FACTORS AFFECTING MALARIA TRANSMISSION BY VECTOR MOSQUITO POPULATIONS IN WESTERN KENYA, WITH SPECIAL REFERENCE TO ALTITUDE

BY

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This thesis is dedicated to my father,

the late Thomas Adungo Angalu

DECLARATIONS

This is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University supervisors.

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ABSTRACT

Five populations of *Anopheles* vectors of malaria from different altitudes along a transect in Nyanza Province of western Kenya were studied over a period of two years, January, 1989 through to December, 1990. Each population was sampled by two methods: the pyrethrum-spray-sheet collection (PSC) technique for the day-resting, and the human-bait technique for the biting population (WHO 1975; Service, 1976). The following parameters were then determined:

(1) species composition,

(2) the relative density of indoor day-resting populations in inhabited houses,

(3) the relative density of the biting populations

(4) the man-biting rates and biting cycles between 19.00 and 07.00 hours, and for a full 24 hour day cycle,

(5) Blood feeding preferences,

(6) parity rates, (7) sporozoite rates, and

(8) entomological inoculation rates, i.e. man-biting rate x sporozoite rate.

In the PSC technique, a total of 192 house searches were carried out by sampling once monthly from eight human-inhabited houses at each altitude. The anophelines were identified morphologically with the aid of keys. Siblings of the *An. gambiae* complex were separated by the cytogenetic identification of polytene chromosomes as described by Coluzzi & Sabatini (1967). Bloodmeal types were identified by the bloodmeal enzyme-linked immunosorbent assay (ELISA) technique (Service, 1986).

The human-bait catches were performed at three different altitudinal sites located at 1219 m (Ahero), 1350 m (Rota) and 1524 m (Oriwo). The protocol involved hourly catches for 12 hours of the night, and on six occasions throughout the 24 hours of day and night, by a team comprising two collectors seated indoors and two others seated outdoors with their legs exposed, and collecting mosquitoes from themselves using test-tubes with the aid of torch lights (WHO, 1975). The data was used for the analysis of the man-biting rates, biting cycles, longevity and parity rates. Sporozoite rates were determined by the ELISA technique described by Burkot *et al.* 1984 and Wirtz *et al.* 1985 and also by dissection for comparison; and were used to calculate the entomological inoculation rates. All the parameters were correlated to altitude, seasonal and climatic changes.

The anopheline composition along the transect varied in species diversity and reduced in population abundance with rising altitude. Member species of the *Anopheles gambiae* complex *An. gambiae* s.s. and *An. arabiensis* were caught at all altitudes. *An. funestus* was not found in collections above 2100 m above sea level. At the lower altitude of 1219 m (Ahero), *An. arabiensis* existed as a homogeneous population, whereas at higher altitudes this species and *An. gambiae* s.s. existed as sympatric (mixed) populations, with the latter tending to be predominant. Small numbers of *An. zeimanni*, *An. pharaoensis* and *An. coustani* were also captured.

The man-biting rates reduced with rising altitude from a peak of 108 bites/man/night for *An. arabiensis* at 1219 m, through 28.3 bites/man/night in a sympatric population, to less than 0.1 bites/man/night in a homogeneous population of *An. gambiae* s.s. at 1524 m. The man-biting rate for *An. funestus*, also reduced from 69.3 bites/man/night to 65.1 bites/man/night and less than 0.1 bites/man/night at the respective altitudes.

Altitude and seasonality affected the parity rates and age composition. *An. arabiensis* at 1219 m had a lower parity rate than *An. gambiae* s.s. at higher altitudes. However, the parity rate for *An. funestus* at 1350 m was higher than that of the same species at 1219 m. Age-composition studies showed that the percentage of age-groups at each gonotrophic cycle differed with altitude, with the duration of the gonotrophic cycle of the three vectors being longer at higher altitude.

Although sporozoite rates were bound to differ with species, it was evident that altitude affected sporozoite development in a given species. While the mean sporozoite rate in *An. arabiensis* from 1219 m was 0.3%, it was significantly higher - 5.3% at 1350 m and 5.5% at 1524 m in its closest relative, *An. gambiae* s.s. The sporozoite rates in *An. funestus* also showed an increasing trend, from 1.9% at 1219 m to 4.2% at 1350 m and 4.0% at 1524 m. These differences occurred also in day resting populations, notably *An. gambiae* s.s. whose sporozoite rates increased from 3.7% at 1524 m to 5.3% at 1829 m and 12.5% at 2134 m. Irrespective of altitude, no sporozoite infections were detected in *An. zeimanni, An. pharoensis* and *An. coustani*.

In contrast to the sporozoite rates, the entomological inoculation rate (EIR) reduced with increasing altitude. The EIR was intermittent and unstable at 1524 m, medium at 1350 m and intense at 1219 m. This phenomenon was consistent with parasitemia rates observed in school children during the same period. A comparison of the three vectors revealed that *An. funestus* consistently had a higher entomological inoculation rate at each altitude than the other two vectors.

Bloodmeal analysis for pooled samples of the three vectors showed that at 1524 m feeding was predominantly on humans in contrast to both human and bovid feeding exibited at 1350 m and 1219 m. Human feeding by *An. arabiensis* was only 28.8% in contrast to 63.5-72% for bovid feeding. However, human feeding was above 90% in both *An. gambiae* s.s. and *An. funestus* without significant variation in altitude.

It is concluded that altitude, besides seasonal, ecological and climatic factors, appeared to have significant effect on malaria epidemiology. In particular, malaria transmission albeit very low and intermittent, occurred also at the high altitudes, formerly known popularly as the "white highlands", contrary to the long held notion that malaria did not occur in these "white highlands" of Kenya. The practical implications of these observations are that malaria control strategies against the mosquito vectors must be carefully selected in relation to altitude and their ecological diversity.

INTRODUCTION

Malaria is a human disease caused by a protozoan organism known as *Plasmodium*, of which so far, four species are known: *Plasmodium falciparum* Welch, 1897, *P. malariae* (Laveran, 1881), *P. ovale* Stephens, 1922 and *P. vivax* (Grassi and Feletti, 1890). The organism develops in both man as the human host, and in the female of some *Anopheles* mosquito species which are also the vectors.

Malaria continues to be a serious health problem in many developing countries of the tropics where it is a major cause of morbidity and mortality to human beings (WHO,1986). Young children and pregnant women are especially vulnerable to severe infection. The World Health Organisation (WHO) estimates that more than 400 million people suffer from the disease, resulting in 1.5 million annual deaths. In sub-saharan Africa alone well over 92 million new clinical cases of malaria occur every year (WHO, 1986). In many parts of Kenya malaria exists as an endemic disease (Diesfield, 1978). In the 1980s, for example, it was estimated that among a population of 18 million, there were 13.5 million (722 per 1000) cases of out-patient hospital attendance annually attributable to malaria (Rept. Min. Health, Kenya Govt, 1989). Malaria, therefore, is a serious health and socio-economic problem in Kenya. In the western areas of Kenya the disease has been described as holoendemic (Roberts, 1974). Inspite of this, there has been very little success to control it, not only in Kenya but also in most other countries affected (WHO, 1986). Indeed, the great progress that had been made in combating this disease has increasingly become frustrated by two main constraints, namely, the increasing resistance of the human stage of the parasite to antimalarial drugs, and of the vector mosquitoes to insecticides.

Malaria is transmitted by mosquitoes of the genus *Anopheles*. In Africa, the main vectors are *Anopheles funestus* Giles and *An. gambiae* Giles species complex (Gillies & De Meillon, 1968). It is, therefore, evident that besides chemotherapy aimed at killing the human stage of the parasite, one other most effective strategy to control malaria involves the killing of both the adult host mosquitoes and their immature stages. For a while insecticides proved to be the most satisfactory means of achieving this goal, as was evident from concerted indoor spraying in western Kenya with fenithrothion, which was able to reduce malaria prevalence among inhabitants from 90% to 15% within 2 years (Fontaine & Pull, 1977). However, it soon became apparent that because of insecticide resistance in the vectors, this method alone could not sustain malaria control without a sound understanding of several other physical and biological factors that may affect the vectors within the environment. For example, malaria prevalence in school children nearest the Lake Victoria shore was shown to be over 90%, whereas in those at a distance only one kilometre further away it was less than 70% (Oster *et al.*, 1986). The reasons for such variation need to be understood in order to formulate cost-effective strategies to control malaria.

Before undertaking malaria control, it is necessary to measure the variable factors that influence the individual malaria transmission situation. These epidemiological factors include, not only the various ecological factors, but also the socio-economic processes of the people that interact with the basic elements in the transmission chain - parasite, vector and infected and uninfected host.

The WHO experts believe that:

(a) anti-malarial operations are likely to succeed if directed against well defined eco-epidemiological strata of small magnitude than the large national or global eradication

campaigns attempted in the early sixties (WHO, 1983).

(b) the factors that could be stratified for any epidemiological study aimed at effective control of malaria must be considered. These were given as:

(i) the distribution, behaviour and vectorial efficiency of vector mosquito species,

(ii) the distribution and relative prevalence of the different *Plasmodium* species,

(iii) the density of malaria vectors and their infectivity, as parameters for the intensity of malaria transmission,

(iv) the ecological, seasonal, topographical and other environmental characteristics of different areas in relation to disease transmission.

Studies on malaria transmission in Kenya carried out by several workers in the early 1960s and 1970s were mainly short term, often covering only one season. The usefulness of the results from these studies with regard to malaria control was limited because the dynamics of the disease transmission would become altered drastically as a result of the adaptation of the vector to the changing environment. This would call for further research for fresh knowledge on the epidemiological and entomological aspects of malaria transmission. In this regard, there appears to be little information from Kenya about the relationships between the prevalence of malaria and the various bionomic factors that are likely to influence its transmission, especially physical features such as altitude. Knowledge of these factors is essential for intervention on the transmission cycle. It was against this background that studies based on a longitudinal transect were undertaken in Nyanza Province of western Kenya, an area reported to be holoendemic for malaria (Roberts, 1974).

GENERAL OBJECTIVE

The general objectives of the study were to monitor the seasonal population densities, age structure and vectorial capacity of malaria mosquito vectors along a longitudinal transect traversing over various altitudinal levels; in order to determine the malaria incidence and its transmission patterns in western Kenya. It is possible that such information would assist in the formulation of effective strategies for malaria control programmes in western Kenya.

1.2 SPECIFIC OBJECTIVES.

1.2.1 To examine variation in anopheline mosquitoes'blood feeding patterns, biting peaks and man-biting rates in relation to the intensity of malaria transmission at the same altitudinal sites.

1.2.2 To determine the *P. falciparum* sporozoite rates and the concomitant inoculation rates in geographically distinct areas of Western Kenya situated at different altitudes.

1.2.3 To examine the seasonal pattern and ecology of the sibling species of the *An. gambiae* complex and their relationship to sporozoite rates and infectivity at the same sites.

1.2.4 To determine seasonal and environmental factors, including, temperature and relative humidity that affect sporozoite rates, blood feeding preferences, survivorship and infectivity of the anophelines at the same sites.

1.2.5 To determine the overall influence of the above entomological factors to the intensity and epidemiological variation of malaria transmission, in Western Kenya.

CHAPTER ONE

1. LITERATURE REVIEW

1.1 Epidemiology of Malaria in Kenya.

Roberts (1974) analysed yearly records of malaria occurrence or epidemiology in Kenya using cases of inflamed spleen rates in children aged between 2 and 9 years, a technique developed by the WHO (1963). From these data malaria occurrence and its annual distribution in the various parts of the country were categorised into three main groups, namely, endemic, epidemic and no transmission. Epidemic transmission is marked by upward fluctuation of malaria incidence leading to an explosive increase in the number of cases of the disease over a short period usually as a result of heavy rainfall. In contrast, endemic malaria is associated with habitual presence of the disease and its transmission agents, the *Anopheles* mosquitoes. Endemic malaria may be further divided into holoendemic, hyperendemic, mesoendemic or hypoendemic to define increasing levels of prevalence as estimated by both surveys of the spleen and parasite rates in particular age groups (Table 1.1). The locality of the present studies fell into the endemic (subdivision holoendemic) category. Adungo *et al.* (1991 a) were concerned with the prevalence of malaria in western Kenya as shown by parasitemia in school children and demonstrated that active transmission of the disease was influenced by the altitude, between 1200 and 2100 m above sea level and its intensity varied with season. Roberts (1974) further reported that the distribution and relative abundance in Kenya of the four known protozoan pathogens that cause malaria also varied as follows: *Plasmodium falciparum* was the most common (80 - 85%), followed by *P. malariae*, (10-15%), *P. ovale* (< 5%) and *P. vivax* was reported as infrequent in the human population. Beier *et al.* (1988) made a similar study in the Kisumu area using the presence of the protozoans in the salivary glands of the vector mosquitoes. She found equally higher rates for *P. falciparum* and lower ones for the other species.

Table 1.1: Malaria Epidemiology by Type and Area. (after Roberts, 1974)

Classification/degree	Spleen rate (age 2-9)	Area
(1) Endemic (a) holoendemic	> 75 %	Coast Province, coastal area, Tana River, Kano Plains, and Taveta.
(b) hyperendemic	50 - 74 %	North Nyanza, Bungoma, Busia, Simba Hills (Coast).
(c) mesoendemic	10 - 49 %	Machakos, Kitui, Thika, parts of North Nyanza, Muranga and Embu, below 1,300 m.
(d) hypoendemic	< 10 %	Meru, Pokot, Samburu, Isiolo, and Baringo
(2) Epidemic	variable	Highland over 1,600 m with high rainfall and dry areas with exceptional rainfall: Masailand Nandi, Kericho, Kisii, NFD, Eastern Kitui, Londiani, and Elgeyo.
(3) No transmission (sometimes anophelism without malaria)	none	At altitude over 2,000 m, Mt. Kenya, Mt. Elgon (forest, moorland, plateaux).

1.2 Mosquito Vectors of Malaria in Kenya

Malaria vectors in the study area were identified over 60 years ago by Symes (1927) and Garnham (1929) who studied mosquitoes of the shores of Lake Victoria and the central highlands of Kenya. These were *Anopheles gambiae* Giles and *An. funestus* Giles. At that time, *An. gambiae* was regarded as a single opportunistic species adapted to a variety of environments. Holstein (1952) was the first person to suspect that this species consisted of two "races". But it was not until ten years later that Patterson (1963) demonstrated the existence of two sibling species in *An. gambiae*. In the same year Patterson *et al.* (1963) working in South Africa, added a third sibling to the list and the three became designated as species A, B and C. Later studies by Davidson *et al.* (1967) showed that *An. gambiae* was in fact a complex of species, and with the advent of cytogenetic techniques for the identification of females, Coluzzi (1968); Green (1972) and Davidson & Hunt (1973) sorted out this complex as containing two sibling species, which Mattingly (1977) named *An. gambiae sensu stricto* Giles (species A), and *An. arabiensis* Patton (species B), while the third, species C was named *An. quadriannulatus* Theobald. Service (1970) studied the ecology of *An. gambiae* s.s. and *An. arabiensis* in Kisumu and concluded that although the two species were sympatric, *An. gambiae* s.s. was dominant in human inhabited hut collections. However, two years later White (1972) working in the same area observed that *An. arabiensis* was the dominant species in indoor collections 50 km away from Kisumu. Prior to a large scale fenitrothion (OMS-43) trial Joshi *et al.* (1975) confirmed Service's findings that *An. gambiae* s.s. was dominant around the Kisumu area. The role of the various anopheline species in the transmission of malaria around Kisumu was further examined by Highton *et al.* (1979). Other anophelines, including *An. pharoensis* Theobald, *An. ziemanni* Grunberg and *An. coustani* Laveran have also been collected in these areas, notably in the Kano Plains and highlands around it (Khamala, 1971), but they do not

appear to be of vectorial importance with regard to malaria transmission.

Other areas in which malaria vectors occur in Kenya include the coastal area as revealed by the studies of Muirhead-Thomson (1951) on what he called salt-water "*gambiae*". Patterson (1962), using the principle of population genetics, differentiated this form from the West African one, and gave the name *An. merus* Donitz. In later studies, Mosha & Mutero (1982) and Mosha & Patrarca (1983) observed that *An. merus*, which is in fact another sibling species of the *An. gambiae* complex was the main vector, accounting for 96% of the vector population in the villages studied. Working in the Mwea Irrigation Scheme Mosha & Subra (1982) and Mutero (1985) demonstrated that *An. arabiensis* was the only sibling species of the *An. gambiae* complex existing in that area along with *An. funestus*.

1.3 Feeding Preferences of the Malaria Vectors

Tempelis (1975) and Washino & Tempelis (1983) are among the recent workers that have shown that survival succes in mosquitoes depends on reproduction, blood feeding frequency and host selection and this may be related to host preference as against opportunistic feeding. Regarding host preference, Gardos & Smek (1976) showed that the number of eggs produced by some mosquitoes, for example, *Ae. cantans* Meigen was related to the source of particular bloodmeals. However, Hess *et al.* (1968); Boreham *et al.* (1975); Chandler *et al.* (1976) and Highton *et al.* (1979) concluded that whatever mosquitoes feed on in any locality appears to be influenced by a combination of host availability and innate host preference. Bruce-Chwatt *et al.* (1966) confirmed earlier findings that *An. gambiae* s.s. was anthropophilic whether biting from indoors or outdoors, and contrasted with *An. arabiensis* which showed a greater tendency to bite other animals (zoophilic) outside houses. They also found *An. funestus* to equally bite both man and domestic animals indoors and outdoors. Working in the Kano Plains, Chandler *et al.*

(1976) used the precipitin technique to illustrate that *An. gambiae* s.s., *An. funestus* and *An. pharoensis* entered houses to bite man in appreciable numbers; whereas *An. arabiensis* and *An. ziemanni* fed on man and domestic animals outdoors. Studies on biting behaviour among malaria vectors within a single gonotrophic cycle were made by Gillies & Wilkes (1974), Boreham *et al.* (1978), Highton *et al.* (1979) and Mutero & Birley (1987). Mutero & Birley (1987) further observed that multiple feeding, that is taking more than one blood meal within a gonotrophic cycle existed. They placed particular attention to the possible existence of differences in the feeding behaviour between *An. gambiae* s.s. and *An. arabiensis*. In this regard, Service (1970) working in Kisumu, Kenya applied the Human Blood Index (HBI) (= "anthropophilic index", "human blood ratio") technique (WHO, 1963) to determine that despite its zoophilic nature, *An. arabiensis* caught indoors had a higher HBI than *An. gambiae* s.s. caught outdoors, because the latter fed on other hosts in the absence of humans. In Garki, northern Nigeria, Molineaux & Gramicia (1980) observed no significant difference in numbers of *An. gambiae* s.l. from human-bait catches conducted indoors and outdoors, suggesting that this vector preferred man in any biotype.

1.4 Sporozoite Rates in the Malaria Vectors

Studies on the sporozoite rates or vectorial capacity of the various malaria vectors have been made by several researchers working in the Kisumu area of Kenya. Notable among them are the studies by Roberts (1974), who found that *An. gambiae* s.l. could have as high as 10% sporozoite rates. Other studies conducted in the same area on the sporozoite rates of malaria vectors were those by Joshi *et al.* (1975), Highton *et al.* (1979) and Beier *et al.* (1987). Joshi and his collaborators confirmed earlier findings by Roberts (1974), but they also reported that *An. gambiae* s.s. and *An. arabiensis* had equal mean sporozoite rates of 8% and 7.8%,

respectively. Highton's group, however, had lower figures of 5.3% and 0.3% respectively for the same species, implying that *An. arabiensis* was of less importance as an effective malaria transmitter compared to *An. gambiae* s.s. In later studies, Beier and his collaborators found sporozoite rates of 10.9% in *An. gambiae* s.l. and also demonstrated that *An. funestus* was equally an efficient malaria transmitter with sporozoite rates of 10.2%. White (1974) and Molineaux & Gramiccia (1980) were concerned with the differences in sporozoite rates of anopheline species found in various studies and concluded that infection rates tended to be higher in *An. gambiae* s.s. than *An. arabiensis* in line with the greater zoophily of the latter species. Similar observations were made by Service (1985), who attributed the high sporozoite rates to the higher survivorship and longevity of *An. gambiae* s.s., which would enhance the efficiency of this vector.

1.5 Entomological Inoculation Rate (EIR) and Malaria Prevalence

Macdonald (1952) defined sporozoite rate as the prevalence of female *Anopheles* mosquitoes with sporozoites in their salivary glands and applied it as the most sensitive and reliable parameter for describing the epidemiology of malaria. Later, Macdonald (1957) defined the entomological inoculation rate of the *Plasmodium* pathogens as the product of man-biting rate and sporozoite rate. Recently, Burkot *et al.* (1987) were concerned with the entomological inoculation rates of *P. falciparum* and *P. vivax* and showed that they had strong positive correlations with parasite prevalences in children. These findings were later shown to be consistent with the Ross-Macdonald model (Birley & Charlwood, 1987). Using blood smears for malaria parasites in Kisumu, Oster *et al.* (1986) reported that the incidence of reinfection of children with *P. falciparum* was as high as 8.9% per day, and this appeared to result from a corresponding high entomological inoculation rate of 0.39 infective bites/person/day. Adungo

et al. (1991 a) also re-examined the malaria prevalence rates, especially in children below the age of six years. They found that the prevalence rates gradually reduced with rising altitude. At Ahero, an irrigation site situated at a low altitude, the parasite rates were as high as 90%, whereas at high altitude, the rates fell to as low as 3.0%, implying transmission reduced with rising altitude.

