23 nights in the tent trap and 94% of the 1130 mosquitoes collected over 6 nights in the light trap. As in 2009 (Charlwood *et al*., 2011), the majority of the specimens identified to species and form were *An. gambiae* M form; however, a larger proportion of the individuals collected in 2010 were S form. Of 107 *An. gambiae* females collected in the light trap and identified to form, 17 were S form (16%). Similarly, 10 of 116 (9%) mosquitoes collected in the tent trap were S form. No statistical difference in the samples collected was observed (Fisher's exact test, $P = 0.105$) and 12% of the combined sample consisted of S form mosquitoes (S form, $n = 27$; M form, $n = 196$). A sample of *An. gambiae* collected from puddles in a fallow rice field showed a similar composition, with 8% of the sample comprising S form insects (S form, $n = 6$; M form, $n = 74$), whereas a sample collected from a puddle in the village was composed entirely of M form individuals ($n = 48$). Moreover, all of the individuals collected in the middle of the village by sweep net from swarms or as mating pairs and identified to molecular form were M form (110 males, eight females). Thus a limited amount of M form mating occurred within the village itself, but swarming sites for S form insects remained undiscovered. Twice the proportion of blood-fed and gravid females was caught in the light trap compared with the tent trap, but the other possible vector, *An. funestus* Giles, was not common in either trap and only 44 *An. funestus* were caught during the whole study period.

The most severe flooding of the previous 19 years occurred in the days following a day of heavy rain on 21 June 2010. This inundated much of the surrounding area and many of the rice fields close to the village. The water in the flooded fields took 10 days to disperse and the affected fields were not brought into production during the study. Unaffected fields

were in different phases of production. With the exception of a single evening, when there was sufficient wind to disrupt swarming, conditions after the rain were uniformly calm. Mean temperatures were 27.14 ± 2.13 °C indoors from 29 June to 3 July and 26.63 ± 3.66 °C outdoors during 3–12 July. Hence the observed changes in the population reflect rates under apparently 'ideal' conditions, rather than responses to environmental perturbation, as was the case in 2009 (Charlwood *et al.*, 2011). Despite the possible elimination of many potential breeding sites by the flooding, populations of *An. gambiae* increased 4.5-fold (from a mean of 163 per night in the first 3 nights of collection to a mean of 740 in the last 3 nights). Populations of *Anopheles pharoensis* increased 10-fold (from 1.33 to 13.3) during the study; populations of *Culex tritaeniorhynchus* (Diptera: Culicidae) declined (from 10.6 to 2.6) and those of *Culex quinquefasciatus* remained stable. Although the population of *An. pharoensis* continued to increase, that of *An. gambiae* declined in the last 3 days of collection. The increase in *An. pharoensis* was best described by an exponential function, whereas that of *An. gambiae*, excluding the final decline, was described by a linear function. In line with its status as a growing population, some *An. pharoensis* were multiparous (of fewer than 15 mosquitoes, dissected, two insects were three-parous and one was five-parous).

The age profiles of the samples of *An. gambiae* collected from the tent trap ($n = 1605$) and the light trap ($n = 182$) were similar ($\chi^2 = 1.161$, $P = 0.281$); most (54%) insects were taking their first feed. Overall, 75% of the first-time feeders were either virginal or had a mating plug and the remainder were *N-I* (Table 1).

On the 4 days during which live and dead insects were sorted and dissected separately, 2119 of the 3133 (68%) unfed females collected were alive at the time of collection. These proportions were similar for the other abdominal stages of *An. gambiae* and for *Cx. quinquefasciatus*. However, a greater proportion of *An. pharoensis* (63%) had died before collection ($\chi^2 = 54.01$, $P = 0.0001$). Other than an excess number of *N-I* among the live *An. gambiae* [Fisher's two-tailed test comparing (*V* + *Plug*) and *N-I* ($P = 0.0431$)], the 166 females collected when dead and dissected and the 206 dissected after live collection were similar in age (Table 2).

The observed rate of increase of *An. gambiae* in this study was lower than that described in 2009 (Charlwood *et al.*, 2011). The estimated rate of increase was higher in the first-feeding population than in the other age groups. The increase in the number of first-time feeders was also more regular than that observed in either second-time or subsequent feeders (Fig. 1). The Pearson correlation coefficients between rank order and sequence (date) of collection were 0.69 for first-time feeders, 0.38 for second-time feeders and 0.49 for subsequent feeders (Fig. 1). Among the first-feeding insects, cross correlations were highest in the *N-I* group ($r^2 = 0.65$), but were similar in the *V* ($r^2 = 0.54$) and *Plug* ($r^2 = 0.55$) groups.

