7

Factors affecting the vectorial competence of *Anopheles gambiae*: a question of scale

Willem Takken[#] and Steve W. Lindsay[##]

Abstract

Malaria transmission in Africa is without doubt governed by the existence of a group of highly efficient vectors, of which *Anopheles gambiae* Giles *sensu stricto* is predominant. The endophilic and anthropophagic behaviours of this mosquito create an intimate association between the human reservoir and insect vectors of malaria. In this paper several mosquito-related and environmental factors that modulate the transmission intensity of malaria in Africa are discussed, in order to illustrate the plasticity of the vectors' responses to malaria interventions. The *An. gambiae* group of species serves as an example of the highly complex interactions between humans, malaria parasites and mosquitoes. Larval and adult characteristics are described that affect the geographic distribution, phenology and longevity of the mosquito vectors. It is shown that spatial and temporal variations in the environment have considerably more effect on these characteristics than is commonly assumed. It is suggested that epidemiological studies pay greater attention to such variations, in particular when estimating the entomological inoculation rate and vectorial competence. When considering the use of transgenic mosquitoes for malaria control, these effects should be studied in order to understand how local variations in vector ecology might affect the outcome of a transgenic release.

Keywords: *Anopheles gambiae*; mosquito; ecology; vectorial competence; behaviour; malaria; transmission; EIR

Introduction

Most attempts to quantify the risk of malaria at the local scale assume that transmission is uniform across space. Typically risk is measured using an estimate of the Entomological Inoculation Rate (EIR), which can be defined as the number of mosquitoes biting people during the transmission season multiplied by the sporozoite rate of the vector population (Macdonald 1957; Bruce-Chwatt 1985). While this approach is satisfactory in producing an overall estimate of the exposure of a population to malaria parasites, it fails to capture the variability in transmission that can occur at the local level which is determined by the habits, genetics and physiology of different vector species, spatial heterogeneity of vector habitats, immune status of

[#] Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands. E-mail: willem.takken@wur.nl
[##] Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, UK. E-mail: s.w.lindsay@durham.ac.uk

the local human population or anti-mosquito strategies employed by local communities. For these reasons the force of malaria transmission, infection and morbidity within one village may be substantially different between neighbouring villages (Thomas and Lindsay 2000; Clarke et al. 2002). These spatial and temporal variations are relevant with respect to predictions of malaria risk and the strategies to be developed for effective interventions.

Many contemporary studies on anopheline genetics focus on the major African malaria vector *Anopheles gambiae* Giles (Holt et al. 2002; Morel et al. 2002), and development of a transgenic mosquito is discussed primarily with this species in mind. As with the basic Macdonald model mentioned earlier, studies on transgenic mosquitoes to date need to consider spatial and temporal variations within the vectors under study, which as we shall see, may have profound effects on disease epidemiology and hence, on the outcome of a transgenic release for malaria control. For this reason we present in this paper an analysis of ecological aspects that govern the role this mosquito plays in malaria risk and epidemiology in tropical Africa. It is realized that in much of its geographic range *An. gambiae* co-exists with other malaria vectors, but the large body of published data on its genetics, biology, ecology and role as malaria vector justify a focus on this important malaria vector and those factors that determine its vectorial competence.

Genetic variation within the *An. gambiae* complex

An. gambiae consists of a complex of seven sibling species, which can be distinguished by cytotaxonomic and molecular means (Coluzzi et al. 2002). Although closely related, in principle all sibling species are apomictic with low levels of hybridization. The chromosomes show fixed paracentric inversion differences between the sibling species, as well as intraspecific inversion polymorphism. Polymorphic chromosomal inversions are most common in *An. gambiae* Giles *sensu stricto* and *An. arabiensis* Patton (Petrarca et al. 1983; Bryan et al. 1987) and are located mostly on chromosome arm 2R. Non-random mating with partial or complete absence of interbreeding between carriers of certain inversion karyotypes has been observed in *An. gambiae s.s.* (Bryan et al. 1982). The chromosomal forms identified to date have been broadly differentiated into Forest and Savanna forms (Coluzzi, Petrarca and Di Deco 1985). Incomplete intergradation between some non-forest populations, characterized by different inversion karyotype frequencies, has led to their recognition as named forms Bamako, Bissau, Mopti, and Savanna. In parts of West Africa, where these forms occur sympatrically, they appear to be segregated environmentally (Coluzzi et al. 1979; Coluzzi 1992; Bayoh, Thomas and Lindsay 2001). The inversion frequency variations were found to be correlated with environmental clines, indicating adaptations to climatic and ecological conditions (Thomson et al. 1997; Coluzzi et al. 2002; Della Torre et al. 2002; Bayoh, Thomas and Lindsay 2001).

Recently it has been shown that *An. gambiae s.s.* exists as two distinct molecular forms, designated M and S (Favia et al. 1997), which are not clearly concordant with the different chromosomal forms (Touré et al. 1998; Della Torre et al. 2001; Gentile et al. 2001). Thus the chromosomal forms may not be reproductively isolated throughout West Africa (Della Torre et al. 2001). Instead they are more likely to represent adaptations to particular habitats since most inversions show distinct seasonal or geographical changes in frequency or both (Della Torre et al. 2001).

Ecological differences between *An. gambiae s.s.* and *An. arabiensis*

Of the seven siblings of the gambiae complex, *An. gambiae sensu stricto* and *An. arabiensis* are the most important vectors of malaria (White 1974). Both species share a continent-wide distribution, from Madagascar to Senegal (Lindsay, Parson and Thomas 1998; Coetzee, Craig and Le Sueur 2000), occur sympatrically in much of this area, and exhibit strong associations with the traditional rural life style of many African communities. Both species are excellent vectors of malaria parasites, mainly *Plasmodium falciparum*, in much of the continent. A third species of significance for malaria transmission in Africa is *An. funestus* Giles, which often co-exists with the other two species and can be an important malaria vector as well. For reasons mentioned above, we will not discuss this species in detail in this paper.