1.6 Malaria Epidemiology in relation to Chromosomal Differentiation as an Adaptation to Environment by Malaria Vectors.

Coluzzi *et al.* (1979) were the first to discover that at the genetic level, ecological and geographic variations affect mosquito cytotypes, causing measurable differences of chromosomal inversion frequencies. Later, Coluzzi *et al.* (1985) found that these variations led to clinal changes in the frequency of various polymorphic inversions in species of *An. gambiae* complex collected from savanna and forest localities along a transect from Sudan and Sahel type savannah and forest to Guinea savannah. They also established that there appeared to be differences in the inversions of the populaton associated with the rice cultivated zone from that of the adjacent savannah. Toure *et al.* (1984) working in Mali recognised intergradation of *An. gambiae* s.s., having apparently been induced by a changing environment, into three forms which they termed *Mopti, Bamako*, and *Savanna. Mopti* and *Bamako* appeared to be reproductively isolated species. White (1974) and Coluzzi *et al.* (1985) found that chromosomal inversion polymorphism arose from adaptation of vector populations to extrinsic factors induced by environmental changes acting selectively on inversion genotypes. They claimed that this selection created biological effects resulting in different behavioural traits which had a direct bearing on malaria transmission or control. They cited the most common of these traits as exophily, zoophily and insecticide tolerance. But, by far the most significant discovery of this

phenomenon in malaria epidemiology was by Kitzmiller (1973) through cytogenetics, of a fixed inversion difference in the sex chromosomes of *An. nuneztovari* Gabaldon, which distinguished malaria vector populations in northern South America from non-vector populations farther south. Carnevale & Robert (1987), while studying the West African *An. gambiae* complex observed that the frequency of certain chromosome inversions appeared to be related to the dynamics of malaria transmission.

1.7 Age Composition of Mosquito Populations and Malaria Transmission

Perry (1912) was the first to observe that age-grading of medically important insects was of epidemiological importance, because it enabled one to establish the age and the proportion of individuals in a local population of vectors living long enough to transmit the infective pathogens. In this regard, in mosquito studies age could be measured by observation of either external characters or changes in some internal organs of the insects.

With regard to external characters, Perry (1912) then proceeded to show that age-grading using external characters, such as the degree of wear on the wing fringes, could distinguish between young and old mosquitoes. In later studies, Corbet (1960 & 1962) reported that external wear of abdominal sternites was a reliable characteristic for detecting nullipars of *Mansonia fraseri* Theobald and *Mansonia metallica* Theobald. In their studies, only 0.9% and 0.1% respectively of these two species were shown by dissection to be parous. The larvae of some hydrachnid mites sometimes parasitise mosquitoes, so Gillet (1957) suggested that they could be used to determine nulliparity, since they detach from female mosquitoes during oviposition. But, Wharton (1959), Detinova (1962), Graham (1969) and Morris & DeFoliart (1970) all expressed doubt on the reliability of this method because a number of parous mosquitoes were found to

be still parasitised by these mites. Corbet (1963) however, emphasised that only hygrobatid mites would indicate nulliparity.

One of the internal characters for age-grading female mosquitoes is meconium, a greenish brown waste product of larval food usually seen in the gut of newly emerged mosquitoes, but which usually lasts 72 hours before disappearing. Corbet (1963) used this method as an indicator of nulliparity or young age in *Mansonia fraseri*. Another internal character used in age-grading of female mosquitoes is the presence of the mating plug placed in the female oviduct by the male at copulation, which Gillies (1956) used to study *An. gambiae*. Also the presence of spermatozoa in the spermatheca and the presence of one or more retained mature eggs found in the ovary on dissection have been employed to determine age in mosquitoes.

Detinova (1945) studied ovarian tracheols and observed that coiled ends indicated nulliparity and uncoiled ends resulting from egg laying indicated parity. He pointed out that this method was simple and more reliable than the above methods and was applicable to all mosquito genera. Later, follicular dilatations were observed by Polovodova (1949) and Detinova (1949) after a mature egg has passed down through the oviduct. Both workers noted that this sac-like structure eventually contracted to leave a small but distinct bead-like swelling in the follicular tube of the ovariole, termed "dilatation" or "follicular relic." The presence of these dilatations could be used to distinguish between nulliparous and parous female mosquitoes, and also to determine the number of times separate batches of eggs have been laid (gonotrophic cycle), and were thus used to estimate the age composition of the mosquitoes. Detinova (1962) and Gillies & Wilkes (1965) used this method to study the East African *An. gambiae*.

1.8 Ecological and geographical factors in malaria transmission

Haddow (1942) and Graham (1945) were the first researchers in this region of Africa to determine that the most important ecological factors governing malaria epidemiology in East Africa were geographical, such as altitude and climatic, including temperature, rainfall, relative humidity and season of the year. This is because some of these factors influenced population size and survivorship of the vectors, and thus malaria transmission. Generally, the underlying ecological factors that restrict insect vectors were outlined by Andrewartha and Birch (1954). They described the distribution and abundance of insect populations and how physical and ecological factors, such as weather affect both reproduction and survival rates of insects. In later years Carnevale & Robert (1987) working in Burkina Faso were able to show that macroclimatic fluctuations result in varied malaria transmission patterns.

CHAPTER TWO

2 STUDY AREAS AND GENERAL METHODS

2.1 STUDY AREAS

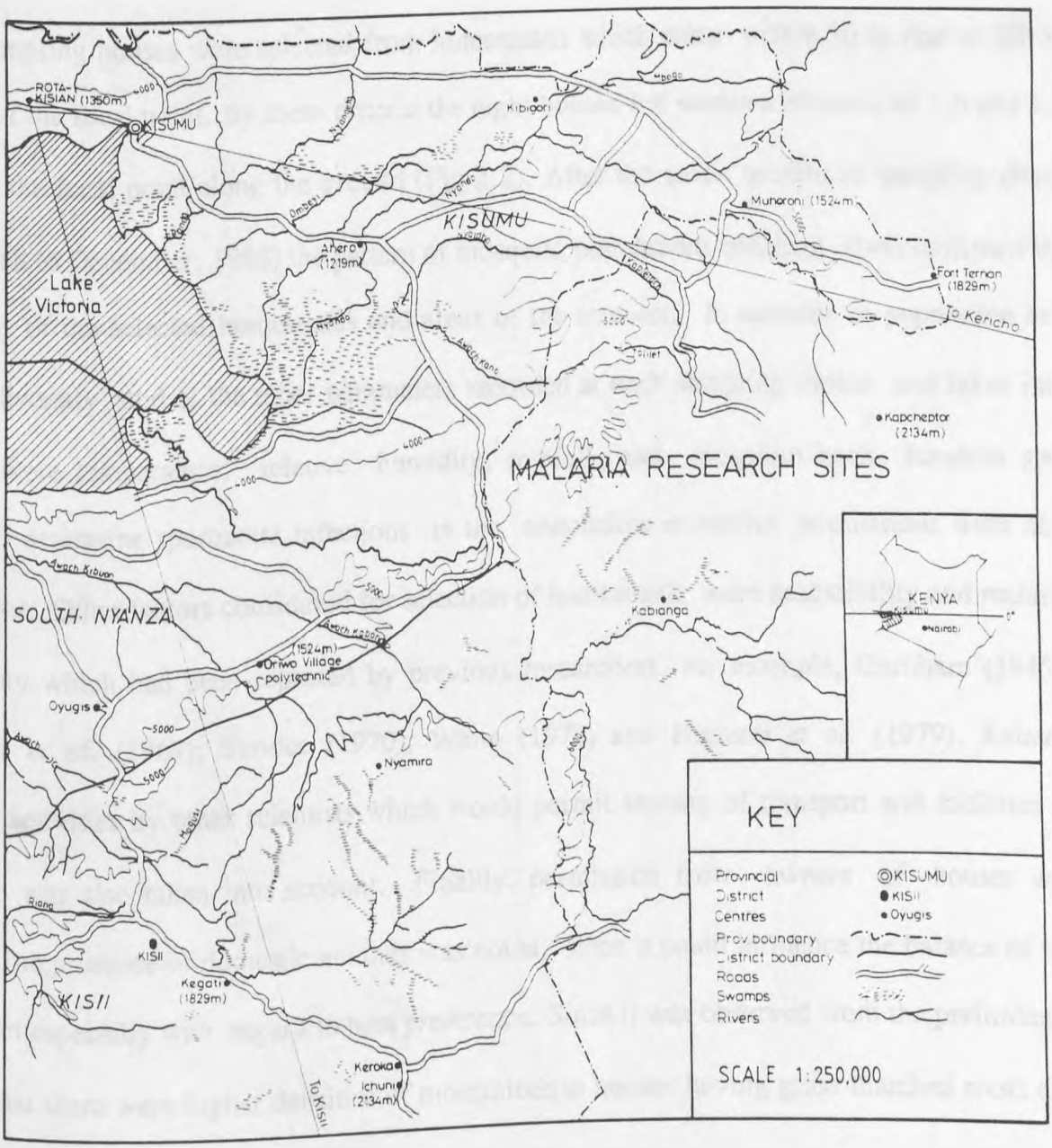
2.1.1 Study Area and Reasons for its Selection

An V-shaped transect line converging at Rota-Kisian, near Kisumu and with one arm running southwards to Kisii along the shores of Lake Victoria and the other arm extending eastwards to Kericho (Fig 2.1) was selected from a contour map. The two directions of the transect were initially chosen in order to duplicate altitude in two different directions, while the transect permitted the study of malaria vectors at different ecological settings. The lowest and highest altitude points along the entire transect were 1212 m to about 2300 m respectively above sea level. The actual study locations were demarcated with the aid of an altmeter and set at intervals of about 300 m rise in altitude between consecutive points convenient for the study. This gave eight localties situated along the two arms of the transect with the necessary variation in height for the observation of changes in mosquito populations, distribution and diversity. The Kisumu - Kericho arm of the transect comprised Ahero, 1219 m, Muhoroni, 1515 m; Fort Ternan, 1818 m and Kapcheptoror, 2121 m. The Kisumu-Kisii arm contained Rota-Kisian, 1350 m, Oriwo, 1524 m; Kegati, 1818 m and Ichuni, 2121 m.

At each altitude a central point was identified and on the basis of the four cardinal points of the compass four homesteads were selected and their demographic data recorded. Those homesteads with a large numerical number of people were selected for study. A typical homestead often comprised of several houses, say two to four for the head of the family, depending on the

Fig.2.1: **Map of Western Kenya showing study sites along the V-shaped transect.**

Source: Survey of Kenya Map: *Lake Basin Development Authority Areas, 1974.*



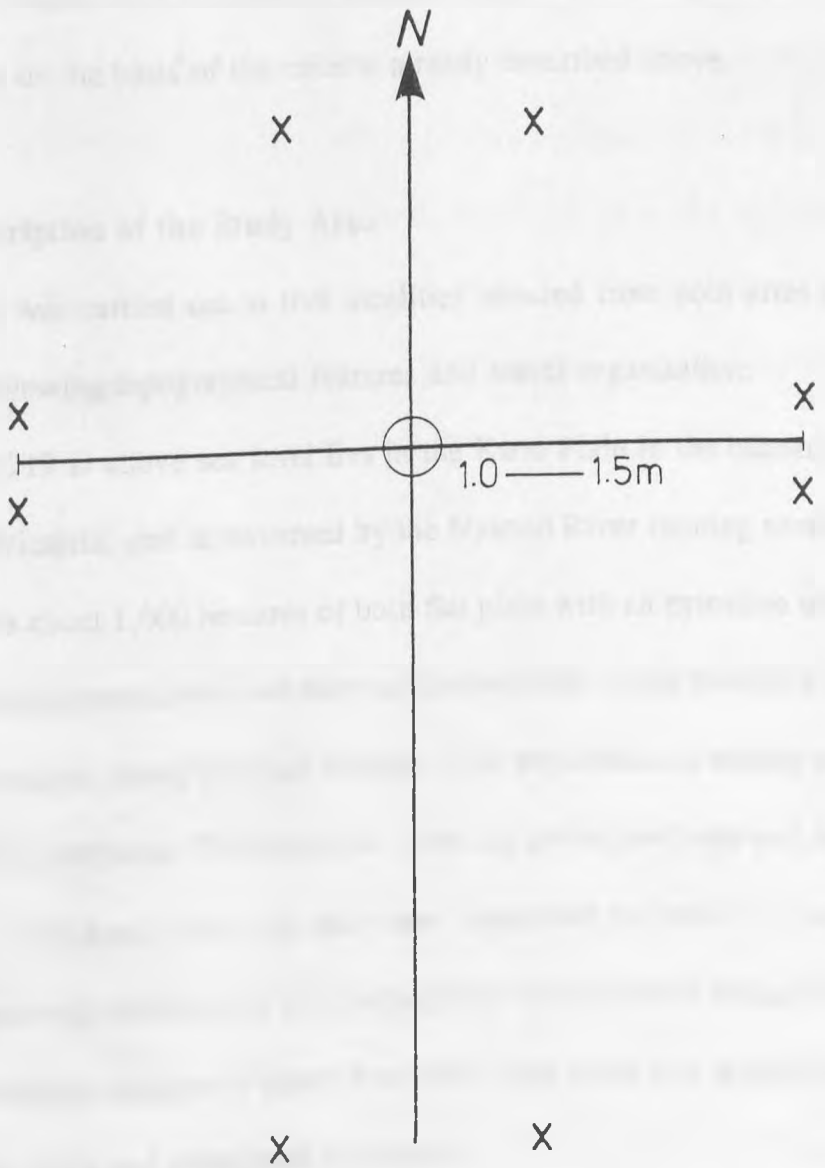
number of wives he has, one for each of the married sons and one for children to sleep in. To select the particular study houses, random mosquito sampling was conducted in the homesteads between June and December, 1988 using Pyrethrum Spray-sheet Collection (PSC) technique once every month. Following these preliminary surveys, a series of eight traditional houses at each site were selected from the homesteads on the basis of the following criteria:

1) harbouring adequate number of anophelines,

2) two of the sampling houses were selected from homesteads situated more or less at each end of the cardinal points of the compass, and

3) the sampling houses were selected from homesteads which were within 50 m rise or fall in altitude of the focal point. By these criteria the eight houses fell within a distance of 1.0 and 1.5 m from the focal point along the ground (Fig 2.2). After the seven months of sampling (from May, 1988 to December, 1988) the pattern of mosquito populations obtained also confirmed the suitability of the selected homesteads and areas of the transect. In addition to population and species diversity studies the other parameters recorded at each sampling station and taken into account were temperature, relative humidity, rainfall and mosquito hosts. Random spot checks to determine sporozoite infections in the anopheline mosquito populations were also carried out. Other factors considered for selection of homesteads were accessibility and malaria endemicity which had been reported by previous researchers, for example, Garnham (1945); Davidson *et al.* (1967); Service (1970); White (1972) and Highton *et al.* (1979). Related research activities by other scientists which would permit sharing of transport and facilities to cut costs was also taken into account. Finallly, permission from owners of houses was sought. The presence of domestic animals was noted, since it could influence the balance of the ecosystem especially with regard to host preference. Since it was observed from the preliminary studies that there were higher densities of mosquitoes in houses having grass thatched roofs and

Fig 2.2: Organisation of a sampling site showing the distribution of houses within homesteads (X) sampled at each altitude



mud walls than in houses having corrugated iron sheet roofs; only houses with grass thatched roofs and mud walls were selected for sampling purposes. This also minimised un-necessary microclimatic or environmental variables. Most of these houses were usually round-shaped, at a diameter of 2.0 to 2.5 m, and with open eaves. One or two windows were round openings of 0.25 m diameter within the wall structure. The main study was finally carried out in houses from the homesteads which had been selected as a result of the preliminary studies. The presence of rivers or swamps if any was noted. Some homesteads had livestock. Each study site was ultimately divided into 8 sampling stations (human inhabited huts) selected from the various homesteads on the basis of the criteria already described above.

2.1.2 Detailed Description of the Study Area

The definitive study was carried out in five localities selected from both arms of the transect. The sites had the following topographical features and social organisation:

(i) Ahero: altitude 1219 m above sea level lies in the Kano Plain in the eastern margin of the Winam Gulf, Lake Victoria; and is traversed by the Nyando River running southwards into the gulf. The area covers about 1,000 hectares of both flat plain with an extensive irrigation scheme for rice growing around Ahero town, and bare scrub land without any extensive tree growth but with intermittent swamps along the lake margin. The population is mainly engaged in rice growing facilitated by irrigation. Two crops of rice are grown per year and are harvested in July and January. The area which has also been described in detail by Surtees (1970) is intermittently covered with paddocks of rice fields grown through canal irrigation at intermittent periods. Human dwellings consist of either huts with mud walls and grass thatched roofs or houses made of mud walls and corrugated iron roofs.

(ii) Rota-Kisian: altitude, 1350 m is situated 15 km west of Kisumu. The vegetaion is the shrub (savannah) type. The population is predominantly occupied in fishing but cattle rearing is practised in a few homesteads. Flooding is very common because of high water retention by the mainly black cotton soil. Most housing consists of huts with mud walls and grass thatched roofs.

(iii) Oriwo; altitude 1524 m above sea level is approximately 80 km south of Kisumu on the main Kisumu-Kisii road. It has well drained sandy loam soils with gentle undulating topography. There is one large permanent swamp and many temporary ponds and rock pools. The vegetation is of savannah (shrub) type with a few eucalyptus, guava and *Lantana camara.* There are two rainy seasons; the long rains from March to June and the short rains from October to November. The temperature ranges from 18-27 C. The relative humidity fluctuates around 50%. The community of approximately 7000 people practices subsistence farming of bananas, potatoes, maize, millet and cassava. Coffee and sisal are grown as cash crops on a small scale. Livestock is kept in most home steads.

(iv) Kegati; 1818 m above sea level is situated 150 km south of Kisumu. Commercial farming of tea is the main occupation of the population. The area has steep slopes often ending in small rivers. Most houses have round mud walls and grass thatched roofs.

(v) Ichuni; 2121 m above sea level has sharp slopes ending in small valleys. There is not much retention of water by the sandy soil. Commercial farming of tea is the main activity of the inhabitants of this area. Most houses are constructed with mud walls and corrugated iron roofs.

2.2 GENERAL MATERIALS AND METHODS

2.2.1 Validation of Laboratory and Sampling Procedures.

Material from mosquitoes caught during the preliminary studies were also used for the validation of the methods to be used. One of these was a comparison between dissection and sporozoite ELISA for the determination of the sporozoite rate, which were carried out in order to determine reproducibility of the two methods. The results of this study are published in a separate report (Adungo *et al.*, 1991).

Methods for dissection of ovaries of malaria vectors for dilatations were developed as described by Polovodova (1949) and Detinova (1949). These methods were validated for accuracy by comparing follicular dilatations observed in the ovaries of mosquitoes from laboratory maintained colonies which had been allowed to lay eggs at least three times with those of wild mosquitoes which had been caught from areas of the preliminary studies.

2.2.2 Mosquito sampling methods:

Mosquitoes were sampled from January, 1989 to December, 1990 using two methods, one for day resting mosquitoes and the other for biting mosquitoes.

2.2.2.1 Sampling of day resting mosquitoes.

Daytime sampling inside houses were made by the "PSC" technique (WHO, 1975; Service, 1976). This was performed once monthly in the 8 selected homesteads. The catches were performed at regular times, between 07.00 and 09.00 hours. Household articles such as furniture were first removed, and food items and water pots covered. White sheets were spread on the floors of houses to be sprayed. The houses were then sprayed with 1.0% mixture of pyrethrum and butoxide in paraffin by one person spraying from the outside through the eaves

and one person inside, after which they were kept closed for 10 minutes. The mosquitoes which had fallen on the sheets from each house were picked up with a pair of forceps and placed in numbered petridishes lined with moist filter paper. The specimens were transported to the laboratory in Kisumu for sorting and identifying the species before computing their densities and any further processing.

2.2.2.2 Sampling of biting mosquitoes

Human-bait catches performed fortnightly, were used to sample both indoor and outdoor biting mosquitoes. The method involved two people seated inside a house or just outside it with their legs and arms exposed and catching mosquitoes from themselves and from one another using test-tubes with the help of flashlights (WHO, 1975); for six hours at a time, whence the team was replaced. Sampling usually ran between 19.00 and 07.00 hours. Some sampling was also conducted between 07.00 and 19.00 hours on six occassions in order to collect mosquitoes biting during day time. Each hourly catch was kept in labelled plastic bags for later identification and processing in the laboratory.

2.2.3 Processing of mosquitoes

Mosquitoes from the PSC collection were initially sorted by sex and all male mosquitoes discarded. The females were then identified to species using morphological characters and counted. Culicine mosquitoes and other non-anopheline species were counted and discarded. All *Anopheles* were retained for further processing and separated according to species which in the main comprised member species of the *An. gambiae* complex and *An. funestus*. After noting the ovarian condition each mosquito was assigned a serial number. Mosquitoes were then divided into several categories according to the test or procedure to be undertaken, and processed as follows:

(a) Mosquitoes for sporozoite studies:

For the determination of sporozoite rates and species in mosquitoes from both the PSC and human-bait collections, specimens from each house were divided into two groups initially, one for dissection for sporozoites and the other for the enzyme linked immunosorbent assay (ELISA) technique. It was necessary to use the ELISA technique in order to determine sporozoite species. Both techniques are described in later chapters. For sporozoite examination using ELISA the mosquitoes were dried in glass dessicators and then stored individually in vials in a deep freeze at -70^{0}C until use. On occassions when sporozoite infections were to be determined by microscopy alone due to lack of ELISA reagents, the salivary glands were dissected out from fresh mosquitoes and examined for sporozoites under a compound microscope.