Cross correlations between virgin and recently mated females were significant at a lag of 1 day ($r^2 = 0.51$), whereas that between (*V* + *Plug*) and *N-I* was significant at 1 and 2 days ($r^2 = 0.60$ and $r^2 = 0.50$, respectively). Significant cross correlations occurred at 4 days between (*V* + *Plug*) and *N-II* ($r^2 = 0.54$) and at 3 days between *N-I* and *N-II* ($r^2 = 0.46$). Cross-correlation coefficients between the number of parous mosquitoes and the total collected were lower than between parous and total collected less newly emerged (pre-gravid) insects. In the latter case, cross correlations were significant at lags of 1 and 2 days ($r^2 = 0.76$ and $r^2 = 0.71$, respectively).

The overall parous rate was 0.36 and the estimated duration of the mean feeding (oviposition) cycle, derived from Eqn (1), was 2.7 ± 0.2 days. Rates and estimates of feeding cycle were similar for light- and tent-trap collections and did not differ between live and dead mosquitoes dissected (2.6 ± 0.2 days vs. 2.7 ± 0.2 days; χ^2 test, $P = 0.197$). A significant cross correlation between *Sac* and *No-sac* mosquitoes occurred at a lag of 1 day ($r^2 = 0.50$). These results indicate that *An. gambiae* fed on days 2, 4, 7, 10 and 13... after emergence and oviposited for the first time between days 4 and 7. In other words, they followed cycle number 5 described by Garrett-Jones & Grab (1964). With such a feeding schedule and a parous rate of 0.36, the estimated daily survival rate obtained from the published curve is 0.84.

With the exception of one windy night, the mean proportion of the most recently emerged insects (i.e. *V* and *Plug*) with a mating plug was 0.63 ± 0.04 (Fig. 2). After the one evening during which windy conditions at sunset prevented males from swarming, only three of the nine newly emerged insects collected the following morning had mating plugs. Although this difference from the expected proportion does not quite achieve significance (Fisher's exact test, $P = 0.094$), this was the lowest proportion observed for any night (four times this number would normally have been collected). The following day, 18 of 21 newly emerged insects had mating plugs. This was the highest proportion (0.86) observed on any night.

Table 1. Age structure of the *Anopheles gambiae* collected in Okyereko, Ghana, during June and July 2010 and subsequently dissected.

Collection	Virgin, *n*	Plug, *n*	*N-I*, *n*	*N-II*, *n*	Parous, with sac, *n*	Parous, no-sac, *n*
Light trap	28	46	20	18	27	43
Tent trap	241	388	233	163	218	362
Total	269	434	253	181	245	405

N-I, nulliparous mosquitoes, Stage I; *N-II*, nulliparous mosquitoes, Stage II.

Table 2. Age structure of *Anopheles gambiae* found to be dead or alive at the time of collection in the tent trap in Okyereko, Ghana.

	Virgin, *n*	Plug, *n*	*N-I*, *n*	*N-II*, *n*	Sac, *n*	No-sac, *n*
Dead	25	48	25	15	23	30
Live	25	49	47	13	22	50
Total	50	97	72	28	45	80

N-I, nulliparous mosquitoes, Stage I; *N-II*, nulliparous mosquitoes, Stage II.