Larval habitats

A characteristic of the *An. gambiae* complex is that these pioneer species occupy temporary aquatic habitats (Gillies, De Meillon and Coetzee 1968). Small, flooded depressions in the soil, hoofprints, tyre tracks and shallow ditches are used for oviposition. This strategy of population development probably reduces the risk of predation for the vulnerable immature stages of the mosquitoes. However, predation can take a heavy toll on mosquitoes (Lacey and Lacey 1990), and the loss of immature stages of *An. gambiae s.s.* can reach almost 100% (Service 1993). Although *An. gambiae s.s.* and *An. arabiensis* commonly share a larval habitat (White and Rosen 1973), *An. gambiae s.s.* usually outcompetes *An. arabiensis* when they occur together (Schneider, Takken and McCall 2000; Koenraadt and Takken 2003). Adults of *An. arabiensis*, however, are better adapted to severely dry environments than *An. gambiae s.s.* (Lindsay, Parson and Thomas 1998; Petrarca et al. 2000), and during extended dry periods *An. arabiensis* tends to be the most dominant member of the complex. Thus, at the beginning of the rainy season the number of *An. arabiensis* rises before *An. gambiae s.s.* has been able to build up to a competitive population (White 1972). In environments with large water surface areas such as irrigated fields in Kenya, *An. arabiensis* dominates presumably because transient larval habitats adequate for *An. gambiae s.s.* are lacking (Mwangi and Mukiama 1992). However, in The Gambia in West Africa, *An. gambiae s.s.* outcompetes *An. arabiensis* in the extensive floodplains, bordering the River Gambia (Lindsay et al. 1991; Bøgh et al. (in press)), and in irrigated rice areas *An. gambiae s.s.* during the rainy season (Lindsay et al. 1991). Adult mosquito numbers increase as soon as the paddies are flooded, rising to a peak when the rice plants are small, before declining when the rice plants cover the surface of the water (Surtees 1970; Snow 1983; Lindsay et al. 1991). After harvesting they may persist in the shallow puddles left after cropping (Klinkenberg et al. 2003). In summary, it is unclear whether the two species occupy separate larval microhabitats, although generally *An. arabiensis* tends to dominate during the dry season in wet and humid parts of Africa and is the major vector all the year round in semi-arid parts of Africa (White 1972; White and Rosen 1973).

Two members of the *An. gambiae* complex favour brackish water for immature development. *An. melas* Theobald in West Africa and *An. merus* Dönitz in East Africa are found in coastal mangrove forests and salt marshes, where they avoid the potential competition of the freshwater members of the complex (Coetzee, Craig and Le Sueur 2000). Locally, both species can be important vectors. *An. bwambae* White is only found in mineral springs in western Uganda, where it co-exists with *An. gambiae s.s.* as adult mosquitoes. All three species can develop in fresh water, but presumably

cannot compete in this environment with the freshwater members of the complex. *An. quadriannulatus* (Theobald) species A in South Africa, and species B in Ethiopia are freshwater species, but may have adapted to lower developmental temperatures than *An. gambiae s.s.* and *An. arabiensis*, providing a competitive advantage above the latter two species.

Mating behaviour

Mating of *An. gambiae* takes place around dusk, when the males sometimes form swarms to which females are attracted by as yet unknown signals (Takken and Knols 1999). Swarms often appear at the same site, but the mechanism of this behaviour is not well understood (Marchand 1984; Takken and Knols 1999). Clearly, mating behaviour of the *An. gambiae* complex remains a black box at present, but should be investigated if the impact of transgenic mosquitoes is to be understood properly (Charlwood 2003).

Biting behaviour

There are considerable differences in biting behaviour among members of the *gambiae*complex, which may cause segregation of the species at this stage of the life cycle. Female *An. gambiae s.s.* are highly anthropophilic, feeding preferentially on humans (White 1974; Coluzzi et al. 1979), although in West Africa they are less discriminating and will feed readily on other animals like horses and cattle (Diatta et al. 1998; Bøgh et al. 2001). In contrast *An. arabiensis* has a more opportunistic feeding behaviour, although *An. arabiensis* can be entirely zoophilic, as recent studies from Madagascar have shown (Duchemin et al. 2001). In general *An. gambiae s.s.* has, on account of its high anthropophily, developed a strong tendency for endophagy and endophily, whereas the more zoophilic *An. arabiensis* tends to be more exophagic and exophilic. Although it is recognized that the anthropophilic behaviour of *An. gambiae s.s.* and the opportunistic behaviours of *An. arabiensis* are genetically fixed (Coluzzi et al. 2002), it is not well understood which factors determine temporal or spatial variations in these behaviours. For these reasons it is necessary to establish the behavioural characteristics of the vector population of specific study areas when measuring epidemiological parameters.

House-entering behaviour

Most people get infected with malaria in the home. *An. gambiae s.s.* is extremely well-adapted for entering houses and feeding on people (Gillies, De Meillon and Coetzee 1968). They do so at night, when individuals are asleep and least able to protect themselves. This ability to get indoors arises from a simple change in behaviour. When most mosquitoes come in contact with a wall they fly off sideways. But when *An. gambiae s.l.* mosquitoes reach a wall, they fly up and are funnelled, by the over-hanging eaves, inside the house through the gap at the top of the wall (see Figure 1, from Snow 1987). The number of mosquitoes entering through open eaves is probably greatly enhanced by attractive warm host odours pouring out of
the openings.

Feeding behaviour

An. gambiae is a nocturnal feeder, with most of the blood-feeding occurring after midnight (Haddow 1954; Lindsay et al. 1989). Once they have reached a host, the mosquitoes will preferentially bite and feed off the lower parts of the body (Braack et al. 1994; De Jong and Knols 1995). During a gonotrophic cycle, normally one blood