(b) Mosquitoes for Bloodmeal studies:

Bloodmeal identification was carried out in order to identify the hosts in each study area. For bloodmeal identification, either dried mosquitoes or filter paper host bloodmeal smears were used as starting material. Procedures for bloodmeal identification are described in detail in the relevant chapter.

(i) For freshly fed mosquitoes, i.e., mosquitoes having fresh bloodmeals, preparation of bloodmeal smears of individual mosquitoes were made on *Whatman* No. 91 filter paper divided into 16 sectors, which were numbered according to house, and area from which the mosquitoes were collected. The smears were stored in a refrigerator for later identification of host blood.

(ii) For dried fed mosquitoes, bloodmeals were also harvested by incubating individualy for one hour the abdomen of individual mosquitoes in 1.0 ml of phosphate buffered saline-Tween 20 solution.

2.2.4 Mosquito Cytogenetic Studies

Fresh females identified morphologically as *An. gambiae* s.l. at or nearing Christopher's stage-III ovaries were selected and preserved until slide preparation of the ovaries for cytogenetics. Sporozoite examination was carried out by dissecting salivary glands of the mosquitoes anteriorly into saline. Similarly, the stomachs of the same specimens containing the bloodmeal were also removed and quashed onto numbered sectors of *Whatman* no.91 filter paper to make bloodmeal smears for host identification. After dissecting out the above, the ovaries were removed posteriorly into dilute Carnoy's fixative (5%) for preservation. Carnoy's fixative was prepared by mixing volume to volume three parts of absolute ethanol with one part of glacial acetic acid. Identification of the *An. gambiae* s.l. species was done by making ovarian chromosome preparations using the method of Coluzzi & Sabatini (1967). *An. gambiae* s.s. and *An. arabiensis* were identified by banding patterns on the X-chromosome of adult ovarian polytene chromosomes by comparison against standard maps published by the above authors.

2.2.5 Determination of Entomological Inoculation Rate

Sporozoite rate is defined as the prevalence of female *Anopheles* mosquitoes with sporozoites in their salivary glands. It is the most sensitive and powerful tool for describing the epidemiology of malaria (Macdonald, 1952 & 1957). From sporozoite rate the entomological inoculation rate can be calculated from the formula below:

Entomological Inoculation Rate (EIR) =

Man Biting Rate (MBR) x Sporozoite Rate (S).

2.2.6 Age-grading of female mosquitoes

Age-grading of female mosquitoes was done by observing the number of dilatations (corresponding to gonotrophic cycles) within the ovarian follicle as described by Polovodova (1949) and Detinova (1949). This would indicate the age pattern of females living long enough for the parasite to develop, i.e. through the extrinsic cycle, (usually about 10 days); at each altitude and how this related to the epidemiology of malaria in the area.

Fresh mosquitoes were dissected in 0.65% saline to remove the ovaries. The ovaries were teased out with fine dissection needles to expose the dilatations in the ovarioles. These correspond to the number of egg batches laid (gonotrophic cycles), which were observed and counted under a compound microscope. The age composition was computed as a product of the number of serial dilatations and interval between successive gonotrophic cycles of a given species. In order to have a representative sample mosquitoes at all the four stages of physiological development, namely, unfed, fully fed, half gravid and gravid were dissected and examied.

2.2.7 Measurent of environmental factors

The metereological data collected were rainfall, temperature and the relative humidity. These were obtained from the metereological station ran by the Kenya Metereological Department nearest to the relevant sampling site. Altitude was read from an altmeter. Micro environmental variations in temperature were checked by using a hygrothermograph kept in one of the houses at each site. The daily minimum and maximum temperatures were read and recorded at 07.00 hours and 19.00 hours. The relative humidity and rainfall were also recorded daily at the sampling sites. The rainfall pattern was divided into three seasons, namely, the short rainy

season from September to November; the dry season, from December to February, and the long rainy season, from March to June. Human activities, particularly irrigation and rice growing were monitored by making weekly visits to the study areas. The data was stored in an IBM-PC computer using a *Symphony* (1985) database from which relationships of various parameters were determined graphically using the *Symphony Graphics Program* and computed using *Minitab* (1982) statistical package.

CHAPTER THREE

3 MOSQUITO SPECIES COMPOSITION, SEASONAL ABUNDANCE AND HOUSE DENSITIES OF DAY RESTING *Anopheles* AT DIFFERENT ALTITUDES

3.1 INTRODUCTION

Service (1976) showed that mosquitoes caught from huts may be expressed in terms of mean hut densities and used for the following purposes:

(a) to measure changes in the seasonal and annual abundance,

(b) to compare house resting densities between villages, and

(c) to assess the impact of control measures on the endophilic species. In this study, the technique was extended to also determine the following parameters:

(d) the influence of altitude and other climatic factors on the changes of mosquito populations,

(e) species diversity at a given site,

(f) species distribution both by site, month and season, and

(g) the relationship to the epidemiological pattern of malaria in an area.

3.2 MATERIALS AND METHODS

The preliminary studies gave the strong criteria for selection of the study sites for focussed entomological observations. These were from both the Kisumu-Kisii and Kisumu-Kericho arms of the transect. The study therefore covered Ahero, 1219 m; Rota, 1350 m; Oriwo, 1524 m, Kegati, 1829 m; and Ichuni, 2134 m. The detailed descriptions of these sites are given above in the general methodology section. The selection of these sites was based on the main on entomological observations from the pleminary studies. One of the major considerations among

the entomological observations was the relative density of vector mosquitoes. The other basis for selection was that these sites although not on one arm of the transect, had natural topographical and cultural features and fauna unlike for example, the Kisumu-Kericho arm of the transect which traversed in the main through areas of sugar cane plantations between Ahero and Muhoroni. The three sites with large mosquito populations were further selected for more detailed longitudinal studies such as man-biting rates, inoculation rates, feeding patterns, cytotaxonomy and other parameters. These included Ahero (1219 m), Rota (1350 m) and Oriwo (1524 m), where the two main species of mosquitoes *An. gambiae* s.l. and *An. funestus* were always present. In order to accomplish the overall picture of vector population dynamics on a long term basis, seasonal monitering of the mosquito populations and other studies were continued for two years even at the sites which showed very few or no mosquitoes.

Day resting mosquitoes were caught by the PSC technique once every month at each locality. Eight houses were sampled between 7.00 and 9.00 hours by two teams of collectors. Sampling was done during these hours in order to include in the catch mosquitoes that would leave the houses with the onset of daylight and also in order to minimise time variation factors (Service, 1976). Mosquitoes from individual houses were placed in numbered petridishes lined with moist filter paper and brought to the laboratory for further processing. The male mosquitoes which were easily distinguished by their bushy antennae were separated and discarded along with non-anopheline females. Anopheline females were identified to species on the basis of taxonomic criteria, using keys from Gillies & de Meillon (1968) to separate members of the *An. gambiae* complex from other anophelines, which comprised mainly *An. funestus*. The different species of female anophelines were further classified according to Sella's stages of the ovarian cycle as either fully fed (F), when the abdomen was full of blood; half-gravid (HG), when the

abdomen contained half developed eggs and half digested blood; gravid (G), when the abdomen contained fully developed eggs and no blood and unfed (UF) when the abdomen had neither eggs nor blood. This classification was done in order to determine whether altitude might affect the rate of oogenesis in a given day resting mosquito population, and thus distribution of the abdominal stages of that population; and also to determine which abdominal stages were likely to have higher sporozoite infections. From these PSC catches three indices were computed for both *An.gambiae* s.s., *An. arabiensis* and *An. funestus*. These were:

1) the total number of female mosquitoes caught per month at each altitude,

2) the percentage of females according to Sella's abdominal stages at each altitude, and

3) hut densities as the geometric mean number of each species per house. These geometric means were converted into Williams' means (Wm), a form of the geometric mean such that

$$\log (Wm + 1) = \sum \frac{\log (n + 1)}{N}, \text{ where } n_1, n_2 \ldots\ldots \text{ represent}$$

actual values of individual catches (Haddow, 1960). The results were further converted into Williams' mean percent (Wm %) of the total catch calculated from the monthly sum of Williams' means. These percentage means were used to determine the relative densities and to illustrate graphically the monthly variation and ecological patterns of vector mosquito populations at different altitudes and within the same areas.

Anopheline mosquito densities were measured by collecting the females "knocked-down" inside entire houses by the PSC method. The actual numbers of freshly fed mosquito females caught were increased by 15% for two reasons: to compensate for 5% of females flying out-doors after feeding (Gillies, 1954), and to allow for 10% loses during spray catching (WHO, 1963).

The mean house densities were expressed as geometric means modified by Williams (1937) and designated as Williams' Mean (Haddow, 1960). The equation used is as expressed above.

In order to determine the ratio and distribution of the sympatric sibling species of the *An. gambiae* complex in a given study area ovarian poletene chromosome preparations were made from specimens of the *An. gambiae* complex at Christophers' stage III, using the method of Coluzzi & Sabatini (1967). Christophers' stage III is the third stage of oogenesis characterised by an oval follicle in which the yolk cells take up more than half the follicle and the nucleus measuring about 200 *u*m is obscured. The X-chromosome was used to identify members of the *An. gambiae* complex with the aid of chromosome maps published by Coluzzi & Sabatini (1967). For this purpose, every month a sample of 100 specimens of *An. gambiae* complex from Ahero, between 10 and 20 specimens (total 237) of this species from Rota, and a total of 111 specimens from Oriwo were examined cytogenetically. The numbers examined were determined by the availability of the suitable ovarian stage for chromosome preparation.

Weather data covering rainfall, temperature and the relative humidity for the period of the study were obtained from metereological records maintained by the nearest station of the Kenya Metereological Department and the Directorate of Civil Aviation in Kisumu (for Rota data); and by the former at Ringa Metereological Station for Oriwo data, and also by the National Irrigation Board at Ahero, for Ahero data. All the three metereological stations are situated less than 2 km from the mosquito sampling sites. Seasons were divided into three according to the annual rainfall patterns, namely short rainy season, dry season and long rainy season (Haddow, 1942; Roberts 1974).

3.3 RESULTS

3.3.1 Species Distribution and Diversity in Relation to Altitude

A total of 25,816 specimens of anopheline mosquitoes were collected from 192 house catches made in the period from January, 1989 through to Decenber, 1990. The sample included *An. arabiensis* as a homogeneous species and *An. funestus* at Ahero. At Rota and elsewhere sympatric species of the *An. gambiae* complex and *An. funestus* were the most abundant. These species will be the only ones discussed in further analysis, although however, small numbers of *An. pharoensis, An. ziemanni* and *An. coustani* were also caught occassionally resting indoors, especially in the long rainy season. Moreover, the existence of these minor species seized altogether at 1524 m above sea level, where even then their populations were very limited. Only 169 specimens of *An. gambiae* s.s. and 14 of *An. funestus* were caught at the high altitude of 1829 m (Kegati) above sea level. On the other hand, above 2100 m (Ichuni), only 14 *An. gambiae* s.s. were caught, and no *An. funestus*. Because of the small sample size the data from Kegati and Ichuni could not be further analysed to give any reasonable statistical significance. For these reasons only data from three altitudes; 1219 m, 1350 m and 1524 m was further analysed.

Table 3.1 shows the detailed breakdown of mosquito composition at the various altitudes. Altitude and seasonality appeared to be significant determinants of mosquito abundance. Some species of mosquitoes were also not found in a given locality as marked by altitude. Hence, the epidemiology and magnitude of malaria transmission may relate to these factors. Of all the anopheline species found at the different altitudes of the transect, *An. arabiensis, An. gambiae* s.s. and *An. funestus* were identified as the major vectors of malaria, and their abundance decreased with increasing altitude. For example, only 14 specimens of the *An. gambiae* complex

Table 3.1: The altitudinal distribution of Anopheles mosquito species caught by the Pyrethrum Spray-Sheet (PSC) technique from January 1989 - December 1990 (2 years).

SPECIES							
Locality or Site	Height above sea level (m)	*An.arabiensis*	*An.gambiae* s.s.	*An.funestus*	*An.ziemanni*	*An.pharoensis*	*An.coustani*
Ahero	1219	13,183	0	3717	280	19	1
Rota	1350	1129	1692	3660	71	35	0
Oriwo	1524	622	1282	105	10	4	8
Kegati	1829	-	169	14	0	0	0
Ichuni	2134	-	14	0	0	0	0
Total	14374	3157	7496	361	419	9	9

Variation of *An. gambiae* complex with altitude was significant.

($r = 0.73$, $z = 1.82$, $p = 0.01$)

Variation of *An. funestus* with altitude was significant

($r = -0.82$, z -2.4, $p > 0.05$).

were caught resting above 2100 m. Regression analysis revealed that the relative abundance of the sibling species of the *An. gambiae* complex varied significantly with altitude ($r = -0.73$, $z = 1.82$, $p = 0.01$), as did the abundance of *An. funestus* ($r = -0.82$, $z = 2.4$, $p < 0.05$).

3.3.2 Seasonal Vector Population Abundance and Monthly Density Patterns.

Table 3.2 shows that there was a very large seasonal variation in population abundance of member species of the *An. gambiae* complex and *An. funestus* in correlation with altitude as illustrated by decreasing indoor resting densities with the rise in altitude and change in season For example, only two *An. funestus* were caught at a height of 1524 m above sea level during the dry season as compared with 18 during short rains and 64 during long rains. In particular, no *An. arabiensis* were caught at 1524 m (Oriwo) during any of the seasons. Likewise, *An. gambiae* s.s. was totally absent from Ahero through out all the seasons. The Chi-test revealed that seasonal variation in population abundance by altitude was significant ($p < 0.0001$). For each altitude variation in population abundance by season was also significant ($p < 0.001$). These differences appeared to be attributable to reduced breeding during the dry season. At 1219 m the ratio of *An. arabiensis* to *An. funestus* was 3:1 during the short rains, 2:1 during the dry season and 5:1 during the long rains. At 1350 m the ratio of *An. gambiae* s.l. (predorminantly *An. gambiae* s.s.) to that of *An. funestus* was 3:1 in favour of *An. funestus* during the short rains, but reversed to 3:1 in favour *An. gambiae* during long rains.

Table 3.2: **Seasonal abundance of female mosquitoes of the three commonest species collected from the 8 houses at each altitude by the PSC technique from Jan 1989 to Dec 1990 (2 years).**

Season	Species	Altitude and Site		
		1219 m, Ahero	1250m, Rota	1524 m, Oriwo
Short rains (Oct - Dec)	*An. arabiensis*	4337	0	0
	An. gambiae	0	803	101
	An. funestus	1527	2297	18
Dry season (Jan/Feb)	*An. arabiensis*	1654	0	0
	An. gambiae	0	70	15
	An. funestus	845	268	2
Long Rains (April-June)	*An. arabiensis*	2269	0	0
	An. gambiae	0	500	1000
	An. funestus	506	267	64

Seasonal variation in population abundance by altitude was significant (x^2 test, $p < 0.0001$ for each test).

For each altitude, the variation in population abundance by season was signicicant. (x^2 test, $p < 0.001$ for each test).

Fig 3.1 compares the monthly catches of *An. funestus* and member species of the *An. gambiae* complex. At Oriwo the near homogeneous population of *An. gambiae* s.s. and at Rota which had a predominant population of the same species, high populations were registered during the long rainy season, especially in the months of April and May. In contrast, *An. arabiensis* as a homogeneous species at the lower altitude of Ahero (1219 m) was most abundant shortly after this season, in August. This implied that peak breeding time for *An. gambiae* s.s. and *An. arabiensis* was not synchronous. At Oriwo, 1524 m above sea level *An. funestus* was also most abundant during the long rains, notably in May, while at 1350 m it was most abundant towards the end of the long rainy season (May and June). This contrasted with the situation at Ahero (1219 m), where *An. funestus* did not appear to be most abundant in any particular month. The mean population of the vectors reduced with rising altitude.

The mean monthly house densities illustrated in Fig 3.2 by percentage of Williams' means show that the yearly wave of the population of member species of the *An. gambiae* complex regularly preceded that of *An. funestus* in a sequence at both 1350 m (Rota) and 1524 m (Oriwo). This classic sequence did not appear to be exhibited between *An. arabiensis* and *An. funestus* at the lower altitude of 1219 m where the peaks for the two species appear to coincide. The population dynamics registered more or less two peaks. At 1350 m, the major peak for *An. gambiae* s.s. occurred in April and crashed one month later as the population for *An. funestus* rose. This pattern appears to be repeated in September and October. Similar events were repeated at 1524 m (Oriwo) where *An. gambiae* s.s. was also the dominant species throughout all seasons. At 1219 m the pattern of population dynamics of *An. arabiensis* seemed to indicate more or less synchronous peaking with that of *An. funestus*, occuring during or soon after the rainy season. The relative abundance of both species of vector mosquitoes was highest at Ahero

Fig 3.1: Mean monthly totals of vectors captured by PSC at different altitude having applied the correction factor (Jan 1989-Dec 1990)

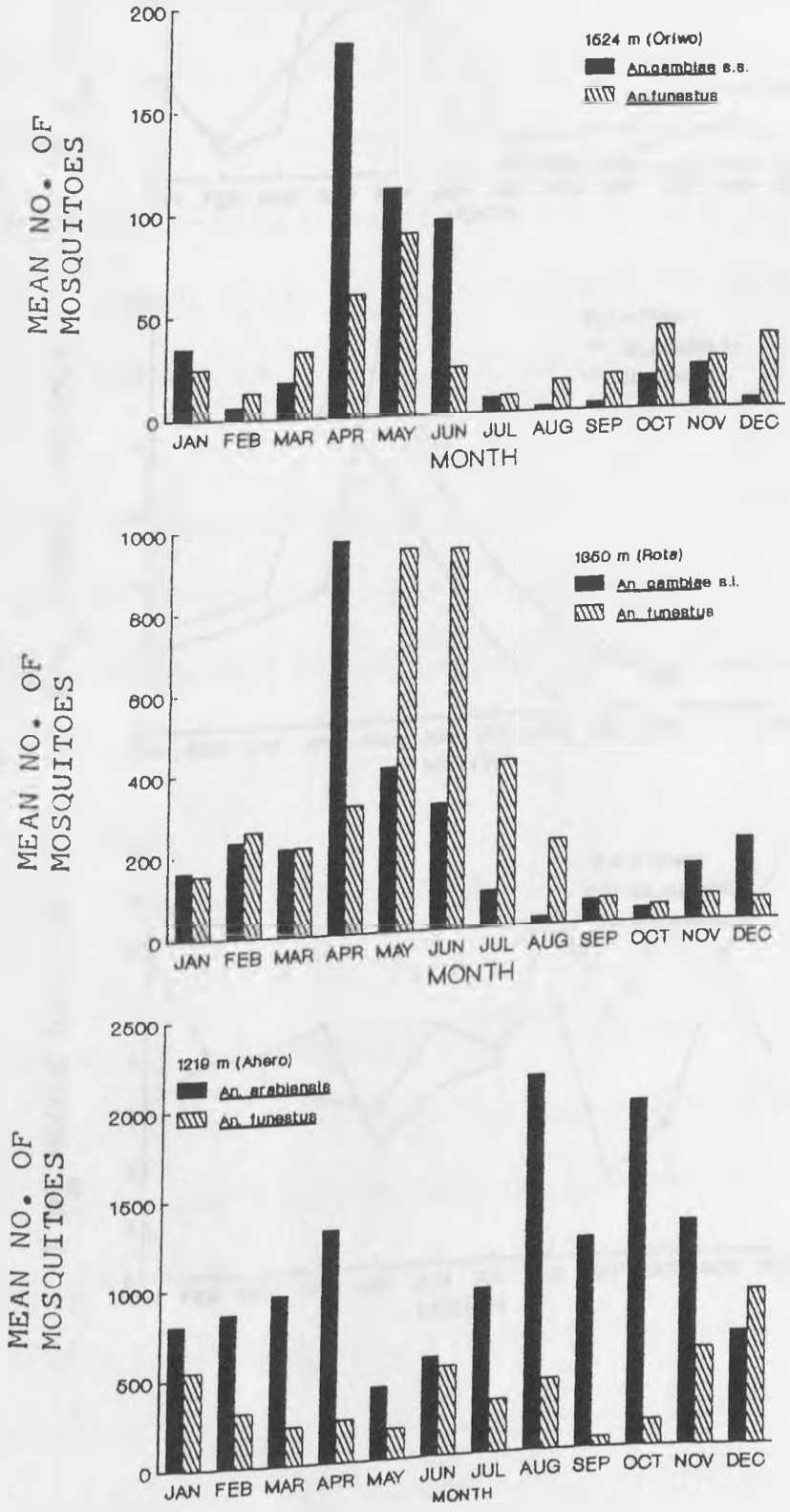
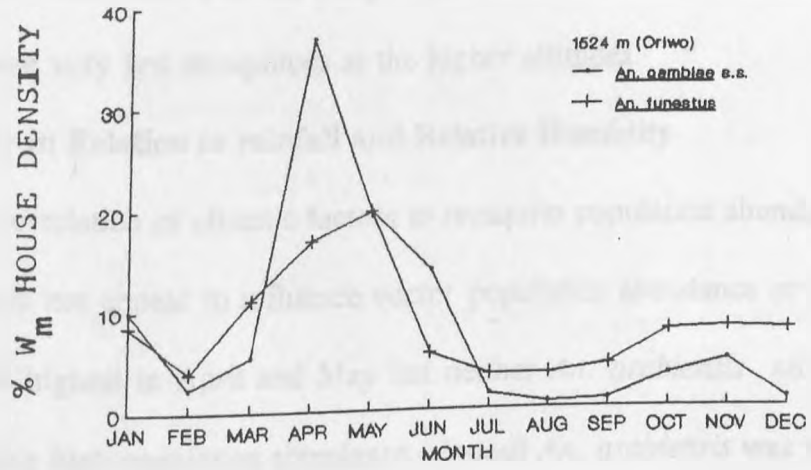
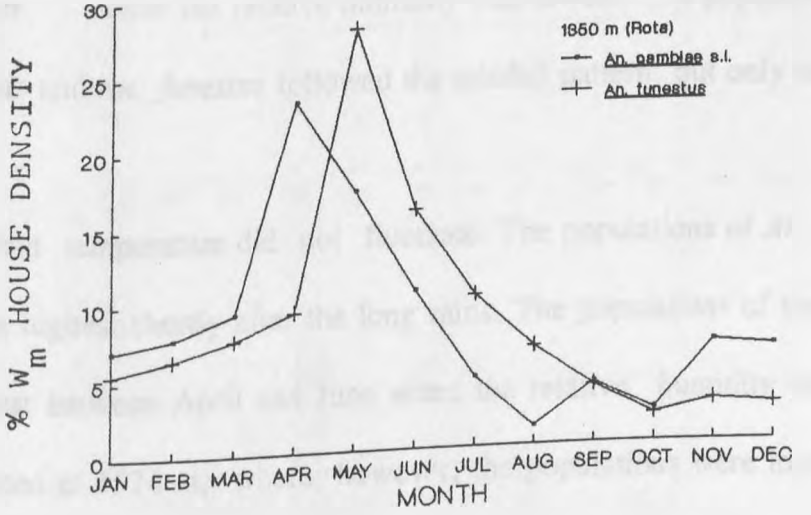
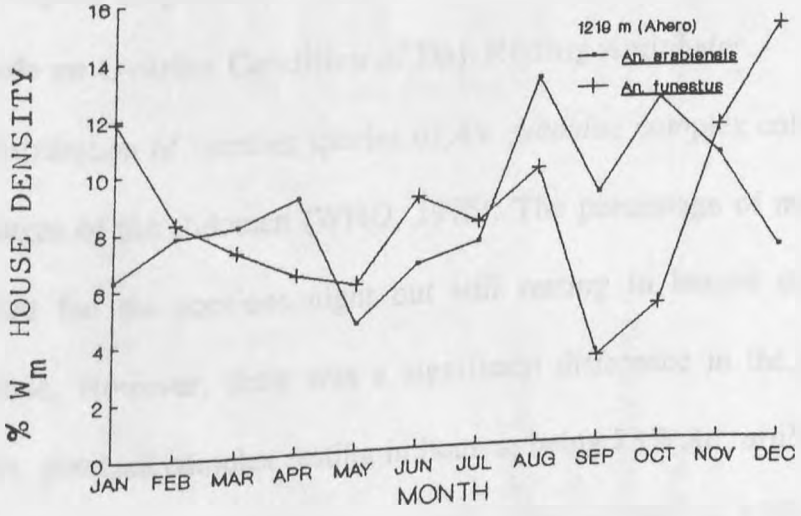