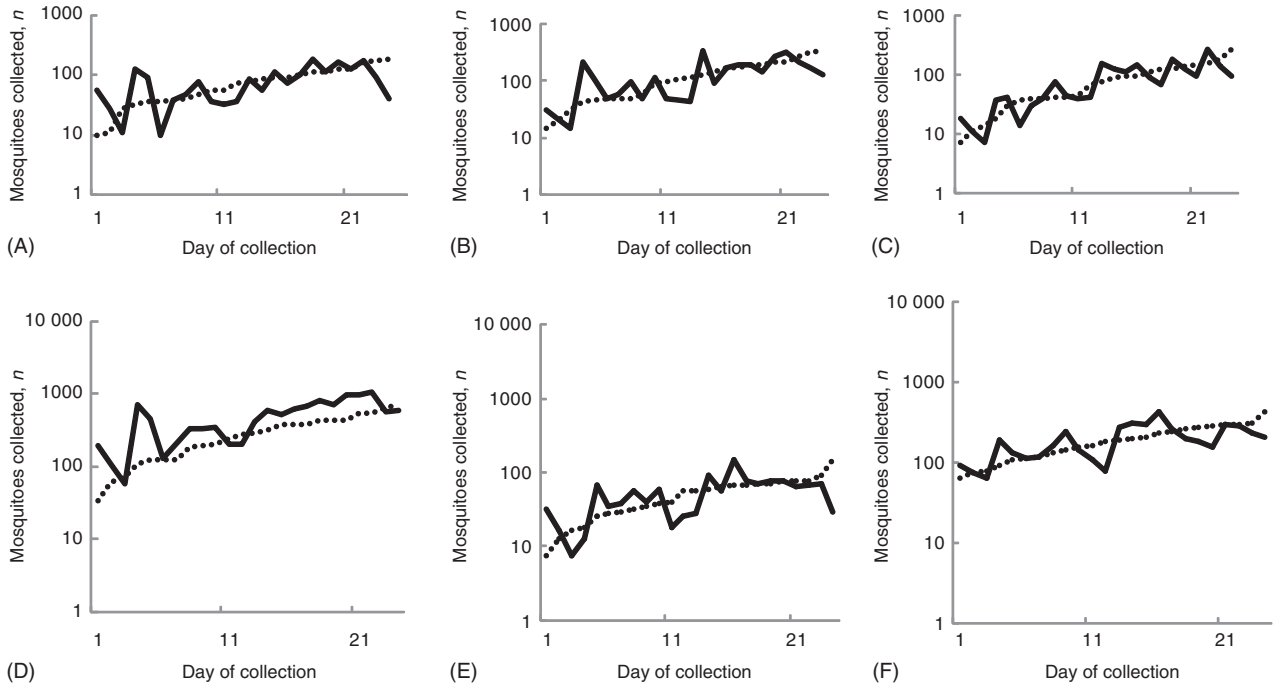

Fig. 1. Estimated numbers of *Anopheles gambiae* by gonotrophic age collected in a Furvela tent trap in the village of Okyereko, Ghana, during June and July 2010. (A) Virginal mosquitoes. (B) Mosquitoes with mating plug, unfed. (C) Nulliparous mosquitoes, Stage I. (D–F) Mosquitoes feeding for (D) the first, (E) the second and (F) a subsequent time.

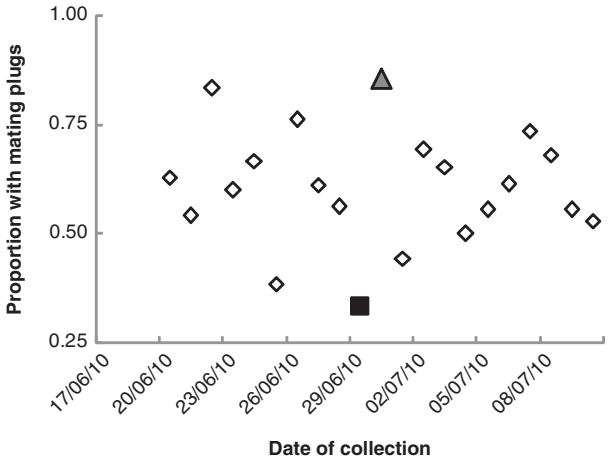

Fig. 2. Proportion of newly emerged *Anopheles gambiae* with mating plugs, by date of collection in Okyereko, Ghana, during June and July 2010. ■, collection after an evening during which wind disrupted swarming behaviour; ▲, collection during the following night.

Eighteen (0.61%) of the 2933 mosquitoes collected over a period of 10 days tested positive for circumsporozoite protein. The rate among tested insects varied considerably from day to day: no positive insects were collected on 6 of the 10 days. The overall mean sporozoite rate in the estimated 1435 parous mosquitoes included in the samples tested by ELISA was 1.25%, similar to that obtained in 2009.