meal suffices to complete egg development, but *An. gambiae s.s.* may require multiple meals because of nutritional stress during immature development (Gillies 1954; Takken, Klowden and Chambers 1998). Although the timing of blood-feeding is genetically fixed, the extensive use of insecticide-impregnated bednets, preventing the mosquitoes from obtaining a blood meal, may select for anophelines that feed at other times, for instance in the early evening when people have not yet gone to bed (M. Yohannes unpublished data). So far the evidence for such a behavioural adaptation is scarce (Takken 2002).
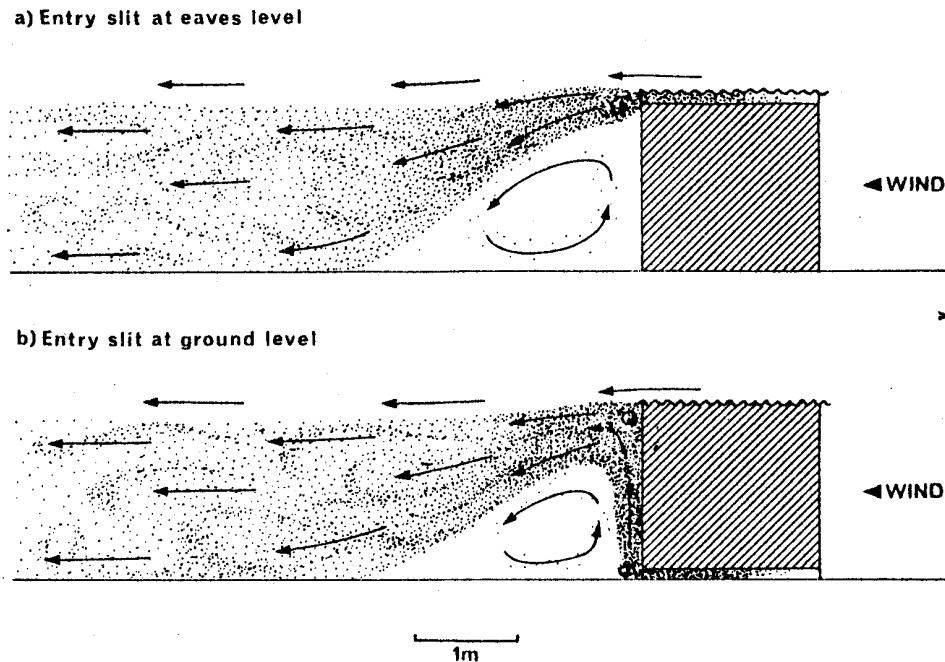
a) **Entry slit at eaves level**

b) **Entry slit at ground level**

1m

Figure 1. Simulations of air and odour streams around a house (from Snow 1987)

Resting behaviour

Following a blood meal, females of *An. gambiae* tend to settle on walls and ceilings of rooms in which they acquired the blood. Eggs are developed during this time, and when these are mature, the female will leave the house in the early evening in search for a suitable oviposition site. This resting phase may take up to 72 hours. Mainly unfed females leave the house in the early morning, presumably to rest in the vegetation nearby. Exophilic species such as *An. arabiensis* tend to spend more time outdoors, while strongly endophilic species like *An. gambiae s.s.* may remain indoors for the duration of egg development and maturation. The conventional insecticidal treatment of houses was designed to kill the indoor resting fraction of the malaria vector population, and often with success (Najera 1989). Ecological and epidemiological studies of malaria vectors may collect indoor-resting females to assess biting behaviour, age structure of the vector population, parasite infection rates and other relevant parameters that might be useful in the assessment of malaria risk. Special attention should be paid to the resting behaviour of female mosquitoes during the dry season. It is assumed that a proportion of adult females survives the often harsh climatic conditions of the dry season, when there is no opportunity to deposit eggs because of lack of larval habitats, inside traditional African huts. Here they find shelter in cracks and crevices in the walls, apparently favoured by a local

microclimate that allows survival(Molineaux and Gramiccia 1980). Others survive by low levels of breeding during the dry season (M. Jawara, personal communication). During the dry season in the Sudan adult *An. arabiensis* have been found near the bottom of wells, which they must have left occasionally to feed, as recently blood-fed females were encountered (Omer and Cloudsley-Thompson 1968). However, few data on dry-season ecology of *An. gambiae* are available and this is, as mating behaviour, an urgent topic to be studied.

Spatial differentiation in optimal habitats
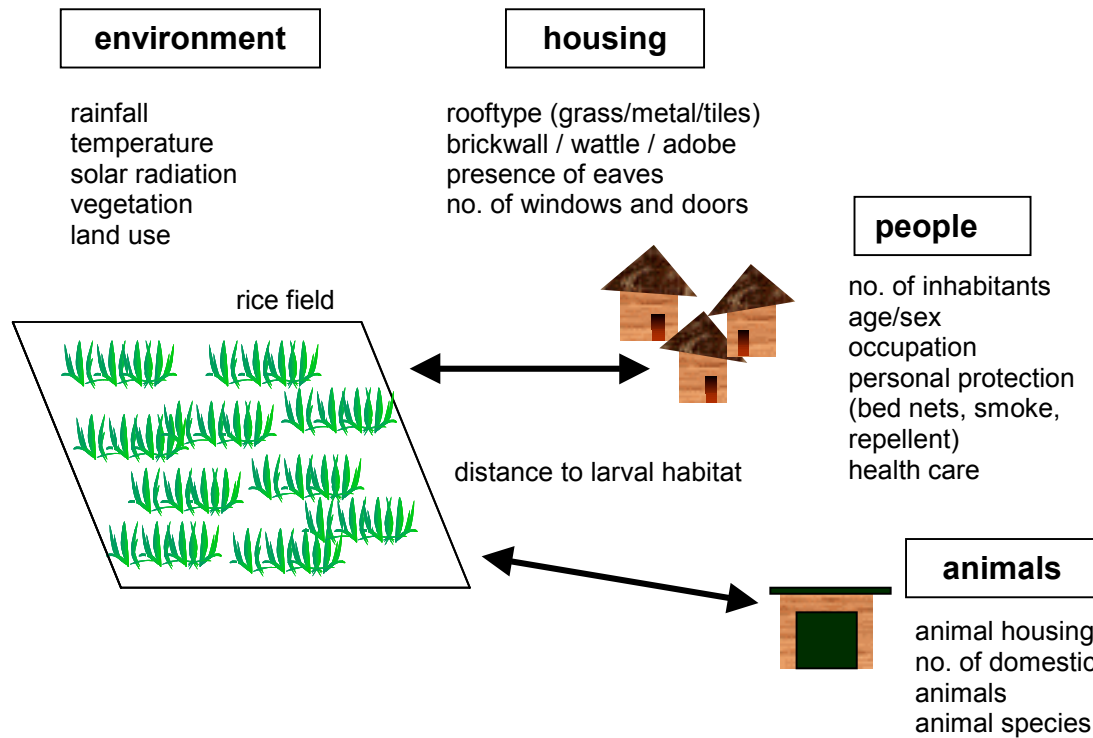
Continental level