Fig 3.2:Mean monthly house densities of female *Anopheles* resting indoors per day at different altitudes (Jan'89-Dec 1990)







as shown by both the total catch and the mean of the seven months; followed by Rota and Oriwo, but there were very few mosquitoes at the higher altitudes.

3.3.3 Vector Density in Relation to rainfall and Relative Humidity

Fig 3.3 a shows the correlation of climatic factors to mosquito population abundance at Ahero. Mean temperatures did not appear to influence vector population abundance or diversity. The relative humidity was highest in April and May but neither *An. arabiensis* nor *An. funestus* showed a corresponding high population abundance. Indeed *An. arabiensis* was most abundant in August and October when the relative humidity was lowest. The population abundance of both *An. arabiensis* and *An. funestus* followed the rainfall pattern but only slightly.

In Fig 3.3 b the mean temperature did not fluctuate. The populations of *An. gambiae* s.l. and *An. funestus* were highest shortly after the long rains. The populations of the two vectors appeared to be highest between April and June when the relative humidity was lowest. A similar situation obtained at 1524 m, where however, the populations were lowest when the relative humidity was highest (Fig 3.3 c).

3.3.4 Effect of Altitude on Ovarian Condition of Day Resting *Anopheles*

Fig 3.4 a shows the distribution of member species of *An. gambiae* complex collected by PSC according to Sella's stages of the abdomen (WHO, 1975). The percentage of member species (about 46%) which had fed the previous night but still resting in houses did not differ significantly with altitude. However, there was a significant difference in the percentage of un-fed species of the *An. gambiae* complex resting in houses, being 23% *An. arabiensis* at 1219 m, and 15% of *An. gambiae* s.s. at 1524 m and only 9% of the complex at 1350 m. The rest of the stages did not appear to differ significantly. The abdominal condition of un-fed *An. funestus* also differed according to altitude between sites, i.e between 1219 m and 1350 m and

Fig 3.3 a: Mean monthly variation of vector populatior abundance at 1219 m (Ahero) in relation to metereological parameters (Jan 1989-Dec 1990

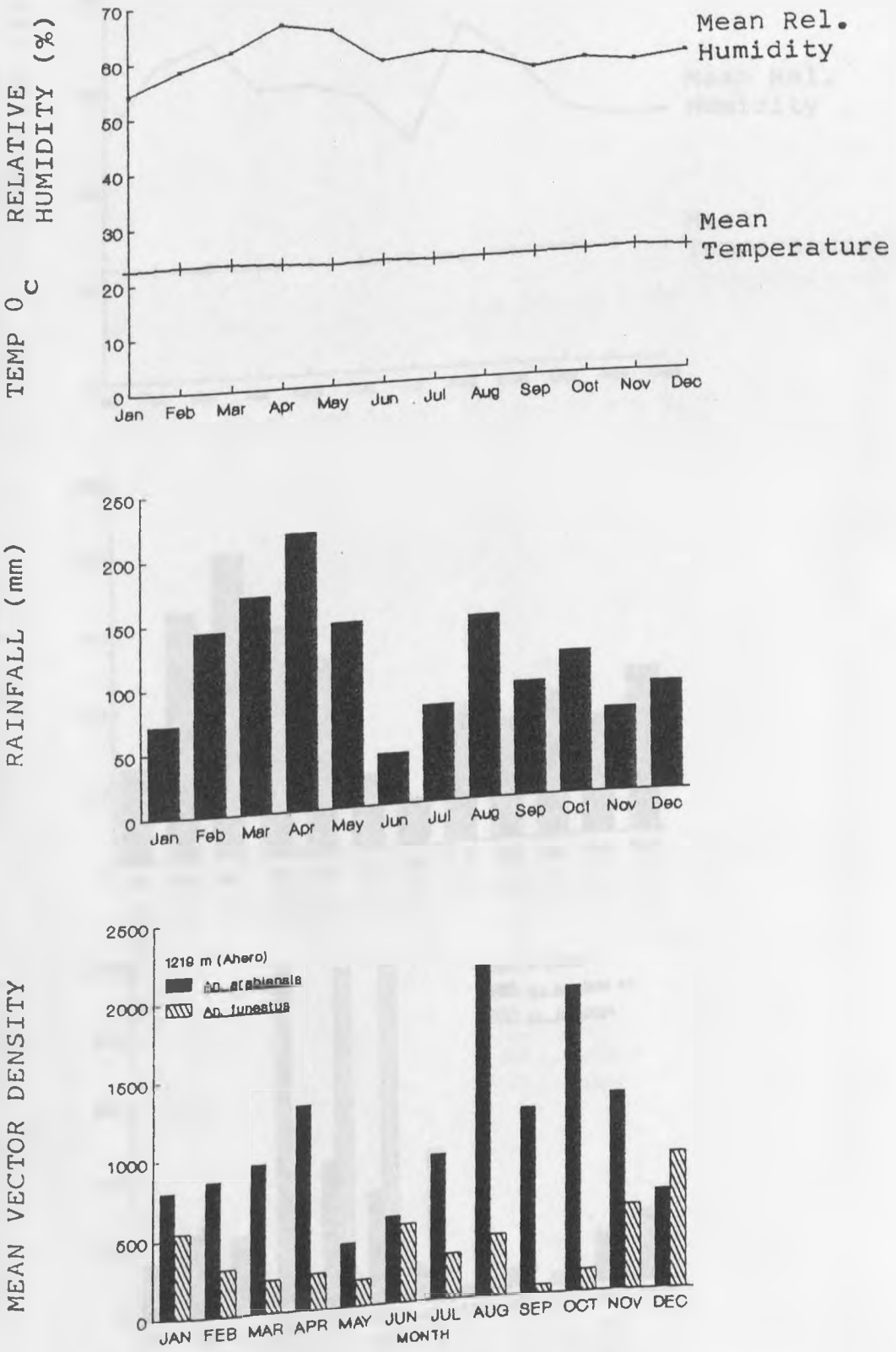


Fig 3.3 b: Mean monthly variation of vector population abundance at 1350 m (Rota) in relation to metereological parameters (Jan 1989-Dec 1990)

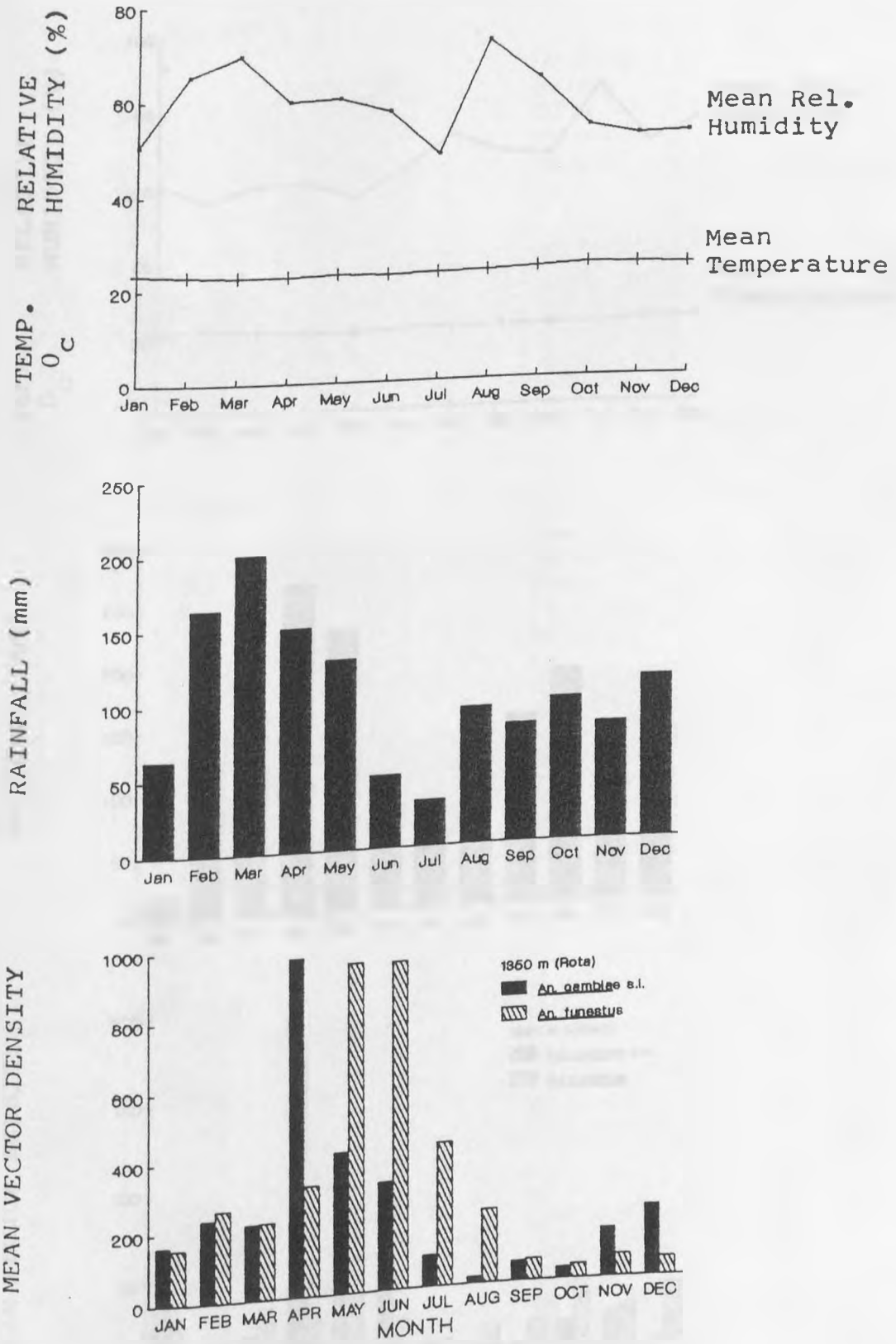


Fig 3.3 c: Mean monthly variation of vector population abundance at 1524 m (Oriwo) in relation to metereological parameters (Jan 1989-Dec 1990)

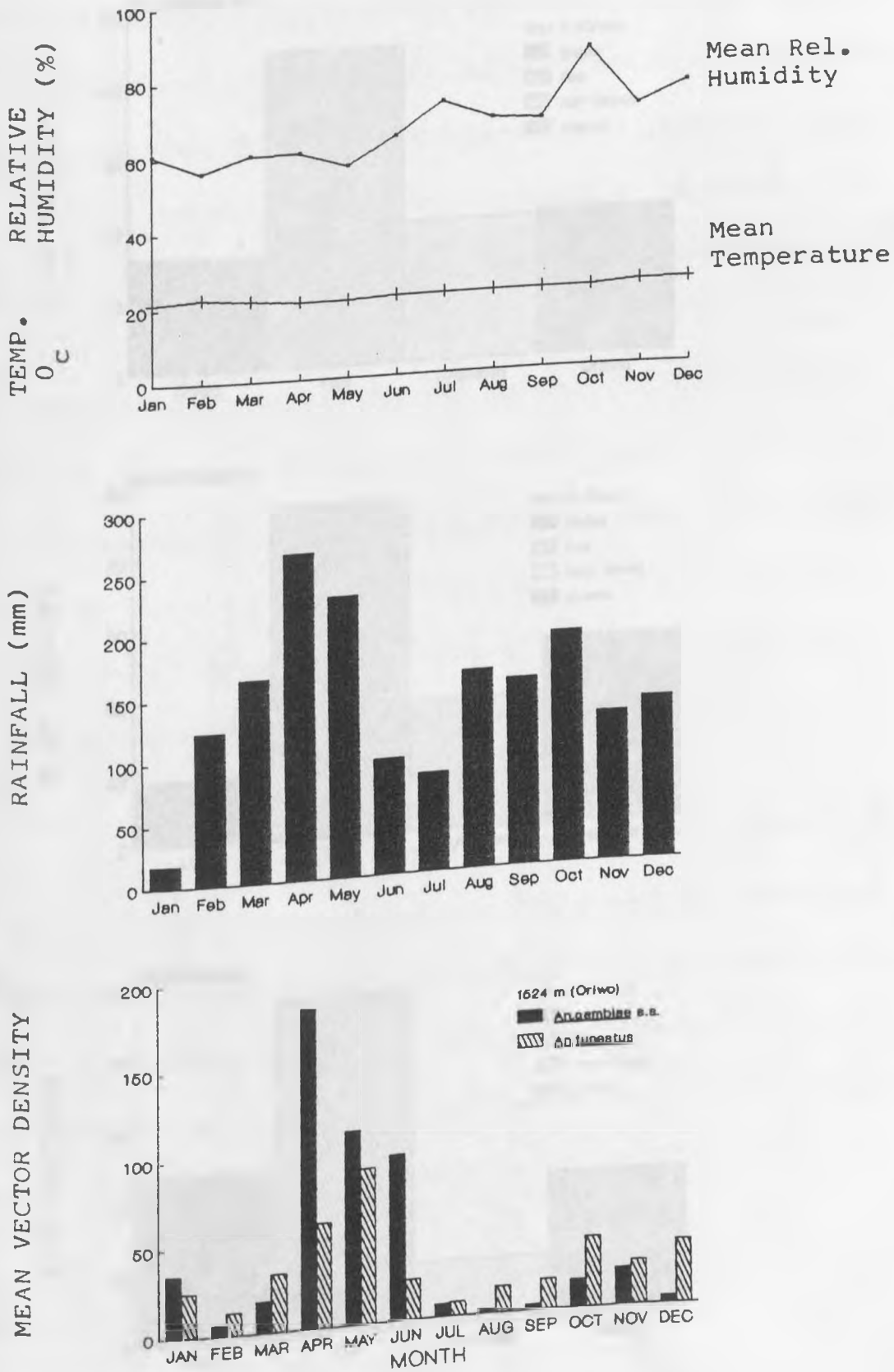
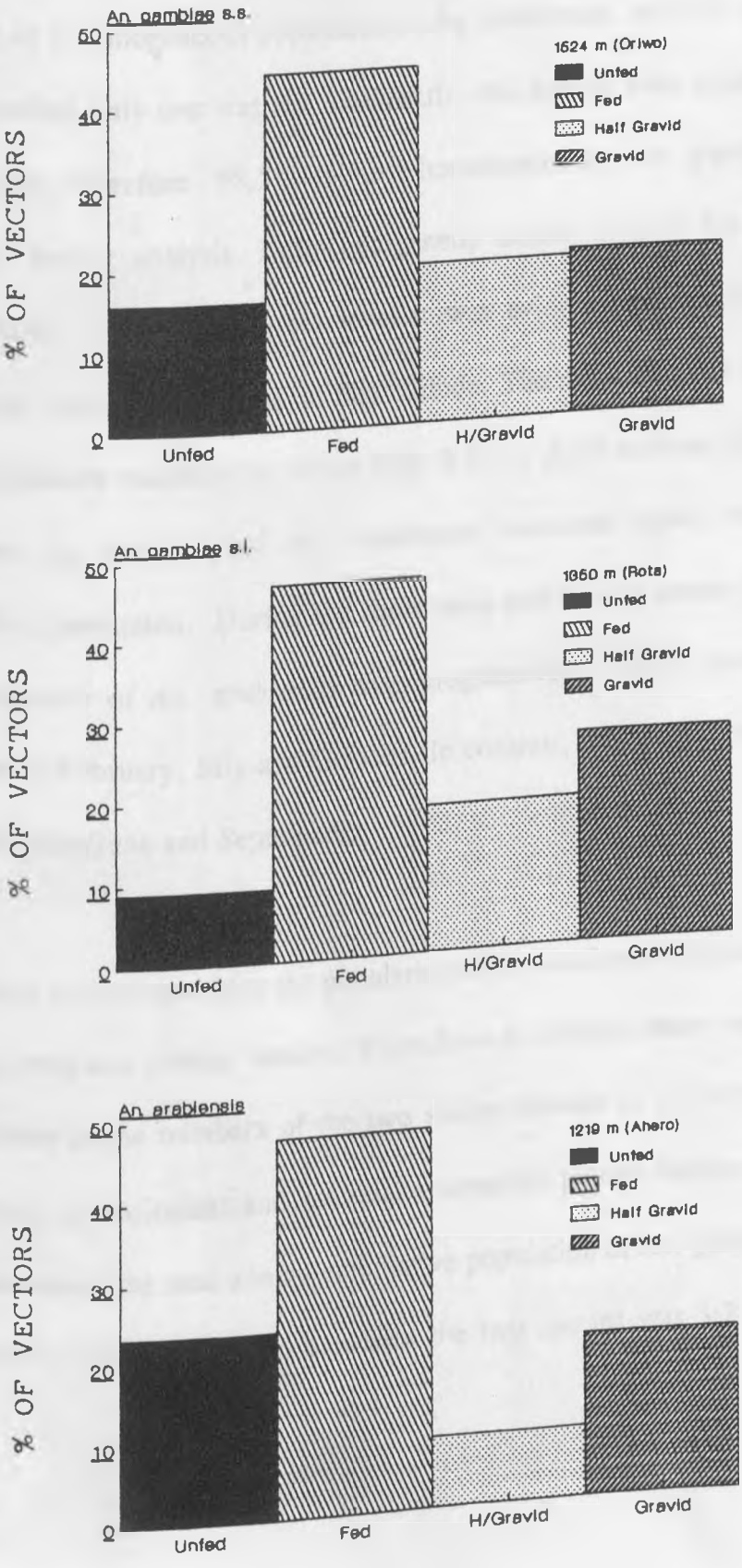


Fig 3.4 a: The influence of altitude on the distribution of the abdominal (Sella's) stages in An. gambiae complex caught at different sites (Jan 1989-Dec 1990)



beween 1524 m and 1350 m on the other (Fig 3.4 b). The difference in unfed condition suggested that altitude played a part at the breeding stage.