Discussion

At the highest observed rate of transmission, one in 100 mosquitoes, or up to 10 insects per night, represents a vector of malaria and, despite the 'paddies paradox' (Ijumba & Lindsay, 2001), the disease remains a problem in Okyereko. Of the 103 malaria cases recorded in June 2010 at the local clinic, which serves over 10 000 people, 40 came from Okyereko (J. Wiafia, personal communication, 2010). The estimated daily survival rate of 84% was similar to estimates obtained for *Anopheles arabiensis* and S form *An. gambiae* from Tanzania (Gillies & Wilkes, 1965; Charlwood *et al.*, 1995). Given the feeding frequency observed in Okyereko, mosquitoes must survive for 13–16 days before they can transmit malaria. The shorter period assumes that the initial pre-gravid meal is infectious. Under such circumstances (and assuming density-independent survival), 10.4% of mosquitoes will survive long enough to be vectors. If the pre-gravid feed is not infectious, only 7.3% will survive the minimum time to be possible vectors. These proportions will decrease if age-specific mortality affects survival (Clements & Paterson, 1981). The low rates of infection observed in the present study probably reflect the fact that many of the mosquitoes had yet to reach the age at which they might transmit, rather than an especially low survival rate. Much of the evidence for age-specific mortality (in which the rate of mortality increases with age) comes from dissections to determine the number of gonotrophic cycles completed by the female mosquito. Dissections depend on an examination of follicles that always fail to develop eggs in any

cycle. This proportion, the so-called 'diagnostic index' (Hoc & Charlwood, 1990), decreases with age, increasing the likelihood of underestimation. Mark–release–recapture experiments tend towards age-independent estimates (exponential fits of population declines being as good as other fits) (Gillies, 1961) and, in a recent extensive study in an isolated oasis, although not mentioned by the authors, the survival of *Anopheles sergentii* determined both by dissection and recapture appeared to be independent of age (Gu *et al*., 2011). The size of the infectious reservoir and the frequency of blood feeding on non-human hosts will also influence transmission.

The rise in the *An. gambiae* population was lower than that observed in 2009 (Charlwood *et al.*, 2011) and lower than that of *An. arabiensis* in Tanzania (Takken *et al.*, 1995; Charlwood, 2003). The population increase was equally well described linearly as exponentially. Changes in the population of new recruits (first-time feeders) are influenced by the larval habitat, whereas factors affecting the adult environment affect changes in second and subsequent feeders. Rates of increase were higher in new recruits than in the population as a whole; the estimated numbers of parous mosquitoes began to decline even as the number of new recruits was rising. The reasons for this decline are unknown because environmental conditions were relatively constant during the study.

Although the *An. pharoensis* population started from a much smaller base, its rise in numbers was exponential. The number of *An. pharoensis* dissected was too small to allow an estimation of survival rates, but the observation of several multiparous insects implies that this species may also be a vector in Okyereko. Interestingly, in their study of filariasis in the village, Dzodzomenyo *et al*. (1999) found that, although *An. gambiae* was the main vector, two of three specimens of *An. pharoensis* examined were infected and one of these was infectious. The relative dearth of *An. funestus* in Okyereko probably reflects the absence of suitable breeding sites.

Govella *et al.* (2009) reported that the efficiency of both light traps and Furvela tent traps for S form *An. gambiae* is density-dependent. It is possible that other traps would have collected even more than the 1000 or so *An. gambiae* collected at peak times in the present study. A comparison between different tent traps in an area like Okyereko might provide useful data.

The history and future of first-feeding insects are, therefore, still difficult to determine. Whether size has a role in mating before feeding, or in feeding before mating, as it does in *An. gambiae* collected in São Tomé (Charlwood *et al*., 2003), is unfortunately unknown, as is the prevalence of sugar feeding in these insects. The mating plug in *An. gambiae* lasts for approximately 24 h (Gillies, 1956). If a mosquito had mated at dawn, the plug may have been absorbed by the time the mosquito was examined. Nevertheless, even in places such as São Tomé, where swarms of *An. gambiae* have been recorded at dawn, dawn activity was a fraction of that observed at dusk (Charlwood *et al*., 2002) and thus mating at dawn is not likely to be the cause. Mated *N-I* insects without a plug had, therefore, presumably mated >24 h before being collected but had not taken a bloodmeal immediately after mating. It is possible that many insects fail to obtain a bloodmeal during the night they mate and therefore the *N-I* observed are basically reminders of the previous nights 'plug-positive' cohort. However, whether they had or had not attempted to feed remains unknown. These insects may have mated away from the village or they may have fed when virginal and subsequently mated and digested their initial bloodmeal before returning to feed again a day later. Virgin mosquitoes that take a bloodmeal are presumably very young. Egg development in mosquitoes is dependent on juvenile hormone levels, which do not rise until day 3 post-eclosion (Noriega, 2004). We also do not know whether engorged *N-I* mosquitoes develop eggs and what effect this might have on survival or vectorial capacity.