At a coarse spatial scale climate is the major factor governing the distribution and relative abundance of insects (Andrewartha and Birch 1954; Sutherst and Maywald 1995). In large areas of tropical sub-Saharan Africa populations of *An. gambiae s.s.* and *An. arabiensis* are sympatric (Coetzee, Craig and Le Sueur 2000), particularly within the 800 mm isohyets of rainfall. Because *An. arabiensis* is better adapted to relatively dry regions, this species is found farther north and south than *An. gambiae s.s.* (Lindsay, Parson and Thomas 1998). For instance, *An. arabiensis* can be found in the Nile valley in northern Sudan, where rainfall is low (Petrarca et al. 2000). Distribution of both species is determined mainly by the capacity of the air to desiccate the insect: *An. gambiae* is more common in wetter areas, whilst *An. arabiensis* is better adapted to drier conditions (Lindsay, Parson and Thomas 1998). The two zoophilic members of the *gambiae* complex, *An. quadriannulatus* species A and species B, have a limited distribution associated perhaps with more subtropical climates than the other members of the complex. *An. quadriannulatus* species A is found in South Africa, Swaziland and Zimbabwe, while *An. quadriannulatus* species B is only found in Ethiopia (Hunt, Coetzee and Fettene 1998). Since both species are considered non-malaria vectors throughout their range, in spite of being susceptible to *P. falciparum* (Takken et al. 1999, Pates, H.V. unpublished data), their existence is mostly important from the evolutionary point of view as they form the root of the phylogenetic tree of the *gambiae* complex (Coluzzi et al. 2002).

The distribution of chromosomal forms of *An. gambiae s.s.* is also governed by climate as they occupy distinct ecological zones in West Africa (Bayoh, Thomas and Lindsay 2001).

Regional level

At a finer spatial resolution other factors become important, such as local climate effects, salinity of the breeding sites and the relative availability of different host species. Local variations in climate can affect the relative abundance of the two main vector species, as seen in the highlands of Kenya, where *An. gambiae s.s.* is more abundant at higher altitudes than *An. arabiensis*, which dominates in the lowlands (Lindsay and Martens 1998; Githeko and Ndegwa 2001).

Differences in adaptation for fresh and saltwater cause spatial segregation between adult members of the *An. gambiae* complex, as shown by the field studies in The Gambia (Bryan et al. 1982). Here *An. melas* occurs in the large pools of brackish water bordering the river, with vegetation dominated by *Sporobolus* sp., *Eleocharis* sp. and *Sesuvium* sp. (Bøgh et al. (in press)). In the same pools *An. gambiae* is found, although it is rarely found in highly saline pools. *An. arabiensis* occurs in small numbers breeding in the rice fields close to the river. Similarly, the saltwater species *An. melas* in East Africa is found only in the coastal areas, with exception of

environment

rainfall
temperature
solar radiation
vegetation
land use

housing

rooftype (grass/metal/tiles)
brickwall / wattle / adobe
presence of eaves
no. of windows and doors

people

no. of inhabitants
age/sex
occupation
personal protection
(bed nets, smoke,
repellent)
health care

rice field

distance to larval habitat

animals

animal housing
no. of domestic
animals
animal species

Mozambique, where it is also found far inland along the Zambezi and Save river systems (Gillies, De Meillon and Coetzee 1968; Coetzee, Craig and Le Sueur 2000).

The propensity of *An. gambiae s.s.* to feed on people explains its absence from game reserves and other areas devoid of human settlement. This species is indeed strongly associated with human settlements (Coluzzi 1992). By contrast *An. arabiensis*, with a more catholic feeding behaviour, can be found from game reserves to urban areas. Recent studies using satellite imagery in the prediction of the distribution of members of the *An. gambiae* complex show the regional segregation of these species caused, mainly, by environmental variables, and allow for estimates of distribution in as yet unstudied areas (Rogers et al. 2002).

Local level

In recent years much knowledge about local variation in temporal and spatial distribution of *An. gambiae s.l.* has been collected. For instance, in the Kilombero valley of southern Tanzania *An. gambiae s.s.* and *An. arabiensis* occur sympatrically during the rainy season, but segregate into different habitats in the dry seasons (Charlwood, Vij and Billingsley 2000). In Burkina Faso spatial and temporal segregation of the chromosomal forms *An. gambiae s.s.* 'Savanna', 'Bamako' and 'Mopti' has been reported within one village, influenced by land use and climatic factors (Della Torre et al. 2002).

Biting intensity, and therefore malaria risk, can vary considerably within a village (see Figure 2). Houses close to larval habitats experience significantly higher biting rates than houses farther away (Lindsay et al. 1995; Smith et al. 1995; Lindsay et al. 2000). House design and personal protection matters may also vary within a village, causing strong variations in biting rates between households and, sometimes, within families (Lindsay, Emerson and Charlwood 2002). Mosquitoes locate their (human) hosts chiefly by host odours, and variability in these odours between humans is a well-known factor determining mosquito biting rates (Schreck, Kline and Carlson

1990; Lindsay et al. 1993; Knols, De Jong and Takken 1995; Lindsay, Emerson and Charlwood 2002).

Human blood index

Epidemiological studies use the avidity with which malaria vectors bite humans as a factor to estimate the daily transmission rate of malaria parasites. This factor depends on the density of hungry adult female mosquitoes and the fraction of them that bite humans (Garrett-Jones 1964). The difference in host preference between *An. gambiae s.s.* and *An. arabiensis*, with the former strongly anthropophilic and the latter opportunistic, makes *An. gambiae s.s.* a better malaria vector (White 1974; Costantini et al. 1996). For this reason epidemiological field studies measure the proportion of blood-fed mosquitoes that have human blood in their midgut. With *An. gambiae* this figure varies from 0.49 to 0.98 (Garrett-Jones, Boreham and Pant 1980), while for *An. arabiensis* this is usually lower. However, there are exceptions. In Senegal, *An. gambiae s.s.* was reported to feed almost exclusively on cattle (Diatta et al. 1998), and on Sao Tomé it was found to prefer dogs above humans (Sousa et al. 2001). In Mali and Burkina Faso *An. arabiensis* has exhibited a high degree of anthropophily (Costantini et al. 1998; Lemasson et al. 1997). It is assumed that environmental

Figure 2. Human and environmental factors that may affect mosquito biting rate

factors influence the observed local variations in anthropophily. For instance the relatively high density of cattle in West Africa coupled with house construction and high use of untreated bed nets may affect the degree of anthropophily (Bøgh et al. 2001).