3.3.5 Ecology of *An. gambiae* and *An. arabiensis* at Ahero and Rota

Cytogenetic identification of mosquito specimens from the different altitudes showed that Ahero 1219 m, comprised of a homogeneous population of *An. arabiensis*. At 1512 m (Oriwo) out of 111 specimens identified only one was *An. arabiensis*, this having been caught in April. The vector population was, therefore 98.5% (almost homogeneously) *An. gambiae* s.s., and is treated as such in further analysis. From cytogenetic examination of the ovaries of 237 mosquitoes from Rota, 1350 m above sea level it was evident that sympatry between *An. gambiae* s.s. and *An. arabiensis* existed at this altitude. The proportions of *An. gambiae* s.s and *An. arabiensis* differed markedly by month (Fig 3.5). In April to June, following the onset of the rainy season, the frequency of *An. arabiensis* decreased rapidly while that of *An. gambiae* s.s rose to a maximum. During the short rains and the dry season (October through February), the frequency of *An. arabiensis* rose progressivelly. Three peaks of 100% *An. arabiensis* appeared in February, July and October. In contrast, there were two peaks of 100% *An. gambiae* s.s. in May/June and September.

Prior to the long rains in April and May the population of *An. arabiensis* declined steadly as that of *An. gambiae* s.s rose in a similar version. From June to October there was a rapid decline and simultaeneous rise in the numbers of the two sibling species in an inverse manner. This resulted in producing an ecological and seasonal succession pattern between the two sibling species. Overall, however, the total annual cumulative population of *An. gambiae* s.s surpassed that of *An. arabiensis*. The overall ratio betweeen the two species was 3:2, in favour of *An. gambiae* s.s.

Fig 3.4 b: The influence of altitude on the distribution of the abdominal appearance (Sellas' stages in An.funestus caught at different heights (Jan 1989 - Dec 1990)

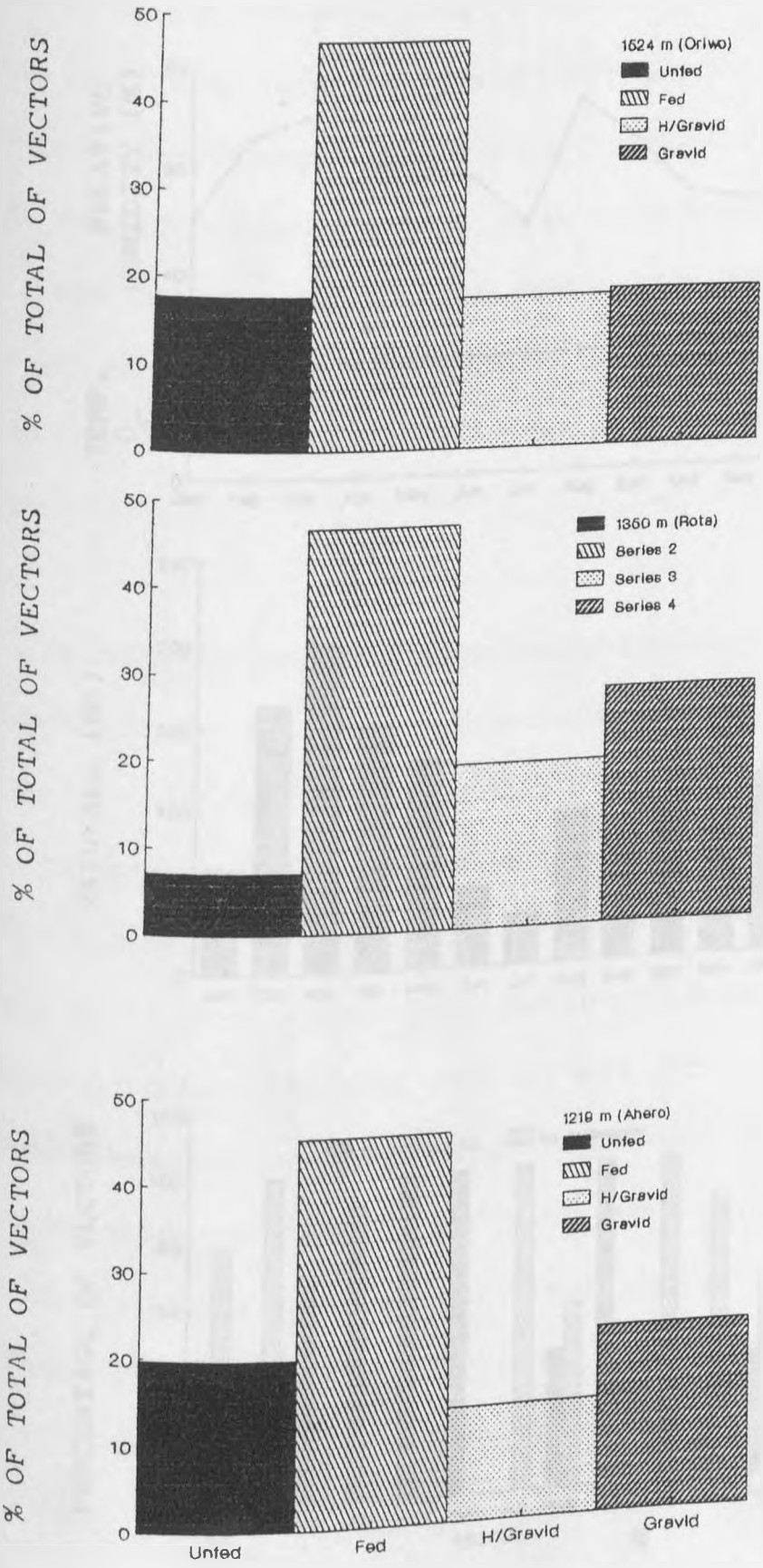
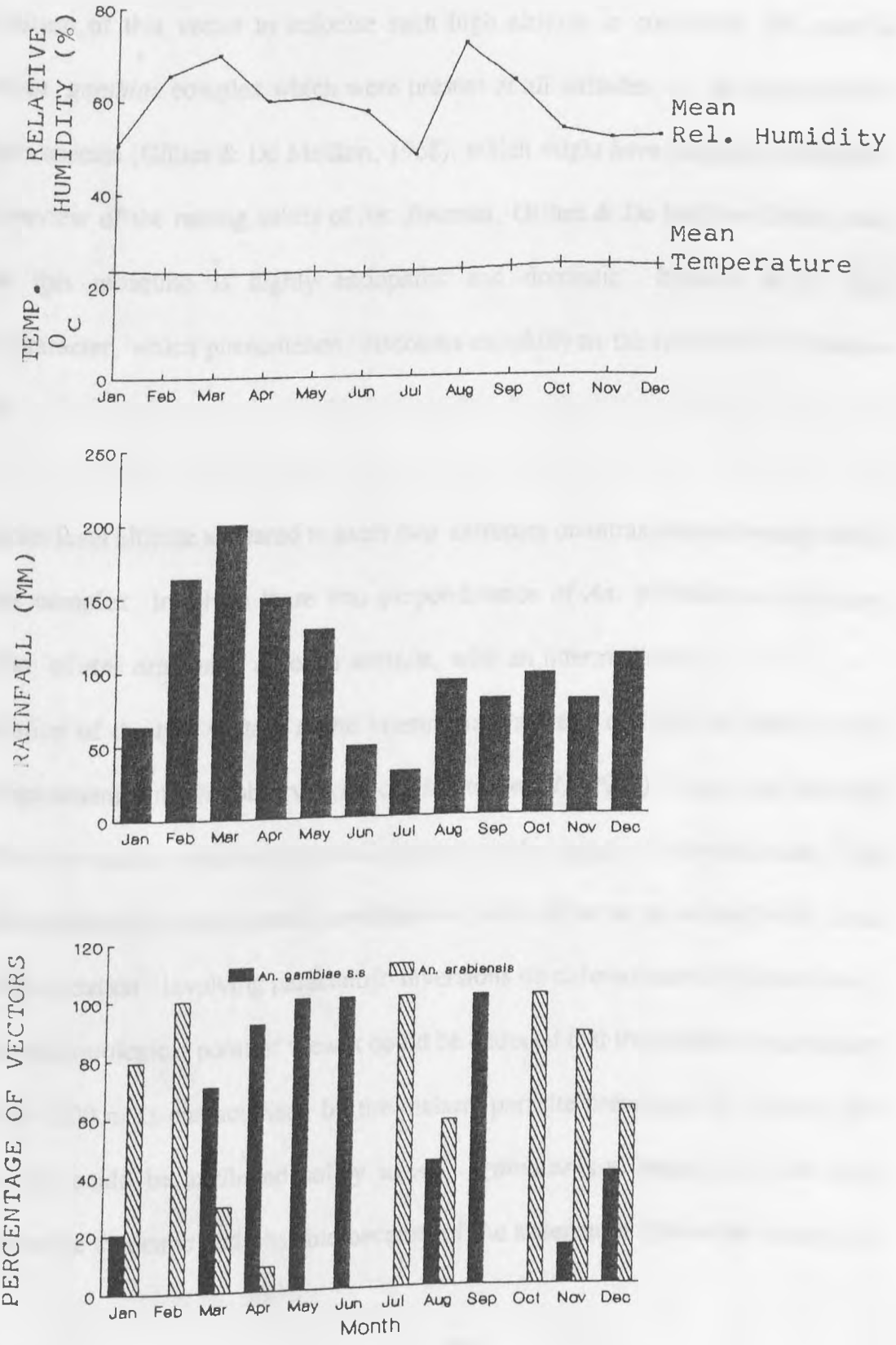


Fig 3.5: Ecological succession between species of the An. gambiae complex at Rota (1350 m) in relation to metereological parameters (Jan 1989-Dec 1990)



3.4 DISCUSSION

From the data presented above it was evident that altitude exerts influence at both interspecies and intraspecies level. At interspecies level, that throughout the two years of study no specimens of *An. funestus* were caught resting indoors above 2100 m (Ichuni) indicated either the probable failure of this vector to colonise such high altitude in contrast to the member species of the *An. gambiae* complex which were present at all altitudes, or the presence of a sub-group of this species (Gillies & De Meillon, 1968), which might have exophilic tendencies. However, in a review of the resting habits of *An. funestus,* Gillies & De Meillon (1968) have concluded that this mosquito is highly endophilic and domestic because of its high anthropophilic character, which phenomenon discounts exophilly as the reason for its absence at high altitude.

At the intra-species level altitude appeared to exert two extremes on intraspecies diversity within the *An. gambiae* complex in which there was preponderance of *An. gambiae* s.s. at higher altitude and that of *An. arabiensis* at lower altitude, with an intermediate sympatric population of the two vectors at the intermediate altitude of 1350 m (Rota). These results were in agreement with the observations of Highton *et al.* (1979). They also indicated the ability of the two sibling species to prevail across a wide range of climatic belts. This capacity to exploit different environmental conditions is believed to be associated with clinal chromosomal differentiation involving paracentric inversions on chromosome-2 (Coluzzi *et al.* 1979). From the epidemiological point of view it could be deduced that the malaria transmission taking place above 2100 m as characterised by the malaria parasite prevalence of 3.0% at this altitude (Chapter 6), could be attributed solely to *An. gambiae* s.s. Malaria in this area, however, is likely to be epidemic and unstable because of the absence of this vector during the

dry season. When the vector population increases dramatically during the long rains, however, epidemics of the so called "highland malaria" (Bruce-Chwatt, 1985); are likely to erupt among the population of such areas who are usually only semi immune to malaria because of very low grade infection risk, leading to serious morbidity and mortality.

The homogeneity of *An. arabiensis* at Ahero, with an altitude of 1219 m, which is an irrigation rice-growing ecosystem is known to prevail in a number of other rice-growing irrigation schemes (White, 1974), and appears to be a phenomenon which is quite wide spread in similar ecological conditions, at least in East Africa (Highton *et al.*, 1979; Mosha & Subra, 1982; Collins *et al.*, 1988). The cyclic phenomenon of sympatry exhibited by *An. gambiae* s.s and *An. arabiensis* at 1350 m (Rota) has also been observed in Nigerian populations by Rishikesh *et al.*, (1985). This author concluded that the seasonal distribution patterns shown by these sibling species were probably governed by different adaptive values related to changes of climatic conditions acting in concert with the patterns of geographical distribution on chromosomal forms as suggested by Coluzzi *et al.*, (1979). The near homogeneity of *An. gambiae* s.s. at 1524 m (Oriwo), may probably suggest a near exclusive tendency of *An. gambiae* s.s. to colonise high altitude in contrast with *An. arabiensis* which similarly seems to be confined exclusively to rice irrigation areas. However, since traces of *An. arabiensis* were also found the mean relative humidity was also likely to play a role.

The observed distribution and diversity of *An. gambiae* s.s. and *An. arabiensis* in the two ecosystems of Ahero and Rota were consistent with the earlier observations of Service (1970). He had studied the ecology of *An. gambiae* s.s. Giles and *An. arabiensis* in Kisumu and concluded that although the two species were sympatric, *An. gambiae* s.s. was predominant in

hut collections. The results were also consistent with the observations made prior to a large scale fenitrothion (OMS-43) trial by Joshi *et al.* (1975), that *An. gambiae* s.s. was predominant around the Kisumu area. From low to higher altitude, however, there was an intergradation in frequency of the two species. This might be associated with clinal changes in the ecosystem, from rice-irrigation to savannah environment as suggested by Coluzzi *et al.* (1985).

Attempts have been made to classify malaria vectors on the basis of feeding and resting sites. Senior-White (1954) termed the tendency of a mosquito to rest outside rather than in domicilliary structures as exophily. Gillies (1956) defined the term further into obligatory, facultative and deliberate exophily. Deliberate exophily is practised by mosquitoes which feed indoors but deliberately avoid to rest indoors, and those that feed outdoors and remain resting outdoors; which behaviour would lead to deliberate avoidance of insecticides used in indoor spraying and thus failure to interrupt the malaria transmission cycle. Coluzzi *et al.*, (1977 and 1979) showed the genetic basis of exophily when he demonstrated the existence of non-random distribution of chromosomal karyotypes which are associated with exophily. On the basis of the observed distribution of Sella's abdominal stages (Figs 3.4 a & b), it would appear a substantial percentage of all the three vectors remain resting indoors after feeding.

In conclusion, the results above suggest that environmental factors, temperature, relative humidity and in particular altitude, may be exerting selective pressures which result in non-random distribution of chromosomal variants in *An. gambiae* mosquitoes. This may influence the successful selection and habitation of a given environment by different karyotypes or lead to divergent behavioural attributes and infection susceptibilities, all of which when combined, may result in different malaria transmission dynamics. Rainfall was the one single factor responsible for population abundance, although at 1219 m intermitent irrigation of rice fields obliterated this pattern.

CHAPTER FOUR

4 MAN-BITING RATES AND BITING CYCLES OF MALARIA VECTORS AND THEIR EPIDEMIOLOGICAL SIGNIFICANCE IN MALARIA TRANSMISSION

4.1 INTRODUCTION

Man-biting rates (MBR) are important parameters in assessing the inoculation risk with malaria sporozoites (Garret-Jones, 1970). However, the relative importance of biting mosquito populations can be influenced by a number of factors including the innate host preference by vector mosquitoes and the relative numbers of different hosts. Human contact with mosquitoes may also be altered through cultural practices which affect numbers and location of both humans and domestic animals. For example, tethering domestic animals near or in houses in savannah Africa is believed to diminish the mosquito attack rate on humans (MacCormack, 1984).

Mosquito attack rates have been shown to be significantly related to time of biting, the species composition and the locality (Haddow, 1945; Haddow & Ssenkubuge, 1965). Biting cycles of the same species were also different in relation to these factors (Lumsden, 1958; van Someren & Furlong, 1964). Such factors are likely to operate simultaeneously with others to determine malaria transmission rates. At the Zika and Mpanga Forests in Uganda, Haddow (1961) further showed that there was a variation in the number of mosquitoes biting according to the vertical height above ground. It was of interest to study the influence of variation in altitude on the above parameters and overall influence on malaria epidemiology. In this regard the man-biting rates were estimated from biting catches at various altitudes, and correlated to factors that are likely to play a role in human-anopheline contact and malaria transmission in the study areas.

4.2 MATERIALS AND METHODS

The study was conducted at the three localties, Ahero, 1219 m; Rota, 1350 m, and Oriwo, 1524 m above sea level. Mosquito sampling at the three altitudinal sites was carried out by the human-bait catch technique, using test-tubes with the help of flashlights (WHO, 1975; Service, 1976). The human-bait collections were performed hourly, starting at 19.00 hours and ending at 7.00 hours, by two teams of volunteers. In all sampling was done on 24 nights. Preliminary studies had shown that malaria vectors were mainly active between these hours. Each team comprised four people; two people seated inside a house and two outside with their legs and arms exposed and catching mosquitoes from themselves and from one another with test tubes for six hours, when the teams were replaced and sampling thus continued throughout the twelve hours of the night.

The sampling collections were made both indoors and outdoors. This would facilitate comparison of the two biotypes, in order to determine whether the vectors caught at a particular altitude were endophagic (prefer feeding indoors), or exophagic (prefer feeding outdoors). Collections were made once monthly between January, 1989 and December, 1990. Each hourly catch of mosquitoes was kept separately in plastic paper bags, and the next morning sorted to species and counted. The results for each species were computed first as geometric means and converted to Williams' means (Haddow, 1960), and plotted graphically as percentages to facilitate comparison of different biting cycles. The man-biting rate (mbr), or the number of bites per person per night was computed as the geometric mean of the cumulative catch of the two years. The biting population density was computed as the arithmetic mean of all vectors caught over the two year period of the study.

Plate 1 (a): Catching mosquitoes with a test-tube by oneself.

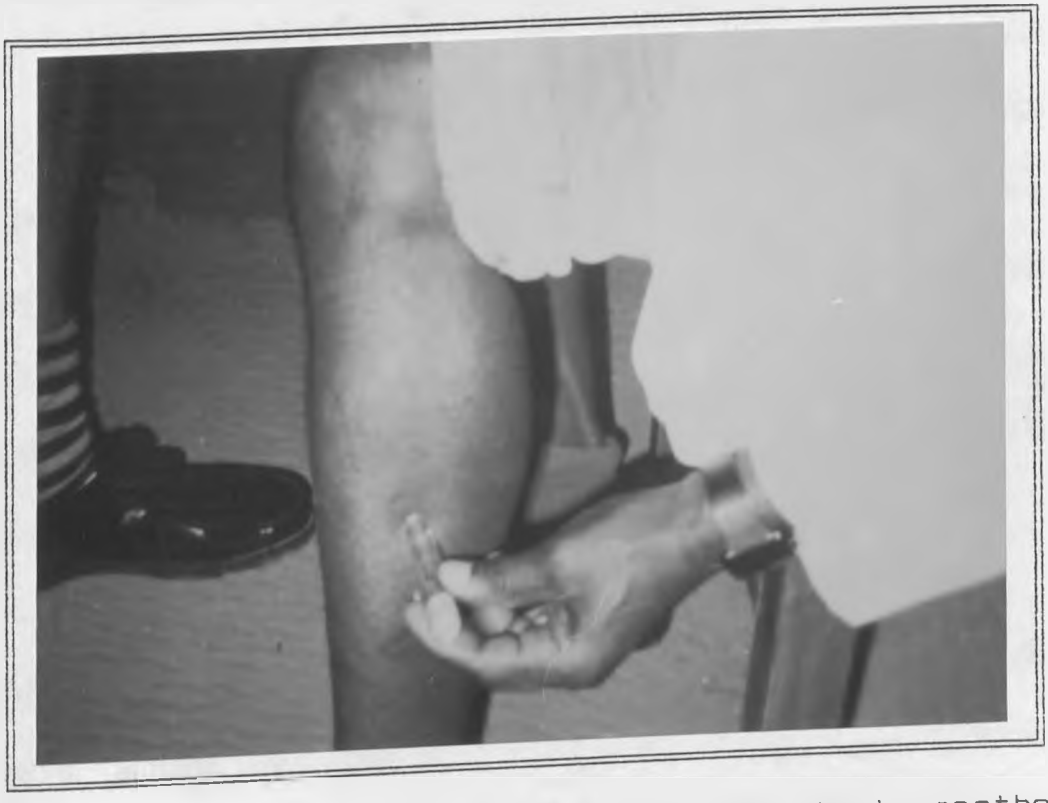


Plate 1 (b): Catching mosquitoes with a test-tube by another person.

4.3 RESULTS

Table 4.1 shows the diversity and proportions of anophelines caught biting at the various altitudes. Eight species of anophelines were caught by human-bait. These came from the three localties which yielded a total of 9738 anophelines in 324 man nights involving 3888 man hours over the period of 24 months. In all the three localities, Ahero, Rota and Oriwo, 72-75% of the anophelines were biting indoors. The two species of the *An. gambiae* complex along with *An. funestus* were, however, the main vectors, and are the subject of the remaining sections of this chapter, germane to the three altitudinal sites. The high indoor biting activity implies very high man-vector contact at all the three altitudes, and as such was significant in malaria transmission. Only 193 mosquitoes were caught at the high altitude of 1524 m (Oriwo).