The population of *An. gambiae* in Okyereko differs from that in São Tomé in a number of respects. The ecological 'templet' (Southwood, 1977) for the two populations differs because, in Okyereko, feeding sites are distant from emergence sites, whereas in São Tomé they are within a few metres of one another. Pairs in copula were rare (in relation to the size of swarms) in Okyereko, but were commonly seen leaving swarms in São Tomé; insects that fed as virgins and returned to feed with a mating plug (*Plug-blood*) were non-existent in Okyereko, but, although relatively under-sampled, were found in São Tomé. Further, mated females with ovaries at Stage I but without a mating plug (*N-I*) were common in Okyereko, but were not recorded in São Tomé. Thus, the environment would appear to have an influence on the ecological characteristics of this important vector of malaria. Further studies on the effects of local conditions on parameters affecting transmission may help in the design of environment-specific control strategies.

Rice fields are often major breeding sites for Anophelines (Mwangangi *et al*., 2010). Dzodzomenyo *et al*. (1999) described a substantial increase in numbers of *An. gambiae* when the fields of Okyereko were irrigated at the end of the dry season. Given the large numbers of new recruits to the population, anti-adult control measures will produce very little perceptible change in the population of human-biting mosquitoes, although they may reduce rates of malaria transmission. Environmental management techniques, such as intermittent irrigation, offer alternatives to adulticide-based interventions and can have a significant effect on mosquito populations (Keiser *et al*., 2005a, 2005b). Frequent draining, drying and subsequent re-flooding of rice fields can greatly increase anopheline larval mortality (Mutero *et al*., 2000), ultimately reducing adult populations, which can sometimes dramatically lower rates of malaria transmission (Keiser *et al*., 2005a, 2005b). Another potential benefit of intermittent irrigation would be the reduction in local mosquito diversity (Mwangangi *et al*., 2010), which might affect both disease-transmitting and nuisance biting mosquitoes. Establishing intermittent irrigation might take several years, during which time test fields located adjacent to one another could be alternatively managed by either intermittent or continuous irrigation and the mosquito breeding and rice yields carefully monitored in each. The high level of interest among villagers in interventions that will reduce rates of malaria may improve the likelihood that intermittent irrigation can be adopted if it can be demonstrated that mosquito breeding is reduced and that rice yields are at least equivalent if not improved (Mutero *et al.*, 2000; Keiser *et al*., 2005a, 2005b). Nevertheless, the most obvious way to reduce transmission is to improve the use of mosquito bednets in the village.

The additional distances involved and the extra energy requirements in Okyereko, compared with São Tomé, may be responsible for the differing ecologies of the respective mosquito populations. How this might be used against the mosquito is, however, uncertain. It may mean that controlling larvae in the fields close to the village will have a greater effect on transmission than controlling those at greater distances, even if all of these are colonized.

Acknowledgements

We would like to thank the villagers of Okyereko and the director of the Biotechnology and Nuclear Agriculture Research Institute, Accra, Ghana, for their hospitality. We also thank Janet Wiafia, senior superintendent midwife at the Okyereko Health Centre, for information on malaria incidence. The study was funded by the International Atomic Energy Agency (IAEA), Vienna, Austria. RJP thanks Dr Laurence Zwiebel and the Vanderbilt University Department of Biological Sciences for financial support through the Mosig Travel Fund.

References

Birley, M.H. & Charlwood, J.D. (1989) The effect of moonlight and other factors on the oviposition cycle of malaria vectors in Madang, Papua New Guinea. *Annals of Tropical Medicine and Parasitology*, **8**, 415–422.

Briegel, H. & Hörler, E. (1993) Multiple bloodmeals as a reproductive strategy in *Anopheles* (Diptera: Culicidae). *Journal of Medical Entomology*, **30**, 975–985.

Charlwood, J.D. (2003) May the force be with you: measuring mosquito fitness in the field. *Ecological Aspects for Application of Genetically Modified Mosquitoes* (ed. by T.W. Scott & W. Takken), pp. 47–62. Kluwer Academic Publishers, Dordrecht.

Charlwood, J.D. & Wilkes, T.J. (1981) Observations on the biting activity of *Anopheles triannulatus bachmanni* from the Mato Grosso, Brazil. *Acta Amazonica*, **11**, 411–413.