Survival strategies

The fitness of malaria vectors depends on their ability to produce large numbers of offspring quickly (Charlwood 2003). To achieve this goal the insects have devised several strategies to avoid environmental extremes. Immature stages of *An. gambiae s.s.* and *An. arabiensis* share a comparatively similar favourite temperature range, which lies between 24 and 29 ^{0}C (Lyimo, Takken and Koella 1992). As adults both species have an optimal temperature range between 22 and 28 ^{0}C (Gillies, De Meillon and Coetzee 1968), although *An. arabiensis* tends to be more tolerant of higher temperatures (Kirby, M. unpublished data). The endophilic behaviour that both species express will favour survival under unusually cold or hot circumstances, because the indoor climate will be moderated and more stable than the daily fluctuations between extreme minimum and maximum temperatures. The species differ in favourite ranges of saturation deficit, *An. arabiensis* being able to survive in drier conditions than *An. gambiae s.s.* This explains why *An. arabiensis* is found biting in the dry season, while *An. gambiae s.s.* is usually absent at that time of the year, even if suitable larval habitats are present (Petrarca et al. 2000).

An. arabiensis has a number of strategies, which allow it to persist in arid conditions. Adult females will lay their eggs on damp surfaces, rather than water, with hatching being delayed in a proportion of eggs (Coluzzi 1965), females will also aestivate during periods of prolonged dryness (Omer and Cloudsley-Thompson 1968; 1970) and dry season refugia exist in which *An. arabiensis* thrive and rapidly colonize other areas at the start of the rainy season (White 1974;

Charlwood, Vij and Billingsley 2000). However, there may be other, as yet unidentified mechanisms that allow this species to survive in dry environments.

Entomological inoculation rate

The central goal of malaria control is to reduce the number of potential new infections that people receive from mosquito bites in order to reduce morbidity and mortality (*Framework for monitoring progress and evaluating outcomes and impact: roll back malaria* 2000). This goal could, in theory, be readily achieved with the advent of DDT in the middle of the twentieth century. The reduction of mosquito survival by the toxic action of insecticides was the most obvious target in the equation of the basic reproductive rate of malaria (Macdonald 1957). For a number of reasons this goal could not be met, and today careful consideration is given to aspects of the parasite-vector-host interaction that might explain malaria risk and unravel specific aspects of this relationship that are more amenable for intervention (Greenwood and Mutabingwa 2002). The EIR is used as the parameter with which the best strategy for malaria interventions can be developed. Annual values of EIR within Africa vary from as low as 0.1 to >1000 depending on the eco-epidemiological conditions of a locality (Hay et al. 2000; Smith, Leuenberger and Lengeler 2001). Generally in Africa when the EIR <10, an area is considered to have unstable malaria and when EIR >100, malaria is stable. Areas with EIR values in between these extremes will vary in malaria endemicity, depending on environmental and demographic conditions such as rainfall, vegetation cover, human-population density and land use patterns.

The pattern of clinical malaria differs according to the level of exposure to malaria parasites (Snow et al. 1994). In areas of intense challenge most children are infected before the age of 6 months, and severe malaria is manifest as anaemia in infants and is responsible for a great many deaths. In areas of less intense transmission cerebral malaria is a greater killer in 2-3-year-old children. Those that survive the early years of childhood develop protective immunity against both clinical malaria and infection. In marked contrast in areas of low transmission both children and adults are at risk of severe complications. In areas where extreme gradients in exposure exist there can be marked differences in clinical malaria, even in rural areas (Clarke et al. 2002). It follows that malaria risk across Africa is highly dynamic, and classical risk maps may need to be adapted to account for local variations caused by environmental conditions.

Malaria interventions are aimed at lowering the force of transmission by a reduction in infectious mosquito bites. Paradoxically, this strategy may enhance malaria risk rather than reduce it, because of a transition from stable to unstable malaria (Greenwood 1996). Recent studies have estimated how much reduction of EIR must be achieved in order to control malaria successfully (Snow and Marsh 1995; 1998; Trape and Rogier 1996; Smith, Leuenberger and Lengeler 2001). However, because the relationship between EIR and malaria morbidity and mortality is determined by factors such as the immune status of the human host, the vectorial competence of the vector and the virulence of the parasite, it has been impossible to make accurate predictions of malaria risk. Nevertheless, for lack of anything better, values of the EIR are widely used to estimate the risk of malaria throughout Africa (Rogers et al. 2002). Outside this continent the EIR is difficult to estimate with any precision because of low vector densities and the predominantly zoophilic nature of many vectors.

Because the ecological factors that determine EIR are not well understood, it cannot be predicted at present what the outcome of a transgenic mosquito release will

be on this epidemiological parameter. In areas with multiple vectors, neutralising one vector species by introgression of a transgenic trait for resistance to the parasite may create a niche for a rare parasite strain, adapted to a different vector species, to exploit, thus removing the advantage created by the introduction of the transgene. The EIR may then not change much, having a minimal impact on malaria risk. On the other hand, a highly successful effect of the transgenic release may reduce the EIR to such low levels that the immune status of the human host is compromised, leading to more severe and complicated malaria than before. In general it is thought that the latter situation would not be serious provided adequate health care is available.

Vectorial competence and spatial variations

The important role of *An. gambiae s.s.* and *An. arabiensis* as vectors of malaria in Africa, as discussed in the sections above, can only be explained by the high competence of these mosquito species for the uptake, development and transmission of *P. falciparum*. This 'vectorial competence' is determined by intrinsic and extrinsic factors such as the insect's physiology, anti-parasite defence system, biting and resting behaviour and the microclimate of its habitat. The intrinsic factors are of course genetically determined, while the extrinsic factors will vary spatially. Thus, at the limit of malaria distribution it will often be the temperatures or absence of rain that affect the vectorial competence. This will create areas with 'anophelism without malaria', mainly because of the high temperatures that do not allow for development of the *Plasmodium* parasite (Lindsay et al. 1991). Geographic-information systems using climate data have been used to model the potential distribution of malaria in Africa, often using the vectorial competence to determine geographic regions where the disease can be transmitted (Sutherst 1993; Martens 1998; Craig, Snow and Le Sueur 1999). Under harsh or unusual climatic and ecological events such as unexpected drought, the mortality rate of the vectors may be very high, causing a sudden reduction in malaria deaths. However, such benefits are often of short duration only. Should this situation become more permanent, then additional scenarios must be modelled in order to predict the potential effects on human health in the area concerned. Although the relationship between anopheline vector and malaria parasite appears fixed, it should be realized that the ability of the mosquito's innate preference for specific climatic systems is more plastic and can be used to good advantage.