Figure 4.1 shows the relative numbers of anophelines biting at different times at all the three sites which differed in altitude, night biting activity started at 19.00 hours and continued throughout the night to 07.00 hours. For *An. arabiensis* peak biting hours for (Ahero) were 02.00 hours which, differed from that of *An. gambiae* s.s. which was at 23.00 and 03.00 hours at 1524 m (Oriwo). Peak biting hours for *An. gambiae* s.s. at 1350 m (Rota) occured at 04.00 hours. For *An. funestus* peak biting at 1219 m and at 1350 m did not vary; occurring from 04.00 to 05.00 hours. However, at 1524 m (Oriwo) two peaks were evident at 01.00 hours and 03.00 hours.

Figure 4.1 further shows that overall, the biting density per hour at 1219 m was twice that at 1350 m and five times that at 1524 m, indicating a clear influence of altitude on the biting activity. Although biting activity is a night time parameter, some observations made during day time in 56 man days of human bait catches involving 672 man hours showed that a total of 600

Table 4.1: **Species composition - Number and percentage of anophelines caught during the 12 hour human-bait collections both indoors and outdoors at various altitudes between Jan 1989 and Dec 1990.**

Species	Ahero, 1219 m			Rota, 1350 m			Oriwo, 1524 m		
	IN	OUT	TOTAL	IN	OUT	TOTAL	IN	OUT	TOTAL
An. gambiae	-	-	-	841 70%	361 30%	1202	77	5	82
An. arabiensis	3660 70.6%	1521 29.4%	5181	-	-	-	-	-	-
An. funestus	1257 85.7%	211 14.3%	1478	1014 79.3%	264 20.7%	1278	66 83.5%	13 16.5%	79
An. coustani	1	0	1	-	-	-	0	8	8
An. pharoensis	5 26.3%	14 73.7%	19	15 42.9%	20 57.1%	35	0	4	4
An. zeimanni	57 20.4%	223 79.6%	280	6 8.5%	65 91.5%	71	1	9	10
An. natalensis	-	-	-	-	-	-	-	9	9
An. pretoriensis	-	-	-	-	-	-	-	1	1
Total	4990 71.7%	1969 28.3%	6959	1876 72.5%	710 27.5%	2586	144 74.6%	49 25.4%	193

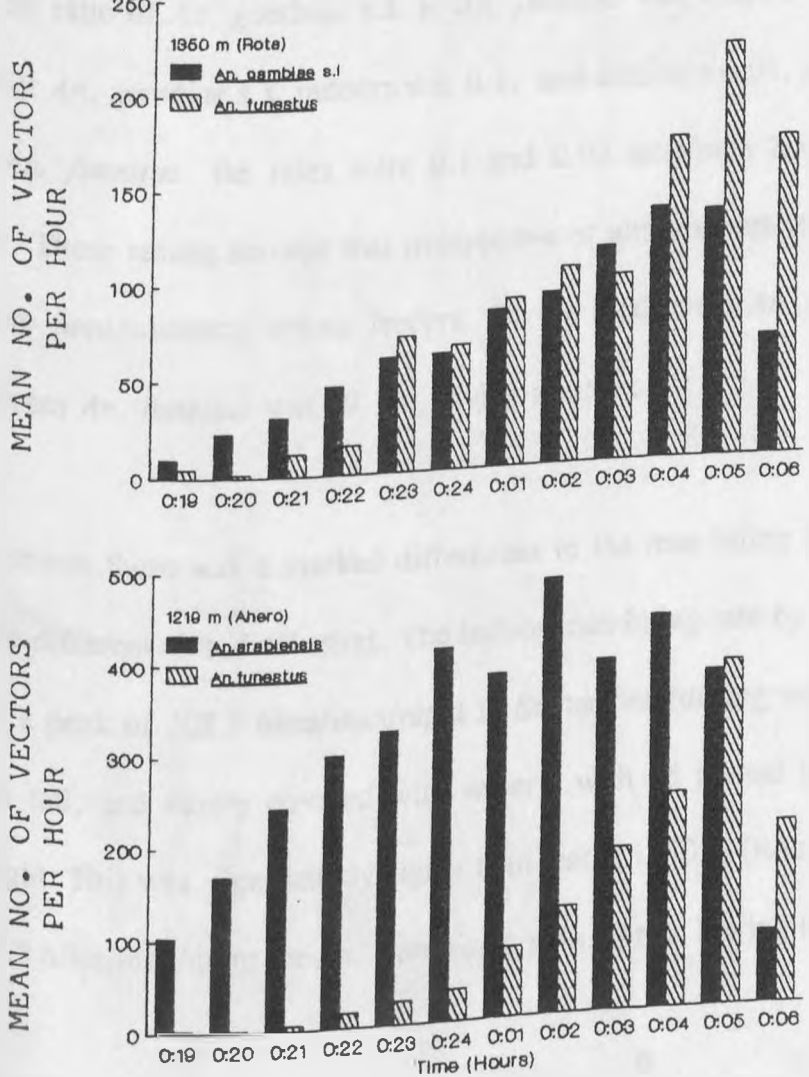
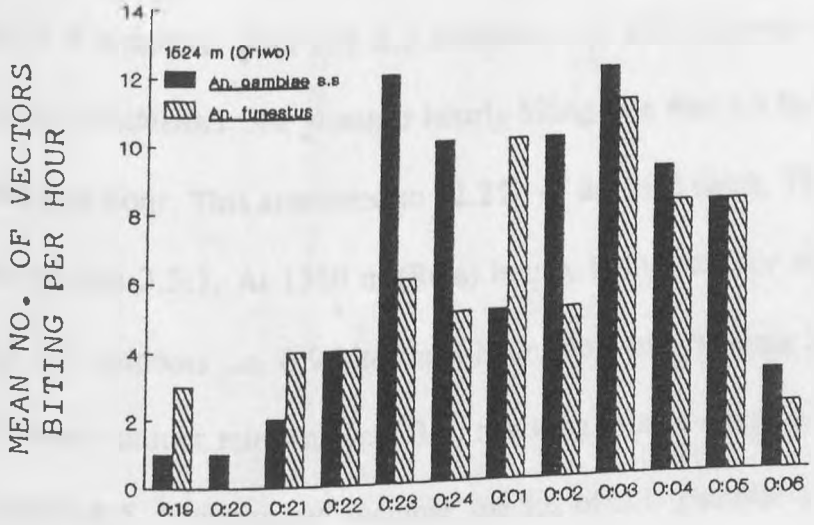

1950 m (Rota)
An. gambiae s.l
An. funestus
MEAN NO. OF VECTORS PER HOUR
0
50
100
150
200
250
0:19 0:20 0:21 0:22 0:23 0:24 0:01 0:02 0:03 0:04 0:05 0:06
1219 m (Ahero)
An.arabiensis
An.funestus
MEAN NO. OF VECTORS PER HOUR
0
100
200
300
400
500
0:19 0:20 0:21 0:22 0:23 0:24 0:01 0:02 0:03 0:04 0:05 0:06
Time (Hours)

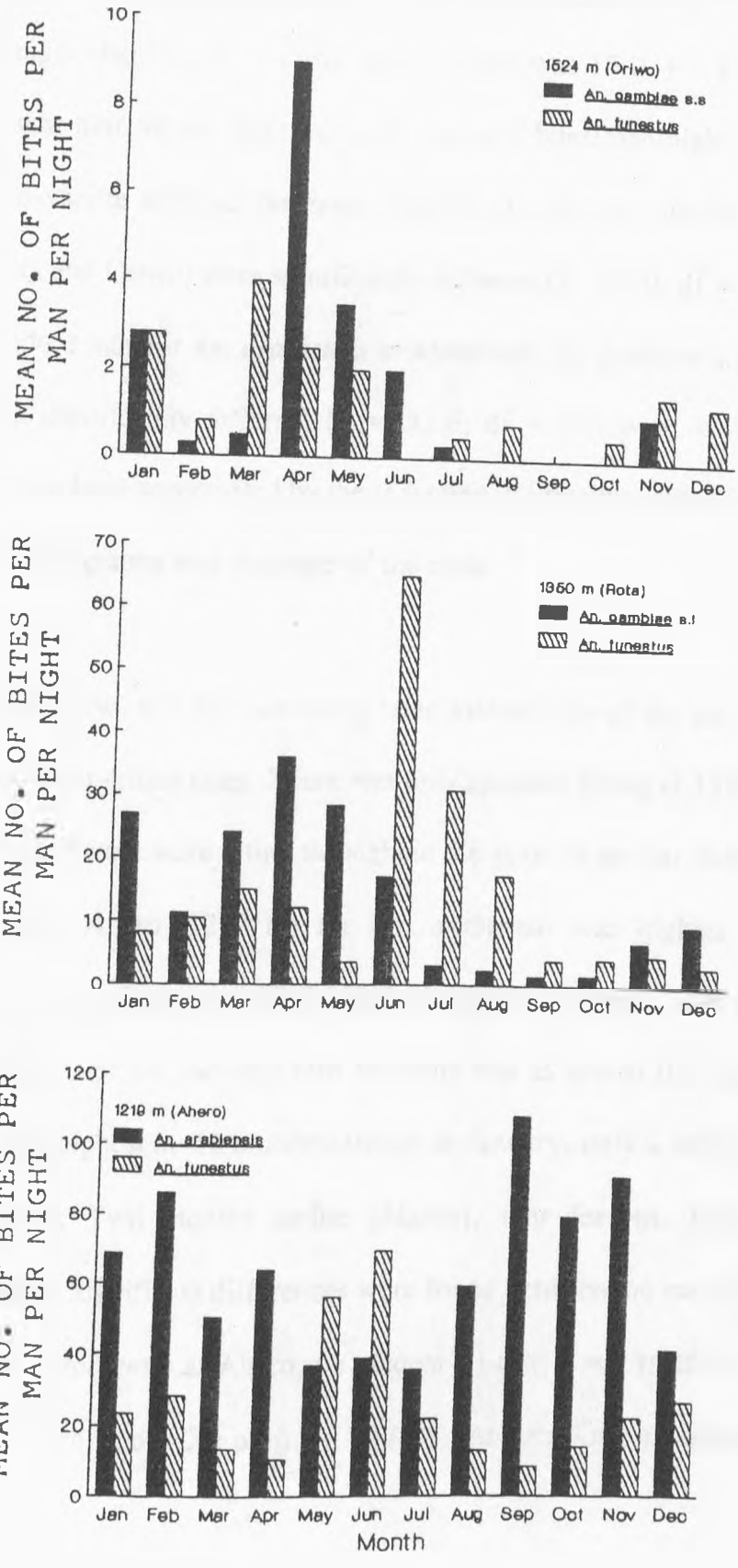
Fig 4.1: Biting cycle and population density of *Anopheles* at different altitudes as shown by mean hourly catches by human-bait over a two year period (Jan 1989-Dec 1990).



anophelines were captured alighting on humans for a bloodmeal. The most abundant day biting species was *An. arabiensis*, of which the activity was highest at 1219 m (Ahero), followed by *An. funestus*. At 1350 m (Rota), where the *An. gambiae* s.s., very few mosquitoes of this complex were captured. At 1219 m (Ahero), the hourly biting rate for *An. arabiensis* rate indoors was 5.4 bites/man hour and 2.3 outdoors, i.e. 3.9 bites/man hour. Of the total catch 77.8% was *An. arabiensis*. *An. funestus* hourly biting rate was 1.9 indoors; and 0.3 outdoors i.e. 1.1 bites/man hour. This amounted to 22.2% of the total catch. The ratio of *An. arabiensis* to *An. funestus* was 3.5:1. At 1350 m (Rota) hourly biting rate for *An. gambiae* s.s. was 1.2 indoors, and 0.5 outdoors i.e. 0.9 bites/man hour. For *An. funestus* indoor hourly biting rate was 1.5, whereas outdoor rate was only 0.4, resulting in an average of 0.9 bites/man hour. Of the total catch 48.5 % comprised member species of *An. gambiae* s.l and 51.5% was *An. funestus*. The ratio of *An. gambiae* s.s. to *An. funestus* was 0.9:1.0. At 1524 m (Oriwo) the biting rate for *An. gambiae* s.s. indoors was 0.1, and outdoors 0.01, averaging 0.07 bites/man hour. For *An. funestus* the rates were 0.1 and 0.02 bites/man hour indoors and outdoors respectively. These results showed that irrespective of altitude both *An. gambiae* s.s. and *An. funestus* were predominantly indoor feeders. Of the total catch *An. gambiae* s.s. comprised 50.9%; whereas *An. funestus* was 49.1%, giving a ratio of 1:1.

Fig 4.2 (a) shows there was a marked differences in the man-biting rates of the three vector species at the different altitudinal sites. The indoor man-biting rate by *An. arabiensis* at 1219 m reached a peak of 108.5 bites/man/night in September (during which month the rice was about 5 mm tall, and barely covered with water), with an annual mean of 63.4 +/- 24.0 bites/man/night. This was significantly higher than that at 1350 m (Rota), where the indoor mbr peaked at 28.3 bites/man/night for *An. gambiae* s.s. in April. During that month the sympatric

Fig 4.2 a: Man-biting rates of Anopheles indoors at different altitudes estimated from night-bite collections by human-bait over a two year period (Jan 1989-Dec 1990).

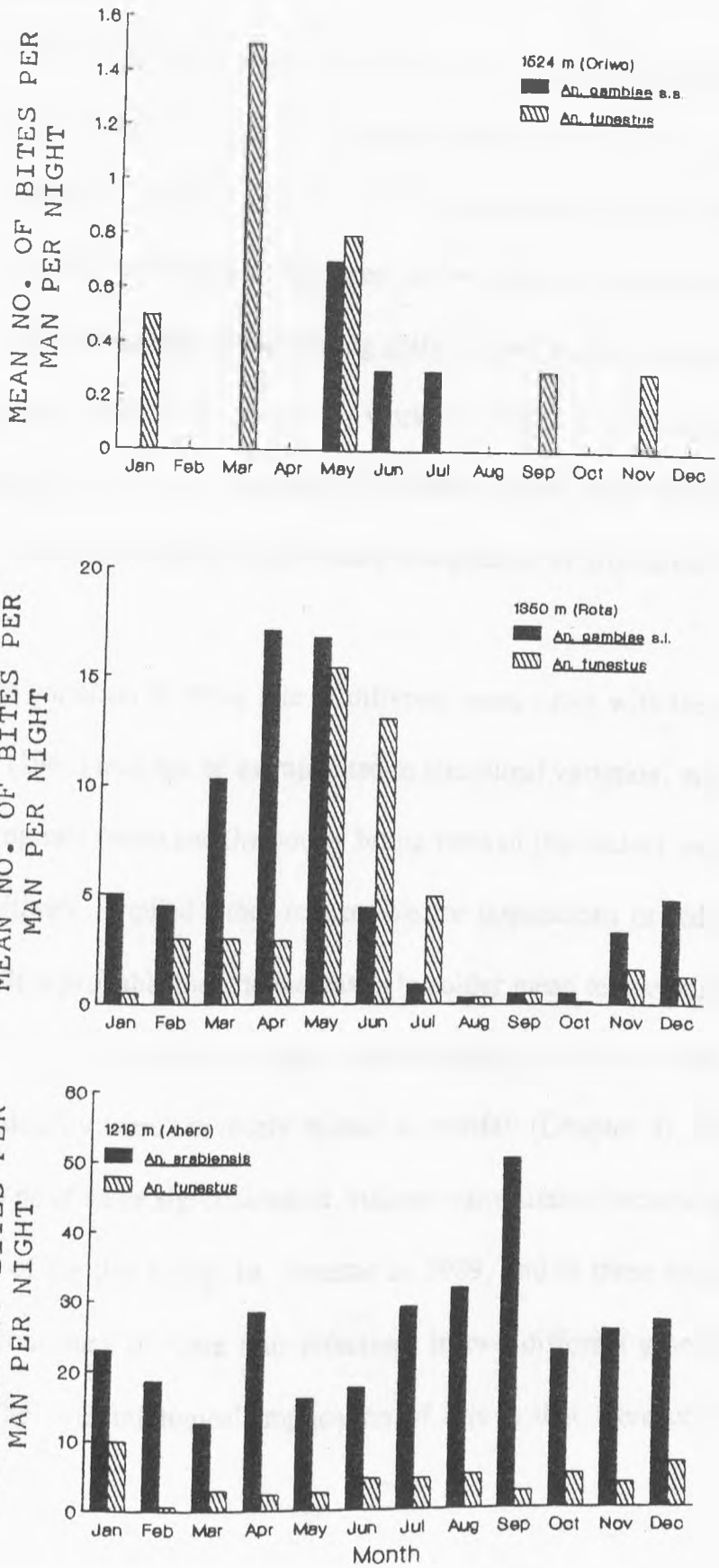


population was 100% *An. gambiae* s.s. The annual mean was 14.1 +/- 12.1. At 1524 m (Oriwo), indoor mbr for *An. gambiae* s.s. peaked at only 0.7 bites/man/night, also in April. At Ahero the indoor mbr for *An. funestus* attained a peak of 69.3 bites in June, with a mean of 25.7 +/- 8.7 bites per man per night. At Rota *An funestus* had a peak indoor mbr of 65.1 bites/man/night also in June, and the annual mean was 15.0 +/- 17.7 bites/man/night. Oriwo had the lowest mbr for *An. funestus*: only about 4 bites/man/night.

Using multi-variate analysis the mean indoor mbr for *An. funestus* at the various altitudes (Ahero, Rota and Oriwo) were significantly different ($F = 8.0$; $df = 33$; $p = 0.001$). Similarly the mean indoor mbr for *An. arabiensis* at Ahero and *An. gambiae* s.s. at Rota and Oriwo were found to be significantly different ($F = 52.6$; $df = 33$; $p = < 10 (-6)$. These differences seemed to have been accentuated by the reduction in breeding sites as altitude increased; thereby altering the topography and drainage of the area.

Figure 4.2 (b) shows that the man-biting rates outdoors for all the sites and species was less than half of indoor man-biting rates. There was only sporadic biting at 1524 m, unlike at 1350 m and 1219 m where vectors were biting throughout the year. In similar fashion to indoor activity, the outdoor mbr at Ahero (1219 m) for *An. arabiensis* was highest, 50.3 bites/man/night in September, having risen from 12.5 bites/man/night in March, with a mean of 25.1 +/- 9.8 bites/man/night. For *An. funestus* mbr outdoors was as low as 0.6 bites/man/night in February although it was highest at 10 bites/man/night in January, only a month prior. Mean mbr = 4.1 +/- 2.4 bites. Two months earlier (March), mbr for *An. funestus* had peaked at 1.5 bites/man/night. Significant differences were found between the mean indoor and outdoor biting rates. For *An. arabiensis* at Ahero, by student's t-test; $T=5.1$; $df=22$; $p=4.1 \times 10 (-05)$; for *An. funestus*, $T=4.0$; $df=22$; $p=6.6 \times 10 (-04)$. At Rota for *An.gambiae* s.s., $T=2.1$; $df = 22$;

Fig 4.2 b: Man-biting rates of Anopheles outdoors at different altitudes estimated from night-bite collections by human-bait over a two year period (Jan 1989-Dec 1990).



p=4.2 x 10 (-2). For *An. funestus*; T=2.1; df=22; p=4.9 x 10 (-02). At Oriwo for *An. gambiae* s.s.; T=2.0; df=22; p=5.9 x 10 (-02). For *An. funestus*, T=2.9; df=22; p=7.8

4.4 DISCUSSION

Information on biting cycles and peak biting time is, fundamentally important for determination and effective utilisation of control measures against malaria in a locality. In this regard, for example, efforts on malaria control have increasingly been directed to the use of bednets (Rozendaal, 1989). Although all the three main vectors of malaria do bite during both day and night time, the finding that greater biting activity took place at night, especially after midnight, as has been also observed by previous workers (Gillies & De Meillon, 1968), underscores the need to emphasise the use of bednets to be effected from about 22.00 hours and to be continued throughout the night to about 06.00 hours irrespective of altitudinal differences.

Although the variation in biting rate of different mosquitoes with the vertical height as reported by Haddow (1961) may not be extrapolated to altitudinal variation, none the less, the finding that the man-biting rate (mbr) and the hourly biting rates of the vectors were drastically reduced with increasing altitude, implied either reduced vector populations or reduced vector biting activity with height. It is probable that the increasingly colder mean temperatures encountered as altitude rises might have impaired both vector multiplication and biting activity, whereas the seasonal increases in density were obviously related to rainfall (Chapter 3). Day biting activity may be considered to be of some significance to malaria transmission because sporozoite infections were found in one of the day biting *An. funestus* in 1989, and in three species of the same vector in 1990. The occurrence of these four infections in two different years of study could hardly be accidental. The epidemiological implication of this is that there could be a risk of malaria

infection and transmission during day time when protective measures are least likely to be applied. During study visits it was observed that it was a common practice to have children and sometimes adults sleeping indoors during day time. These people were vulnerable to infection.

Much interest has often been directed to the possible transmission of malaria by species other than *An. gambiae* and *An. funestus*. It was apparent from the low biting catches that other anophelines may be negligible in malaria transmission in western Kenya. For a mosquito to be of vectorial importance it has to bite in sufficiently high numbers and also have a reasonably high sporozoite rate. Clearly *An. pharoensis* in this area did not satisfy both criteria, since only 58 specimens were caught from the three study sites during the entire study period, although this species is regarded as a vector in Egypt, but usually not in the Afrotropical Region (Gillies & de Meillon, 1968; Gillies & Coetzee, 1987). Moreover, as shown in Chapter 6, none of these were found with sporozoite infections. This, however, contrasted with the observations of Ijumba *et al.* (1990), who reported large numbers of this vector biting at Mwea-Tebere irrigation scheme, Kenya. On the other hand many specimens of *An. ziemmani* were caught biting, albeit more abundantly outdoors, thereby, reducing the chances of man/vector contact. Its contribution to malaria transmission, however, was not crucial because of lack of infectivity with *plasmodium*. *An. coustani* appeared more common only at high altitudes especially at Oriwo, but likewise it was a negligible player in malaria transmission.