Charlwood, J.D., Birley, M.H., Dagoro, H., Paru, R. & Holmes, P.R. (1985) Assessing survival rate of *Anopheles farauti* (Diptera: Culicidae) from Papua New Guinea. *Journal of Animal Ecology*, **54**, 1003–1016.

Charlwood, J.D., Paru, M.H., Dagaro, H. & Lagog, M. (1986) The influence of moonlight and gonotrophic age on the biting activity of *Anopheles farauti* (Diptera, Culicidae) from Papua New Guinea. *Bulletin of Entomological Research*, **76**, 211–227.

Charlwood, J.D., Kihonda, J., Sama, S. *et al.* (1995) The rise and fall of *Anopheles arabiensis* (Diptera, Culicidae) in a Tanzanian village. *Bulletin of Entomological Research*, **85**, 37–44.

Charlwood, J.D., Billingsley, P.F., Takken, W., Lyimo, E.O.K., Smith, T. & Meuwissen, J.H.E.T. (1997) Survival and infection probabilities of anthropophagic Anophelines from an area of high prevalence of *Plasmodium falciparum* in humans. *Bulletin of Entomological Research*, **87**, 445–453.

Charlwood, J.D., Pinto, J., Sousa, C.A., Ferreira, C. & do Rosário, V.E. (2002) The swarming and mating behaviour of *Anopheles gambiae* (Diptera: Culicidae) from São Tomé Island. *Journal of Vector Ecology*, **27**, 178–183.

Charlwood, J.D., Pinto, J., Sousa, C.A., Ferreira, C., Petrarca, V. & do Rosário, V.E. (2003) A mate or a meal—pre-gravid behaviour of female *Anopheles gambiae* from the islands of São Tomé and Príncipe, West Africa. *Malaria Journal*, **2**, 7.

Charlwood, J.D., Tomás, E.V., Salgueiro, P., Egyir-Yawson, A., Pitts, R.J. & Pinto, J. (2011) Studies on the behaviour of peridomestic and endophagic M form *Anopheles gambiae* from a rice growing area of Ghana. *Bulletin of Entomological Research*, **101**, 533–539.

Clements, A.N. & Paterson, G.D. (1981) The analysis of mortality and survival rates in wild populations of mosquitoes. *Journal of Applied Ecology*, **18**, 373–399.

Dzodzomenyo, M., Dunyo, S.K., Ahorlu, C.K., Coker, W.Z., Appawu, M.A., Pedresen, E.M. & Simonsen, P.E. (1999) Bancroftian filariasis in an irrigation project community in southern Ghana. *Tropical Medicine and International Health*, **4**, 13–18.

Garrett-Jones, C. & Grab, B. (1964) The assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the proportion of parous females. *Bulletin of the World Health Organization*, **31**, 71–86.

Gillies, M.T. (1953) The duration of the gonotrophic cycle in *Anopheles gambiae* and *An. funestus*, with a note on the efficiency of hand catching. *East African Medical Journal*, **30**, 129–135.

Gillies, M.T. (1956) A new character for the recognition of nulliparous females of *Anopheles gambiae*. *Bulletin of the World Health Organization*, **15**, 451–459.

Gillies, M.T. (1961) Studies on the dispersion and survival of *Anopheles gambiae* Giles in East Africa, by means of marking and release experiments. *Bulletin of Entomological Research*, **52**, 99–127.

Gillies, M.T. & De Meillon, B. (1968) *The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region)*, 2nd edn, Vol. 54. Publications of the South African Institute for Medical Research, Johannesburg.

Gillies, M.T. & Wilkes, T.J. (1965) A study of age composition of populations of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in northeastern Tanzania. *Bulletin of Entomological Research*, **56**, 129–135.

Govella, N.J., Chaki, P.P., Geissbuehler, Y. *et al.* (2009) A new tent trap for sampling exophagic and endophagic members of the *An. gambiae* complex. *Malaria Journal*, **8**, 1.

Gu, W., Muller, G., Schlein, Y., Novak, R.J. & Beier, J.C. (2011) Natural plant sugar sources of *Anopheles* mosquitoes strongly impact malaria transmission potential. *PLoS One*, **6**, e15996.

Hii, J.L., Birley, M.H. & Sang, V.Y. (1990) Estimation of survival rate and oviposition interval of *Anopheles balabacensis* mosquitoes from mark–recapture experiments in Sabah, Malaysia. *Medical and Veterinary Entomology*, **4**, 135–140.