Discussion

Spatial and temporal variations in vector distribution and vector population dynamics and behaviour affect the current distribution of malaria in Africa. It is not the presence of an infectious reservoir in humans that is the greatest contributing factor to the highly endemic status of much of tropical Africa, but it is rather the extremely high vectorial competence, long survival and preference for human blood of the near-ubiquitous *An. gambiae* that maintain the status quo of malaria. Several decades of interventions for malaria control have attempted to release Africa of the burden of malaria, but to no avail, hampered by weak health systems, poor governance and poverty. Adaptation of the vector(s) to the varied ecological and climatological situations across the continent allows for this situation to continue unabated. We suggest that future interventions should consider the force of ecological associations and the resulting plasticity of the vectors to ecological change, to look for weaknesses in these relationships. Such insights may allow us to develop new

methods for vector control that will reduce the vector population to levels below thresholds of epidemiological significance. Temporal and spatial variations in vector ecology across Africa affect the transmission risk and epidemiology of malaria, and interventions will have to adopt an approach that allows for the consideration of ecological factors that affect the force of transmission in different geographical zones. Transgenic mosquitoes can contribute to this goal, provided base-line information on essential ecological processes such as mating, host-seeking and oviposition are properly understood.

References

Andrewartha, H.G. and Birch, L.C., 1954. *The distribution and abundance of animals*. University of Chicago Press, Chicago.

Bayoh, M.N., Thomas, C.J. and Lindsay, S.W., 2001. Mapping distributions of chromosomal forms of *Anopheles gambiae* in West Africa using climate data. *Medical and Veterinary Entomology,* 15 (3), 267-274.

Bøgh, C., Clarke, S.E., Pinder, M., et al., 2001. Effect of passive zooprophylaxis on malaria transmission in The Gambia. *Journal of Medical Entomology,* 38 (6), 822-828.

Bøgh, C., Thomas, C.J., Clarke, S.E., et al., (in press). Localised breeding of Anopheles gambiae complex mosquitoes along the River Gambia, West Africa. *Bulletin of Entomological Research*.

Braack, L.E., Coetzee, M., Hunt, R.H., et al., 1994. Biting pattern and host-seeking behavior of *Anopheles arabiensis* (Diptera: Culicidae) in northeastern South Africa. *Journal of Medical Entomology,* 31 (3), 333-339.

Bruce-Chwatt, L.J., 1985. *Essential malariology*. 2nd edn. Heinemann, London.

Bryan, J.H., Di Deco, M.A., Petrarca, V., et al., 1982. Inversion polymorphism and incipient speciation in *Anopheles gambiae s. str.* in the Gambiae, West Africa. *Genetica,* 59, 167-176.

Bryan, J.H., Petrarca, V., Di Deco, M.A., et al., 1987. Adult behaviour of members of the *Anopheles gambiae* complex in the Gambia with special reference to *An. melas* and its chromosomal variants. *Parassitologia,* 29 (2-3), 221-249.

Charlwood, J.D., 2003. May the force be with you: measuring mosquito fitness in the field. *In:* Takken, W. and Scott, T. W. eds. *Ecological aspects for application of genetically modified mosquitoes*. Kluwer Academic Publishers, Dordrecht. Frontis series no. 2.

Charlwood, J.D., Vij, R. and Billingsley, P.F., 2000. Dry season refugia of malaria-transmitting mosquitoes in a dry savannah zone of east Africa. *American Journal of Tropical Medicine and Hygiene,* 62 (6), 726-732.

Clarke, S.E., Bogh, C., Brown, R.C., et al., 2002. Risk of malaria attacks in Gambian children is greater away from malaria vector breeding sites. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 96 (5), 499-506.

Coetzee, M., Craig, M. and Le Sueur, D., 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitol Today,* 16 (2), 74-77.

Coluzzi, M., 1965. Biological observations on the *Anopheles gambiae* complex. *Cahiers Office du Récherche Scientifique Outre-Mer, Serie Entomologie Medicale et Parasitologie,* 3, 183-184.

Coluzzi, M., 1992. Malaria vector analysis and control. *Parasitology Today,* 8, 113-118.

Coluzzi, M., Petrarca, V. and Di Deco, M.A., 1985. Chromosomal inversion intergradation and incipient speciation in Anopheles gambiae. *Bollettino di Zoologia,* 52, 45-63.

Coluzzi, M., Sabatini, A., Della Torre, A., et al., 2002. A polytene chromosome analysis of the *Anopheles gambiae* species complex. *Science,* 298 (5597), 1415-1418.

Coluzzi, M., Sabatini, A., Petrarca, V., et al., 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73, 483-497.

Costantini, C., Gibson, G., Sagnon, N., et al., 1996. Mosquito responses to carbon dioxide in a west African Sudan savanna village. *Medical and Veterinary Entomolgy,* 10 (3), 220-227.

Costantini, C., Sagnon, N.F., Della Torre, A., et al., 1998. Odor-mediated host preferences of West African mosquitoes, with particular reference to malaria vectors. *American Journal of Tropical Medicine and Hygiene,* 58 (1), 56-63.

Craig, M.H., Snow, R.W. and Le Sueur, D., 1999. A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitology Today,* 15 (3), 105-111.

De Jong, R. and Knols, B.G., 1995. Selection of biting sites on man by two malaria mosquito species. *Experientia,* 51 (1), 80-84.

Della Torre, A., Costantini, C., Besansky, N.J., et al., 2002. Speciation within *Anopheles gambiae* - the glass is half full. *Science,* 298 (5591), 115-117.

Della Torre, A., Fanello, C., Akogbeto, M., et al., 2001. Molecular evidence of incipient speciation within *Anopheles gambiae s.s.* in West Africa. *Insect Molecular Biology,* 10 (1), 9-18.

Diatta, M., Spiegel, A., Lochouarn, L., et al., 1998. Similar feeding preferences of *Anopheles gambiae* and *A. arabiensis* in Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 92 (3), 270-272.

Duchemin, J.B., Tsy, J., Rabarison, P., et al., 2001. Zoophily of *Anopheles arabiensis* and *An. gambiae* in Madagascar demonstrated by odour-baited entry traps. *Medical and Veterinary Entomology,* 15 (1), 50-57.

Favia, G., Della Torre, A., Bagayoko, M., et al., 1997. Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive isolation. *Insect Molecular Biology,* 6 (4), 377-383.