CHAPTER FIVE

5 A COMPARISON OF AGE-COMPOSITION AND PARITY RATES OF MALARIA VECTORS AT DIFFERENT ALTITUDES

5.1 INTRODUCTION

Lumsden (1952) showed that a single mosquito population may consist of two components represented by two age-groups called nulliparous (young female mosquitoes that have not yet laid eggs), and parous (old mosquitoes that have laid eggs at least once). From an epidemiological point of view it is desirable to know whether biting habits and activity cycles of a mosquito population would be influenced by the age-group. This is because mosquitoes infected with malaria pathogens on their first feeding visit must live long enough to make a second or subsequent feeding visits in order to transmit the disease to a potential victim, i.e only parous mosquitoes would be capable to transmit disease. If such a study revealed that the nulliparous form the greatest feeding population in an area, the danger of malaria would be less in that area. In this connection, Gillies (1954) concluded that although younger female mosquitoes were slightly more active before 22.00 hours, their pattern of feeding did not differ materially from that of older females, and the gross features of the biting cycle were, therefore not being determined by age-groups behaving as distinct populations. However, studies on other families of biting Diptera, have revealed significant differences on the ages of females biting at different times. For example, in Northern Nigeria, both Crosskey (1958) and Lewis (1958) found that more old *Simulium damnosum* Theobald bit shortly before and after midday, the

younger flies biting more in the early morning and late afternoon. To investigate this question further, plans were made to study the ages of anopheline mosquitoes coming to bite at different times, and altitudinal levels on the study transect at Ahero, (1219 m) and Rota (1350 m).

In mosquito studies, age can be measured in several ways, one of which involves observations of external characters, for example, the degree of wear on the wing fringes and parasitisation of mosquitoes by larvae of hydrachnid mites. More reliable methods involve observations of internal characters in female mosquitoes, for example, the presence of meconium, the presence of a mating plug in the female oviduct, the presence of spermatozoa in the spermatheca, coiling of ovarian tracheoles and the presence of sac-like structures known as follicular dilatations left after a mature egg has passed down through the oviduct. This latter method has the advantage of being able to distinguish nulliparous and parous individuals as well as indicating the number of separate batches of eggs laid (gonotrophic cycle). This method was used in the present studies to estimate the age composition, and to assess the percentage of mosquitoes infected at a given gonotrophic age.

5.2 MATERIALS AND METHODS

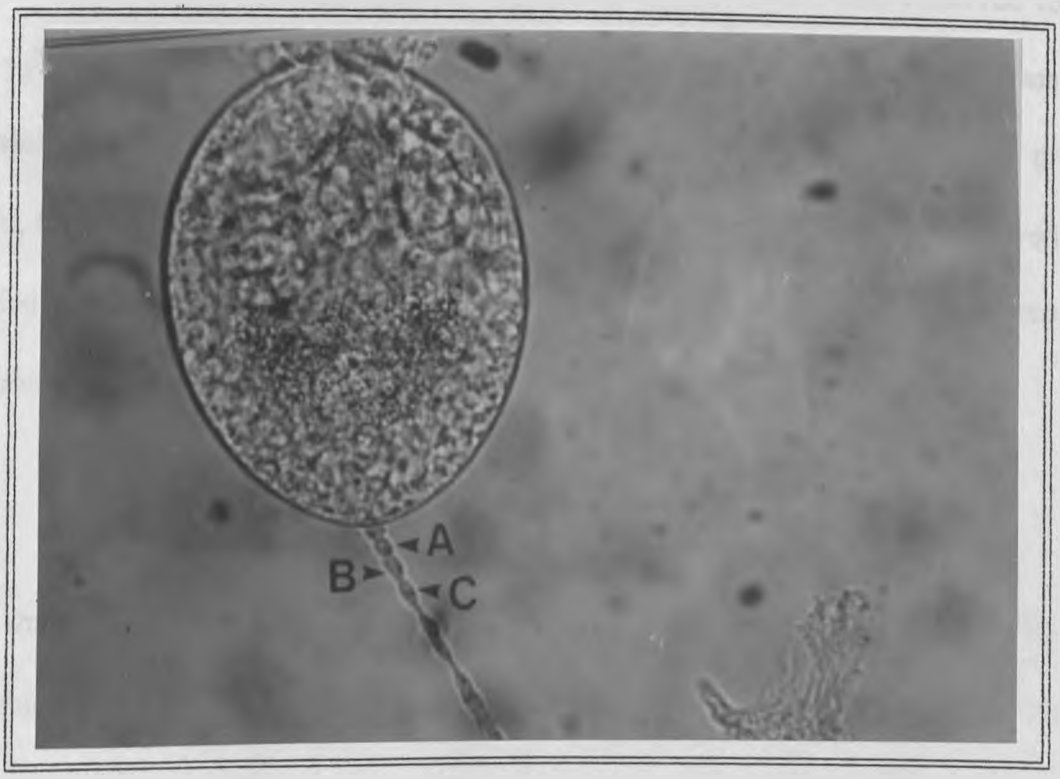
Age-composition studies were carried out on specimens collected mainly from two altitudinal sites, 1219 m (Ahero) and 1350 m (Rota) to determine whether altitude would influence the feeding habits and timing or cycle in young and older mosquitoes. The third site, Oriwo had very few mosquitoes. The first two sites were therefore, examined in more detail because they had adequate numbers of mosquitoes throughout the period of study. The mode of collecting the specimens was by the PSC technique and the human-bait already described above. For PSC samples, different species of mosquitoes from each of the 8 houses described above in the general methods were sorted according to Sella's (defined above) abdominal stages and given

serial numbers. Samples of each stage with odd numbers were picked in order to give a representative sample from each house and Sella's stages. These were dissected under a microscope to remove salivary glands for sporozoite examination, and the ovaries were removed and teased out for dilatation as earlier described. For those collected by the human-biting method, hourly catches of the different anopheline species caught were numbered serially. Where the total hourly catch was less than 10 mosquitoes of a given species, all were dissected for dilatations and also sporozoites. However, where the hourly catch of a given species exceeded 10 mosquitoes, only 10 specimens were selected randomly and dissected for both dilatations and sporozoites.

Dilatations in the ovaries were observed and counted with the aid of a compound microscope, starting from the sac end of individual ovarioles. Those mosquitoes which did not have any dilatations were classified as nulliparous, and those which had one or more dilatations, or the presence of mature eggs retained in the ovarioles were classified as parous. Parity rates were calculated as a percentage of those mosquitoes which had laid once. These rates were used to determine the percentage of old mosquitoes. The actual number of dilatations in the ovarioles of each mosquito, considered to indicate the number of gonotrophic cycles completed was recorded.

The gonotrophic cycle was studied using specimens collected in a given month. Between 50 to 60 freshly-fed females were collected at a time by aspiration inside houses and kept individually in 50 ml vials of which the bottoms were lined with moist filter paper. The vials were plugged with cotton wool and left at ambient temperature in one of the grass thatched houses from which the mosquitoes had been aspirated. Regular visual observations were made on the progress of

Plate 2: An ovariole of a mosquito showing the dilatations (A,B,C...) corresponding to the gonotrophic cycles (age-grading).



bloodmeal digestion and concomitant developmment of eggs. Initially these observations were made every six hours, thereafter reduced to three hour intervals. As the mosquitoes became gravid they were watched every half hour until they laid eggs. The approximate time of egg laying by individual mosquitoes was recorded. In this way, the duration taken between feeding and laying of eggs, gonotrophic cycle, and the number of times this had occurred, was used to estimate and compare the approximate gonotrophic age of a group of mosquitoes at a given altitude. Unfed females collected during biting catches were fed on the arm of a volunteer and reared until egg laying. Those which did not lay eggs were dissected for dilatations and parity.

In the study protocol, a total of 180 *An. arabiensis* were caught from Ahero and observed for the gonotrophic cycle during the short rains (October). In Febrruary, during the dry season, about a month before the onset of the long rains 54 female *An. arabiensis* were observed for the gonotrophic cycle, and during the long rains in June, 142 female *An. arabiensis* were observed. Fifty three fully fed *An. funestus* captured at Ahero were reared for the gonotrophic cycle. From Rota 83 *An. gambiae* s.s. females were studied for the gonotrophic cycle.

5.3 RESULTS

Table 5.1 shows that seasonal parity rates for the various vectors increased with altitude. This trend was more evident with *An. funestus* at Ahero which was only about 33% during the long rainy season. Vectors at Rota showed a higher parity rate despite the expected high output of nullipars as a result of enhanced breeding following the long rains, but was not so obvious with *An. gambiae* s.s. caught at the high altitude of Oriwo (1524 m), because of small numbers of specimens sampled. *An. gambiae* s.s. at Rota, exhibited higher seasonal parity rates than the population of *An. arabiensis* at Ahero.

Table 5.1: **The influence of altitude on parity rates of *Anopheles* vectors caught by human-bait techniques from houses between Jan 1989 and Dec 1990.**

Season	Species	Altitude and Site		
		1219 m, Ahero	1350 m, Rota	1524 m, Oriwo
Short rains (Oct.-Dec)	*An.arabiensis*	189/630 30%	0	0
	An.gambiae	0	16/113 54%	2/2 100%
	An.funestus	7/177 39%	44/69 63.8%	0
Dry season (Jan/Feb)	*An. arabiensis*	167/382 43.7%	0	0
	An. gambiae	0	99/187 52.9%	13/15 86.7%
	An. funestus	71/155 45.8%	48/187 55.2%	2/2 100%
Long rains (Apr-Jun)	*An. arabiensis*	197/434 45.4%	0	0
	An. gambiae	0	395/644 61.3%	18/40 45%
	An. funestus	152/451 33.7%	359/579 62.0%	7/19 36.8%

Seasonal variation in parity rate by altitude was significant.

(χ^2 test, $p < 0.0001$ for each test).

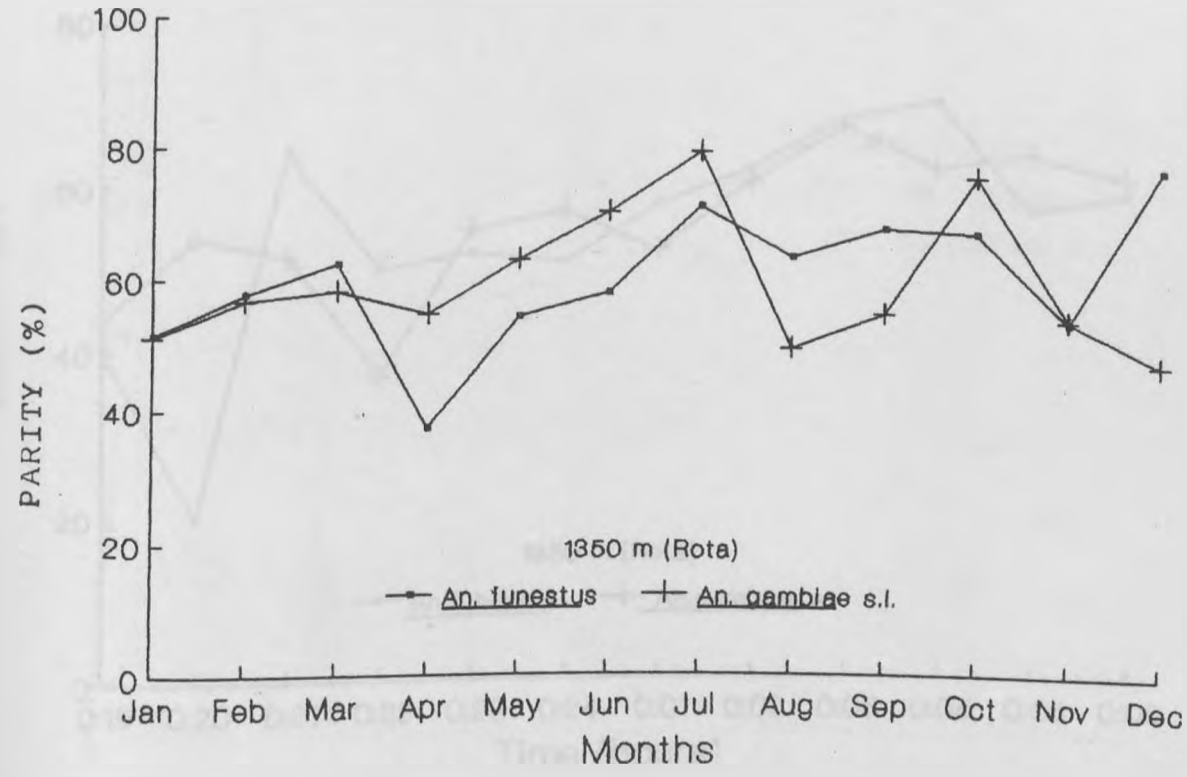
For each altitude the variation in parity rate by season was indeterminate because of few samples.

Fig 5.1 (a) shows that the mean monthly parity rates of *An. gambiae* s.s. and *An. funestus* at Rota (1350 m) were 60%. These parity rates did not differ significantly between the two vectors. In contrast, the mean parity rates for *An. arabiensis* and *An. funestus* at Ahero were 45%. During the short rainy season the parity rates of the two vectors differed, that of *An. funestus* being higher than that of *An. arabiensis*. Thus there was significant difference in the parity rates of the vectors according to altitude. The significance of the higher parity rates at Rota was that there was a likelihood of older and thus more infected vectors feeding at this altitude than at 1219 m, and hence enhanced malaria transmission.

Fig 5.1 (b) shows the parity rates of vectors biting at different hours of the night differed by altitude. At individual altitudes vectors with lower parity rates tended to feed in the early hours of the night and those with higher parity rates fed in the late hours of the night. This suggested that younger mosquitoes tended to feed in the early hours and older mosquitoes in the late hours.

Fig 5.2 shows the influence of altitude on gonotrophic age. While Ahero mosquito population was 60% nulliparous, the Rota population was 40% nulliparous for each species of vectors. The decline in gonotophic age was more rapid at Ahero, implying probably a higher mortality of the vectors at the lower altitude, or imergence of young mosquitoes from breeding places at a higher rate. In general, a consistently higher percentage of vectors lived through more gonotrophic cycles at Rota than at Ahero. Both species of the *An. gambiae* complex lived through four gonotrophic cycles. At 1219 m, the gonotrophic age of *An. arabiensis* decayed faster than that of *An. gambiae* s.s. *An. funestus* lived up to 8 gonotrophic cycles at Rota. Altitude appeared to have a significant influence on the rate of gonotrophic decay, i.e., probably the rate at which older mosquitoes die.

Fig 5.1 a: Parity rates by month of An.gambiea s.l., An. funestus and An.arabiensis caught at different altitudes by human-bait over a two year period (Jan 1989 -Dec 1990)



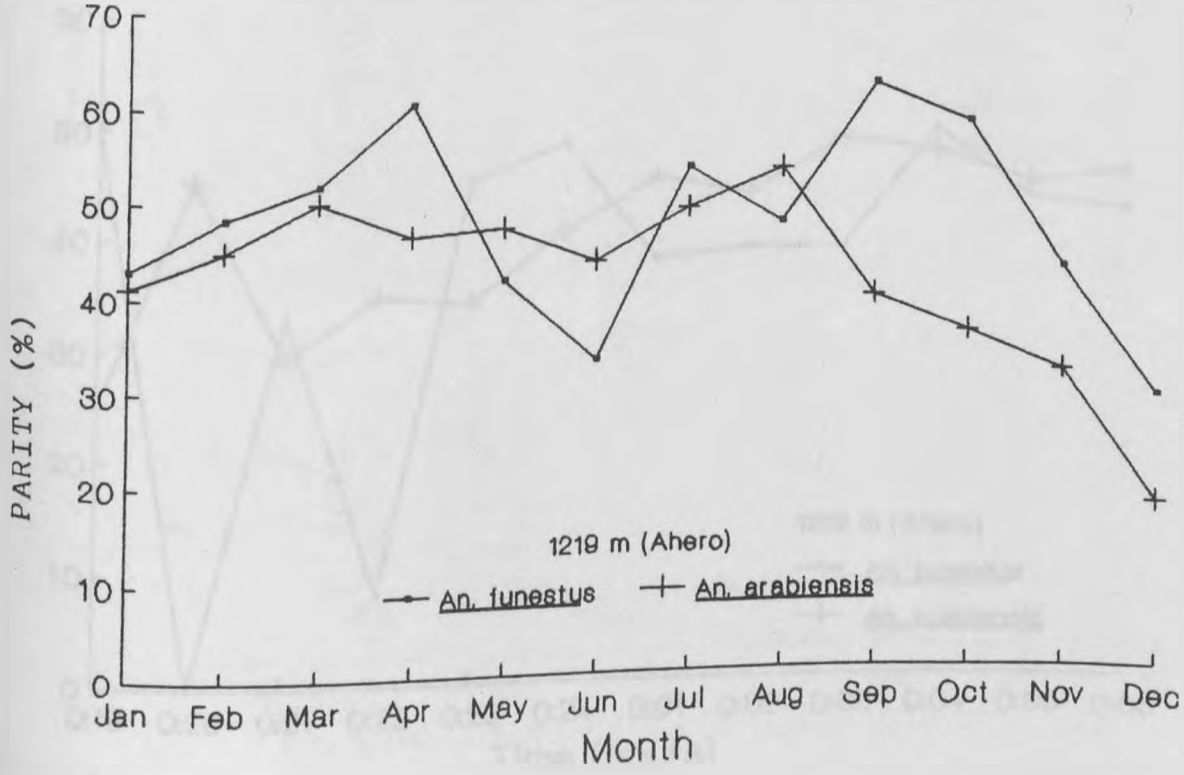
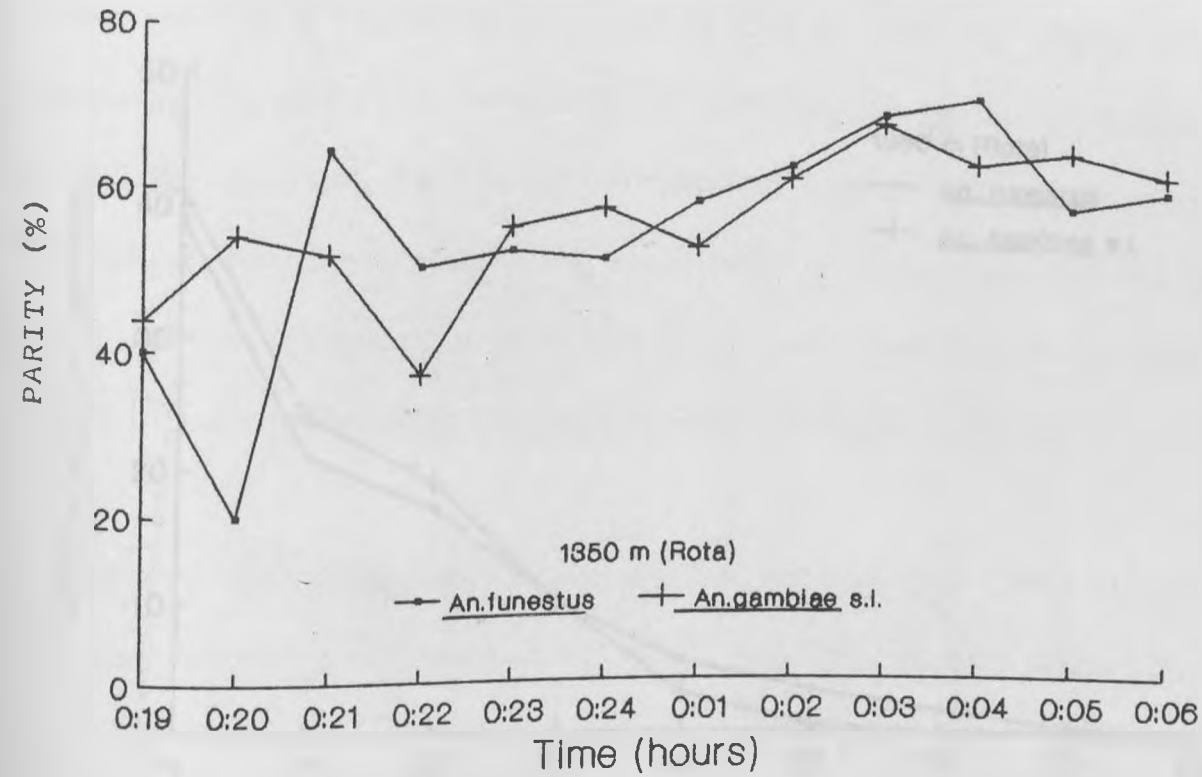


Fig 5.1b: Parity rates by hour of An.gambiae s.l., An. funestus and An.arabiensis caught at different altitudes by human-bait over a two year period (Jan 1989 -Dec 1990)