Hoc, T.Q. & Charlwood, J.D. (1990) Age determination of *Aedes cantans* using the ovarian oil injection technique. *Journal of Medical and Veterinary Entomology*, **4**, 227–233.

Holmes, P.R. & Birley, M.H. (1987) An improved method for survival rate analysis from time series of haematophagous Dipteran populations. *Journal of Animal Ecology*, **56**, 427–440.

Ijumba, J.N. & Lindsay, S.W. (2001) Impact of irrigation on malaria in Africa: paddies paradox. *Medical and Veterinary Entomology*, **15**, 1–11.

Kampango, A., Cuamba, N. & Charlwood, J.D. (2010) Does moonlight influence the biting behaviour of *Anopheles funestus* (Diptera: Culicidae)? *Medical and Veterinary Entomology*, **25**, 240–246.

Keiser, J., Singer, B.H. & Utzinger, J. (2005a) Reducing the burden of malaria in different eco-epidemiological settings with environmental

management: a systematic review. *Lancet Infectious Diseases*, **5**, 695–708.

Keiser, J., De Castro, M.C., Maltese, M.F., Bos, R., Tanner, M., Singer, B.H. & Utzinger, J. (2005b) Effect of irrigation and large dams on the burden of malaria on a global and regional scale. *American Journal of Tropical Medicine and Hygiene*, **72**, 392–406.

Klowden, M. (1999) The check is in the male: male mosquitoes affect female physiology and behaviour. *Journal of the American Mosquito Control Association*, **15**, 213–220.

Lord, C.C. & Baylis, M. (1999) Estimation of survival rates in haematophagous insects. *Medical and Veterinary Entomology*, **13**, 225–233.

Mutero, C.M. & Birley, M.H. (1989) The effect of pre-gravid development on the estimation of mosquito survival rates. *Journal of Applied Entomology*, **107**, 96–101.

Mutero, C.M., Blank, H., Konradsen, F. & van der Hoek, W. (2000) Water management for controlling the breeding of *Anopheles* mosquitoes in rice irrigation schemes in Kenya. *Acta Tropica*, **76**, 253–263.

Mwangangi, J.M., Shililu, J., Muturi, E.J. *et al.* (2010) *Anopheles* larval abundance and diversity in three rice agro-village complexes Mwea irrigation scheme, central Kenya. *Malaria Journal*, **9**, 228.

Noriega, F.G. (2004) Nutritional regulation of JH synthesis: a mechanism to control reproductive maturation in mosquitoes? *Insect Biochemistry and Molecular Biology*, **34**, 687–693.

Okoye, P.N., Wilson, M.D., Boakye, D.A. & Brown, C.A. (2005) Impact of the Okyereko irrigation project in Ghana on the risk of human malaria infection by *Anopheles* species (Diptera: Culicidae). *African Entomology*, **13**, 249–253.

Santolamazza, F., Mancini, E., Simard, F., Qi, Y., Tu, Z. & della Torre, A. (2008) Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. *Malaria Journal*, **7**, 163.

Scott, J.A., Brogdon, W.G. & Collins, F.H. (1993) Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *The American Journal of Tropical Medicine and Hygiene*, **49**, 520–529.

Southwood, T.R.E. (1977) Habitat, the templet for ecological strategies? *Journal of Animal Ecology*, **46**, 337–365.

Takken, W., Charlwood, J.D., Billingsley, P.F. & Gort, G. (1995) Dispersal and survival of *Anopheles funestus* and *An. gambiae s.l.* (Diptera, Culicidae) during the rainy season in southeast Tanzania. *Bulletin of Entomological Research*, **88**, 561–566.

Takken, W., Klowden, M.J. & Chambers, G.M. (1998) Effect of body size on host seeking and bloodmeal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. *Journal of Medical Entomology*, **35**, 639–645.

Wilkes, T.J. & Charlwood, J.D. (1979) A rapid gonotrophic cycle in *Chagasia bonneae* from Brazil. *Mosquito News*, **39**, 137–139.

Wirtz, R. (1987) Comparative testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. *Bulletin of the World Health Organization*, **65**, 39–45.

Yawson, A.E., Weetman, D., Wilson, M.D. & Donnelly, M.J. (2007) Ecological zones rather than molecular forms predict genetic differentiation in the malaria vector *Anopheles gambiae* s.s. in Ghana. *Genetics*, **175**, 751–761.

Accepted 31 August 2011
First published online 17 November 2011