Framework for monitoring progress and evaluating outcomes and impact: roll back malaria, 2000. World Health Organization, Geneva, WHO/CDS/RBM/2000.25. 28. [http://mosquito.who.int/cmc_upload/0/000/012/168/m_e_en.pdf]

Garrett-Jones, C., 1964. The human blood index of malaria vectors in relation to epidemiological assessment. *Bulletin of the World Health Organization,* 30, 241-261.

Garrett-Jones, C., Boreham, P.F.L. and Pant, C.P., 1980. Feeding habits of anophelines (Diptera: Culicidae) in 1971-78, with reference to the human blood index: a review. *Bulletin of Entomological Research,* 70, 165-185.

Gentile, G., Slotman, M., Ketmaier, V., et al., 2001. Attempts to molecularly distinguish cryptic taxa in *Anopheles gambiae s.s. Insect Molecular Biology,* 10 (1), 25-32.

Gillies, M.T., 1954. The recognition of age-groups within populations of *Anopheles gambiae* by the pre-gravid rate and the sporozoite rate. *Annals of Tropical Medicine and Parasitology,* 48, 58-74.

Gillies, M.T., De Meillon, B. and Coetzee, M., 1968. *Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region).* 2nd edn. South African Institute for Medical Research, Johannesburg. Publication of the South African Institute for Medical Research no. 54.

Githeko, A.K. and Ndegwa, W., 2001. Predicting malaria epidemics in the Kenyan highlands using clmate data: a tool for decision makers. *Global Change and Human Health,* 2, 54-63.

Greenwood, B., 1996. Fever and malaria. *Lancet,* 348 (9023), 280-281.

Greenwood, B. and Mutabingwa, T., 2002. Malaria in 2002. *Nature,* 415 (6872), 670-672.

Haddow, A.J., 1954. Studies of the biting habits of African mosquitos: an appraisal of methods employed, with special reference to the twenty-four hour catch. *Bulletin of Entomological Research,* 45, 199-242.

Hay, S.I., Rogers, D.J., Toomer, J.F., et al., 2000. Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, internet access and review. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 94 (2), 113-127.

Holt, R.A., Subramanian, G.M., Halpern, A., et al., 2002. The genome sequence of the malaria mosquito *Anopheles gambiae. Science,* 298 (5591), 129-130,141-149.

Hunt, R.H., Coetzee, M. and Fettene, M., 1998. The *Anopheles gambiae* complex: a new species from Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 92 (2), 231-235.

Klinkenberg, E., Takken, W., Huibers, F., et al., 2003. The phenology of malaria mosquitoes in irrigated rice fields in Mali. *Acta Tropica,* 85 (1), 71-82.

Knols, B.G.J., De Jong, R. and Takken, W., 1995. Differential attractiveness of isolated humans to mosquitoes in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 89, 604-606.

Koenraadt, C.J. and Takken, W., 2003. Cannibalism and predation among larvae of the *Anopheles gambiae* complex. *Medical and Veterinary Entomolgy,* 17 (1), 61-66.

Lacey, L.A. and Lacey, C.M., 1990. The medical importance of riceland mosquitoes and their control using alternatives to chemical insecticides. *Journal of the American Mosquito Control Association,* 2 (Suppl), 1-93.

Lemasson, J.J., Fontenille, D., Lochouarn, L., et al., 1997. Comparison of behavior and vector efficiency of *Anopheles gambiae* and *An. arabiensis* (Diptera:Culicidae) in Barkedji, a Sahelian area of Senegal. *Journal of Medical Entomology,* 34 (4), 396-403.

Lindsay, S., Ansell, J., Selman, C., et al., 2000. Effect of pregnancy on exposure to malaria mosquitoes. *Lancet,* 355 (9219), 1972.

Lindsay, S.W., Adiamah, J.H., Miller, J.E., et al., 1993. Variation in attractiveness of human subjects to malaria mosquitoes (Diptera: Culicidae) in The Gambia. *Journal of Medical Entomology,* 30 (2), 368-373.

Lindsay, S.W., Armstrong-Schellenberg, J.R., Zeiler, H.A., et al., 1995. Exposure of Gambian children to *Anopheles gambiae* malaria vectors in an irrigated rice production area. *Medical and Veterinary Entomolgy,* 9 (1), 50-58.

Lindsay, S.W., Emerson, P.M. and Charlwood, J.D., 2002. Reducing malaria by mosquito-proofing houses. *Trends in Parasitology,* 18 (11), 510-514.

Lindsay, S.W. and Martens, W.J., 1998. Malaria in the African highlands: past, present and future. *Bulletin of the World Health Organization,* 76 (1), 33-45.

Lindsay, S.W., Parson, L. and Thomas, C.J., 1998. Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae sensu stricto* and *An. arabiensis,* using climate data. *Proceedings of the Royal Society of London. Series B. Biological sciences,* 265 (1399), 847-854.

Lindsay, S.W., Shenton, F.C., Snow, R.W., et al., 1989. Responses of *Anopheles gambiae* complex mosquitoes to the use of untreated bednets in The Gambia. *Medical and Veterinary Entomolgy,* 3 (3), 253-262.

Lindsay, S.W., Wilkins, H.A., Zieler, H.A., et al., 1991. Ability of *Anopheles gambiae* mosquitoes to transmit malaria during the dry and wet seasons in an area of irrigated rice cultivation in The Gambia. *Journal of Tropical Medicine and Hygiene,* 94 (5), 313-324.

Lyimo, E.O., Takken, W. and Koella, J.C., 1992. Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae. Entomologia Experimentalis et Applicata,* 63, 265-271.

Macdonald, G., 1957. *The epidemiology and control of malaria.* Oxford University Press, London.

Marchand, R.P., 1984. Field observations on swarming and mating in *Anopheles gambiae* mosquitoes in Tanzania. *Netherlands Journal of Zoology,* 34, 367-387.

Martens, P., 1998. *Health and climate change : modelling the impacts of global warming and ozone depletion.* Earthscan, London. Health and the environment series.

Molineaux, L. and Gramiccia, G., 1980. *The Garki project: research on the epidemiology and control of malaria in the Sudan Savanna of West Africa.* World Health Organization, Geneva.

Morel, C.M., Touré, Y.T., Dobrokhotov, B., et al., 2002. The mosquito genome - a breakthrough for public health. *Science,* 298 (5591), 79.

Mwangi, R.W. and Mukiama, T.K., 1992. Irrigation scheme or mosquito hazard: a case study in Mwea Irrigation Scheme. *Hydrobiologia,* 232, 19-22.

Najera, J.A., 1989. Malaria and the work of WHO. *Bulletin of the World Health Organization,* 67 (3), 229-243.

Omer, S.M. and Cloudsley-Thompson, J.L., 1968. Dry season biology of *Anopheles gambiae* in the Sudan. *Nature,* 217, 879-880.

Omer, S.M. and Cloudsley-Thompson, J.L., 1970. Survival of female *Anopheles gambiae* Giles through a 9-month dry season in Sudan. *Bulletin of the World Health Organization,* 42 (2), 319-330.

Petrarca, V., Carrara, G.C., Di Deco, M.A., et al., 1983. Il complesso *Anopheles gambiae* in Guinea Bissau. *Parassitologia,* 25 (1), 29-39.

Petrarca, V., Nugud, A.D., Ahmed, M.A., et al., 2000. Cytogenetics of the *Anopheles gambiae* complex in Sudan, with special reference to *An. arabiensis*: relationships with East and West African populations. *Medical and Veterinary Entomolgy,* 14 (2), 149-164.

Rogers, D.J., Randolph, S.E., Snow, R.W., et al., 2002. Satellite imagery in the study and forecast of malaria. *Nature,* 415 (6872), 710-715.

Schneider, P., Takken, W. and McCall, P.J., 2000. Interspecific competition between sibling species larvae of *Anopheles arabiensis* and *An. gambiae. Medical and Veterinary Entomolgy,* 14 (2), 165-170.

Schreck, C.E., Kline, D.L. and Carlson, D.A., 1990. Mosquito attraction to substances from the skin of different humans. *Journal of the American Mosquito Control Association,* 6 (3), 406-410.

Service, M.W., 1993. *Mosquito ecology : field sampling methods*. 2nd edn. Elsevier Applied Science, London.

Smith, T., Charlwood, J.D., Takken, W., et al., 1995. Mapping the densities of malaria vectors within a single village. *Acta Tropica,* 59, 1-18.

Smith, T.A., Leuenberger, R. and Lengeler, C., 2001. Child mortality and malaria transmission intensity in Africa. *Trends in Parasitology,* 17 (3), 145-149.

Snow, R.W., Bastos de Azevedo, I., Lowe, B.S., et al., 1994. Severe childhood malaria in two areas of markedly different falciparum transmission in east Africa. *Acta Tropica,* 57 (4), 289-300.

Snow, R.W. and Marsh, K., 1995. Will reducing *Plasmodium falciparum* transmission alter malaria mortality among African children. *Parasitology Today,* 11, 188-190.

Snow, R.W. and Marsh, K., 1998. New insights into the epidemiology of malaria relevant for disease control. *British Medical Bulletin,* 54 (2), 293-309.

Snow, W.F., 1983. Mosquito production and species succession from an area of irrigated rice fields in The Gambia, West Africa. *Journal of Tropical Medicine and Hygiene,* 86 (6), 237-245.

Snow, W.F., 1987. Studies of house-entering habits of mosquitoes in The Gambia, West Africa: experiments with prefabricated huts with varied wall apertures. *Medical and Veterinary Entomolgy,* 1 (1), 9-21.

Sousa, C.A., Pinto, J., Almeida, A.P.G., et al., 2001. Dogs as a favored host choice of *Anopheles gambiae sensu stricto* (Diptera: Culicidae) of São Tomé, west Africa. *Journal of Medical Entomology,* 38 (1), 122-125.

Surtees, G., 1970. Effects of irrigation on mosquito populations and mosquito-borne disease in man, with particular reference to ricefield extension. *International Journal for Environmental Studies,* 1, 35-42.

Sutherst, R.W., 1993. Arthropods as disease vectors in a changing environment. *In:* Lake, J. V., Bock, G. R. and Ackrill, K. eds. *Environmental change and human health*. Academic Press, London, 124-145. Ciba Foundation Symposium. No. 175.

Sutherst, R.W. and Maywald, G.F., 1995. Predicting insect distributions in a changed climate. *In:* Harrington, R. and Stork, N. E. eds. *Insects in a changing environment : 17th symposium of the Royal Entomological Society of London, 7-10 September 1993 at Rothamsted Experimental Station, Harpenden*. Academic Press, London, 59-61.

Takken, W., 2002. Do insecticide-treated bednets have an effect on malaria vectors? *Tropical Medicine and International Health,* 7 (12), 1022-1030.

Takken, W., Eling, W., Hooghof, J., et al., 1999. Susceptibility of *Anopheles quadriannulatus* Theobald (Diptera: Culicidae) to *Plasmodium falciparum. Transactions of the Royal Society of Tropical Medicine and Hygiene,* 93 (6), 578-580.

Takken, W., Klowden, M.J. and Chambers, G.M., 1998. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae sensu stricto*

(Diptera: Culicidae): the disadvantage of being small. *Journal of Medical Entomology,* 35 (5), 639-645.

Takken, W. and Knols, B.G., 1999. Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annual Review of Entomology,* 44, 131-57.

Thomas, C.J. and Lindsay, S.W., 2000. Local-scale variation in malaria infection amongst rural Gambian children estimated by satellite remote sensing. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 94 (2), 159-163.

Thomson, M.C., Connor, S.J., Milligan, P., et al., 1997. Mapping malaria risk in Africa: what can satellite data contribute? *Parasitology Today,* 8, 313-318.

Touré, Y.T., Dolo, G., Petrarca, V., et al., 1998. Mark-release-recapture experiments with *Anopheles gambiae s.l.* in Banambani Village, Mali, to determine population size and structure. *Medical and Veterinary Entomology,* 12 (1), 74-83.

Trape, J.F. and Rogier, C., 1996. Combating malaria morbidity and mortality by reducing transmission. *Parasitology Today,* 12, 236-420.

White, G.B., 1972. The *Anopheles gambiae* complex and malaria transmission around Kisumu, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 66 (4), 572-581.

White, G.B., 1974. Anopheles gambiae complex and disease transmission in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 68 (4), 278-301.

White, G.B. and Rosen, P., 1973. Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Diptera: Culicidae). II. Ecology of species A and B in savanna around Kaduna, Nigeria during transition from wet to dry season. *Bulletin of Entomological Research,* 62, 613-625.