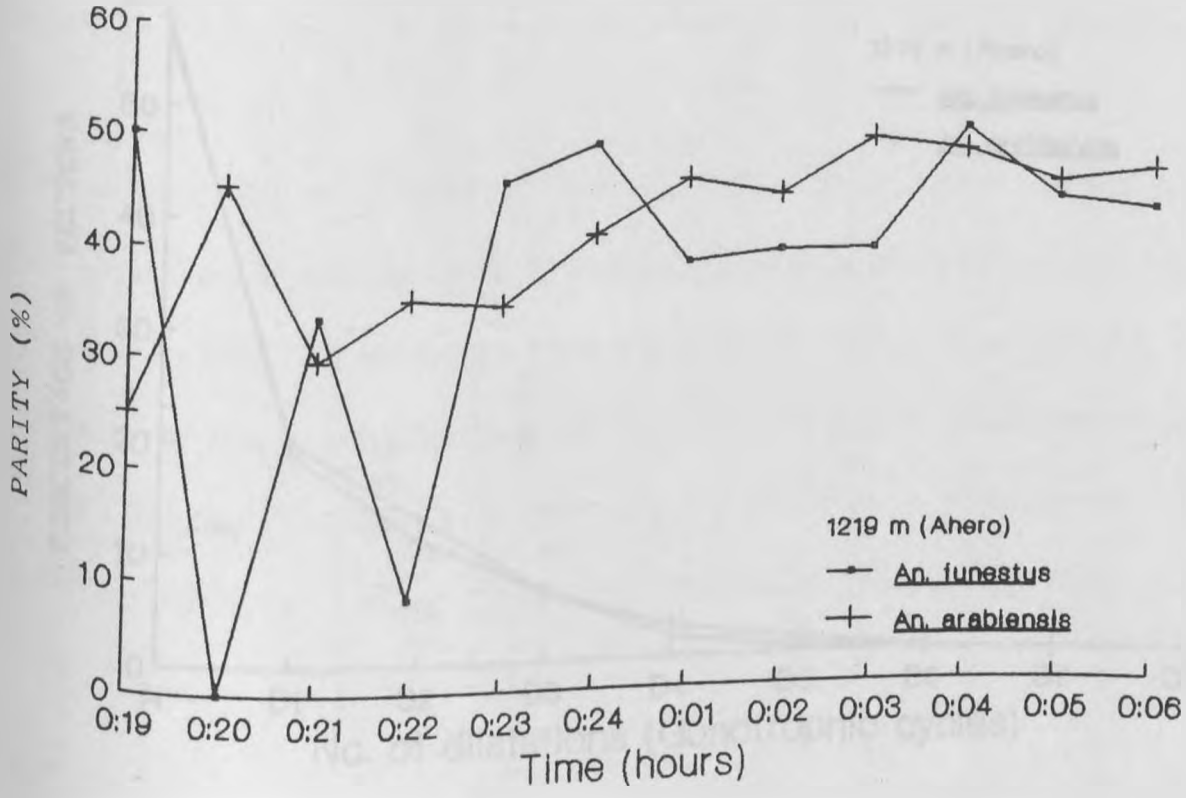


Fig 5.2: Gonotrophic age composition of An.gambiae s.l., An.funestus and An.arabiensis caught at different altitudes by human-bait over a two year period (Jan 1989 -Dec 1990)

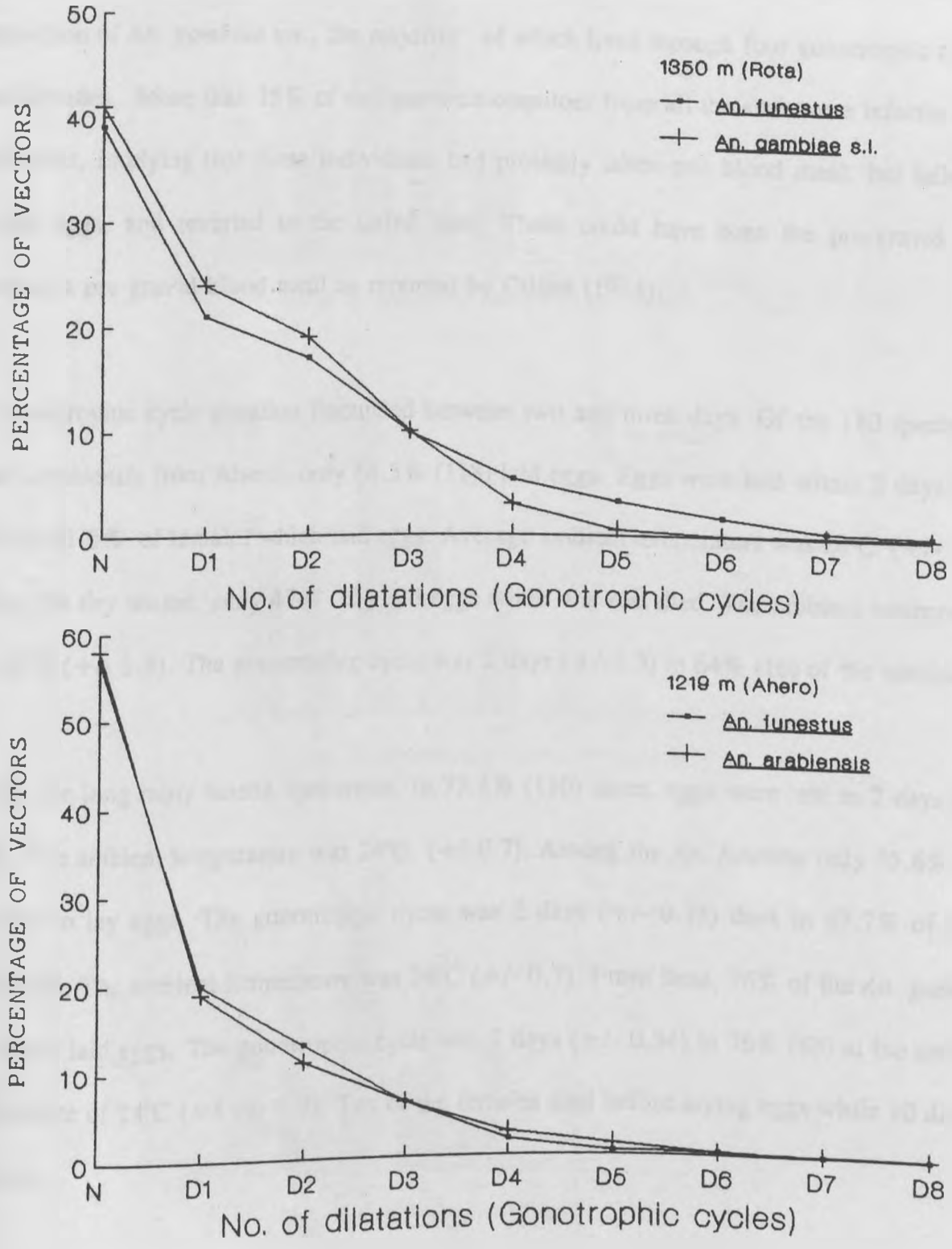
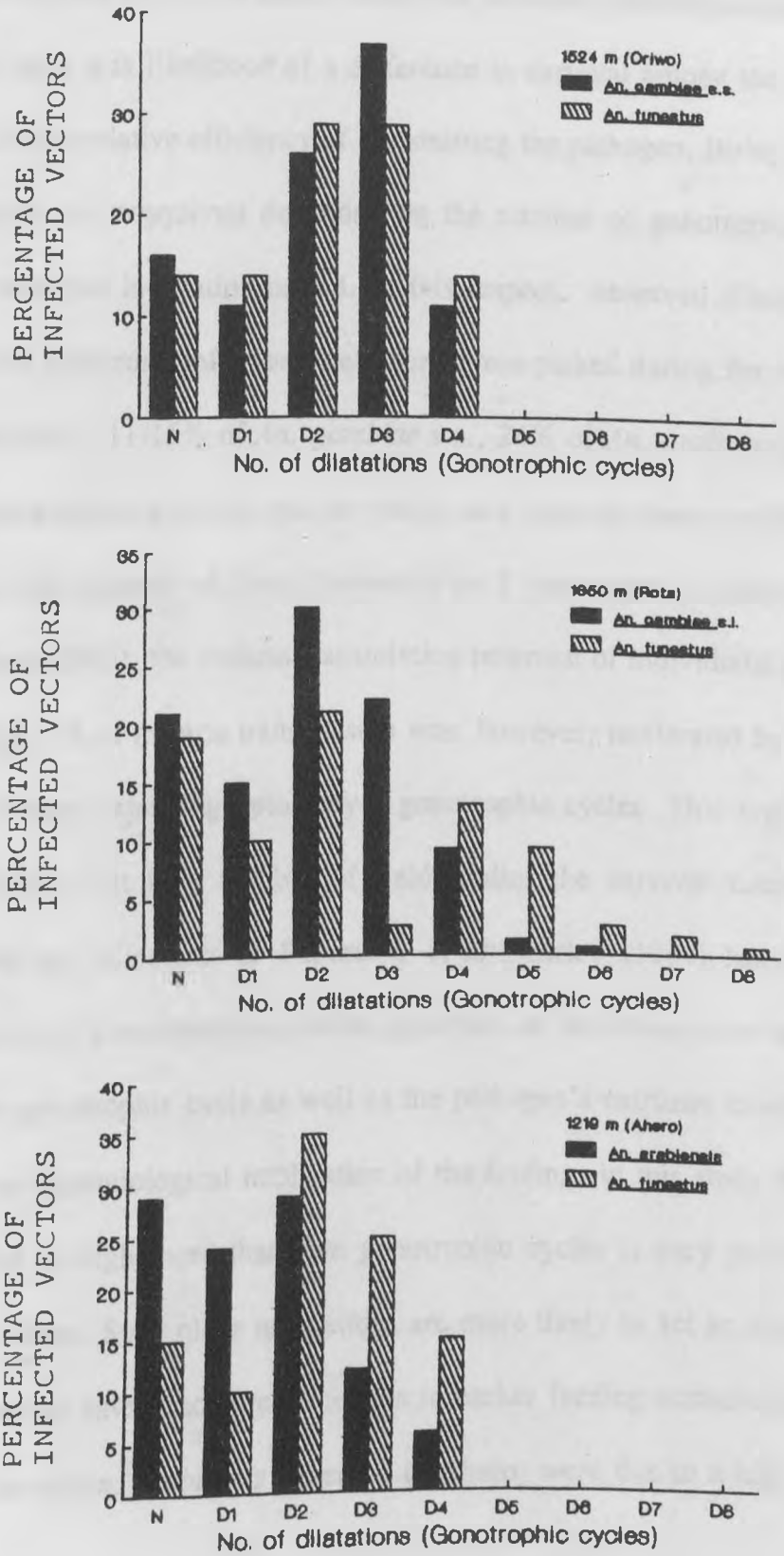


Fig 5.3 shows the distribution of infected vectors according to age and altitude at the three sites of Oriwo, Rota and Ahero. At the higher altitude of Oriwo, the majority of infected *An. funestus* lived through four gonotrophic cycles, whereas those caught at Rota, 1350 m lived up to eight gonotrophic cycles. At Ahero this species also lived up to four gonotrophic cycles. Infected *An. arabiensis* lived up to four gonotrophic cycles at 1219 m. This was similar to the age composition of *An. gambiae* s.s., the majority of which lived through four gonotrophic cycles at all altitudes. More than 15% of nulliparous mosquitoes from all the sites were infected with sporozoites, implying that these individuals had probably taken one blood meal, but failed to develop eggs, and reverted to the unfed state. These could have been the pre-gravid, i.e. requiring a pre-gravid blood meal as reported by Gillies (1954).

The gonotrophic cycle duration flactuated between two and three days. Of the 180 specimens of *An. arabiensis* from Ahero, only 64.5% (118) laid eggs. Eggs were laid within 2 days (+/- 0.63) by 81.5% of females which laid eggs. Average ambient temperature was 28°C (+/- 1.5). During the dry season, only 47% 25) laid eggs while the rest died. The ambient temperature was 31°C (+/- 1.3). The gonotrophic cycle was 2 days (+/-1.3) in 64% (16) of the specimens.

Among the long rainy season specimens, in 77.4% (110) cases, eggs were laid in 2 days (+/- 0.69). The ambient temperature was 24°C (+/-0.7). Among the *An. funestus* only 55.6% (30) survived to lay eggs. The gonotrophic cycle was 2 days (+/- 0.75) days in 67.7% of those which laid. The ambient temperature was 24°C (+/- 0.7). From Rota, 76% of the *An. gambiae* specimens laid eggs. The gonotrophic cycle was 2 days (+/- 0.34) in 76% (63) at the ambient temperature of 24°C (=4+/- 0.7). Ten of the females died before laying eggs while 10 did not lay eggs.

Fig 5.3: Gonotrophic age-composition of sporozoite infected vectors captured by human-bait at different altitudes over a two year period (Jan 1989 -Dec 1990)



5.4 DISCUSSION

The fact that the three vectors, *An. gambiae* s.s., *An. arabiensis* and *An. funestus* had a gonotrophic cycle of between 2 and 3 days, which was influenced by the ambient temperature, confirms that in reality not all the three vectors had the same gonotrophic cycle. The significance of this is that there was likelihood of a difference in survival among the three vectors, which could determine the relative efficiency of transmitting the pathogen. Birley (1984) suggested the vectorial capacity in mosquitoes depended on the number of gonotrophic cycles required to complete the extrinsic incubation period. In this respect, observed dilatations in the ovaries meant that since sporozoite infections were sometimes picked during the very first gonotrophic cycle, albeit by only 11-15% of *An. gambiae* s.s., 24% of *An. arabiensis* and 10-14% of *An. funestus* the transmission potential was very high, as a result of these very high infections. Since these vectors were capable of living beyond 8 or 9 gonotrophic cycles as also observed by Gillies & Wilkes (1965), the malaria transmission potential of individudal mosquitoes was very high. Their high risk of malaria transmission was, however, moderated by the fact that most of them had a life-span extending upto only 5 gonotrophic cycles. This supported recent studies which have shown that in a number of field studies the survival rates of malaria vectors decreased with age (Clements & Patterson, 1980). Birley (1984) has also shown that the vectorial capacity of a mosquito population depended on the vector survival rate and the actual duration of the gonotrophic cycle as well as the pathogen's extrinsic incubation period inside the vector. The epidemiological implication of the findings in this study that a big percentage of vectors went through more than five gonotrophic cycles is very profound with regard to malaria transmission. Such older mosquitoes are more likely to act as vectors, because of the higher likelihood of having acquired infection in earlier feeding occassions.

Low parity rates of *An. arabiensis* observed at Ahero, were due to a high recruitment rate of

nullipars as a result of increased breeding area through the irrigation flooding of the rice fields. This was postulated by comparing the low numbers of nulliparous forms found at Rota in a savannah area away from the abundant breeding places in the irrigation scheme. Carnevale (1987) made similar observations in Burkina Faso. This also explained the higher survival rates found among the Rota specimens despite the more or less similar duration of the gonotrophic cycles of the vectors from Ahero and Rota. It was also likely that there was intense interspecies and intra species competition for bloodmeal resources resulting in less robust individuals which could be susceptible to higher mortality at Ahero unlike Rota. Under such adverse conditions only a small minority of mosquitoes could have lived through the sporogonic cycle of the parasite and thus transmit disease. Furthermore the reproduction rate of mosquitoes may have been reduced as there was reduction in output from the breeding places. The total impact of these complex events on malaria transmission has been computed mathematically as vectorial capacity (Garret-Jones, 1964).

Infected mosquitoes were reported to probe more than once before taking a bloodmeal (Wekesa, 1990). Since a good percentage of mosquitoes were infected right from the nulliparous stage it would appear that either the probing activity was high, or the pre-gravid rate was high, thus leading to acquisition of sporozoite infections by a sizeable proportion of individuals. With regard to the latter possibility, Mutero & Birley (1987) have reported a pre-gravid rate of 7 to 70% among members of the *An. gambiae* in Kenya. The epidemiological implication of this phenomenon is that the vectorial capacity of the individual species in the study areas could be very high. Rota was likely to have a higher transmission potential by a particular age group than Ahero and Oriwo, because a good number of vectors went up to eight gonotrophic cycles, coupled by the higher sporozoite rates in both *An. gambiae* s.s. and *An. funestus* in this area.

CHAPTER SIX

6 SPOROZOITE AND INOCULATION RATES OF *Anopheles* MOSQUITOES AND MALARIA PREVALENCE IN SOME AREAS OF WESTERN KENYA

6.1 INTRODUCTION

When a mosquito feeds on a person infected with malaria, it may take up sexual forms of the malaria parasite known as gametocytes, which undergo a process of development into oocysts within the mosquito. This process of sporogony ultimately leads to subdivision of the oocysts into infective forms called sporozoites, which migrate to the salivary glands of the mosquito, ready for injection into the next victim. The sporozoite rate is a measure of the proportional number of female *Anopheles* mosquitoes in a population with sporozoites present in the salivary glands. This entomological parameter facilitates,

(a) the identification of primary and secondary vectors of human malaria and,

(b) the evaluation of malaria control programmes.

It has been described as the most sensitive and powerful tool for describing the epidemiology of malaria (Macdonald, 1952 & 1957; Onori & Grab, 1980; Burkot *et al.*, 1987 & 1988). The classical method to estimate sporozoite rate is the microscopic examination of salivary glands in the mosquitoes (Macdonald, 1952). However, limitation of this technique is twofold: the sporozoites can not only be morphologically distinguished, but also can be missed in low grade infections. To overcome these shortfalls, the development of species specific

monoclonal antibodies to a surface protein in the sporozoite, known as the circumsporozoite (CS) protein have permited the development of immunoradiometric assays (IRMA) (Zavala *et al.*, 1982), and enzyme-linked immunosorbent assays (ELISA) (Burkot *et al.*, 1984; Wirtz *et al.*, 1985), that detect CS proteins of the specific *Plasmodium* species. Evaluation of ELISA for the identification of sporozoites in wild Afrotropical *Anopheles* from Kenya was carried out by Beier *et al.* (1987) and Adungo *et al.* (1991 a) and found to be highly sensitive and comparable to dissection. In this study the ELISA technique was used to determine the sporozoite rate.

The entomological inoculaton rate was defined as the product of the sporozoite rate (s) and the mosquito biting density on humans (ma) (Garret-Jones, 1964). The parameter ma is the absolute mosquito density per person (m), and the rate at which a single mosquito bites humans (a). The parameter (a) is a composite of several factors including, host preference, life expectancy, oviposition cycle duration and the human blood index. The entomological inoculation rate was computed by Garret-Jones (1964) by dividing the human blood index by the oviposition cycle duration. Thus the equation for entomological inoculation rate was given as follows:

Entomological Inoculation Rate (E.I.R) = ma x s

6.2 MATERIALS AND METHODS

The anopheline mosquitoes used for the determination of sporozoite infections were samples of indoor day-resting collected by the PSC technique from the five altitudinal points of the entire transect. Samples of biting specimens were collected by the human-bait technique from Ahero (1219 m), Rota (1350 m) and Oriwo (1524 m described in Chapter 4 above. Hourly collections were separately analysed in the laboratory to detect sporozoites in identified *Anopheles* species. The ELISA method was used for the detection of sporozoites. The sporozoite rate was calculated from the cumulative total of both day-resting and night-bite collections. The entomological inoculation rate of individual mosquito species was calculated using the sporozoite rate of night-bite specimens collected from the three sites. ELISA method was used because it had an advantage in that it could also detect, identify and quantitate sporozoite species in either fresh or dried mosquitoes. It was thus more reliable in assessing infection susceptibility and infectivity by a given species of *Plasmodium* in the various vector mosquitoes, namely, members of the *An. gambiae* complex and *An. funestus*.

The basic ELISA method for sporozoite detection has been described in detail by Burkot *et al.* (1984 a & b) and Wirtz *et al.* (1987). In the ELISA method only the head and thoracic regions of the mosquitoes are used to prepare triturates for testing so as to minimise detection of the oocyst stage, normally found on the abdomen. The triturates thus prepared in 50 *u*l of blocking buffer (BB), which for 1 litre contained: 10 g of bovine serum albumin; 5.0 g casein; 0.1 g thimerosal and 0.01 g phenol red; and 1,000 ml phosphate buffered saline (PBS) *p*H 7.4) with Nonindent P-40 (NP-40) (5 *u*l NP-40/1 ml of BB) using glass grinders, were made up to 250 *u*l by addition of 200 *u*l BB in order to bring the final volume to 250 *u*l per

mosquito. Portions of 50 *ul* of this final preparation were then used for ELISA tests as described by Wirtz *et al.* (1987). Briefly, this test detects sporozoites by employing the **2 A10** monoclonal antibody which recognises **Asn-Ala-Asn-Pro** (Nardin *et al.*, 1982). The assay used in this study is that described by Wirtz *et al.* (1987) which is 10 times more sensitive than the original *P. falciparum* ELISA of Burkot *et al.* (1984 b). Each test was carried out in 96-well soft polyvinyl micro titre plates by performing the series of incubations and washings according to the technique described in detail by Beier *et al.* (1987). The negative controls consisted of field collected *An. gambiae* s.l. and *An. funestus* males from the study areas. Positive ELISA reactions were determined both visually and by reading from an ELISA reader (Titertek Multiskan MCC/340; U.S.A.), of ELISA absorbance cut off values after 30 minutes of twice the mean of 4 negative controls on the same plate as recommended by Beier *et al.* (1988). The ELISA reactions of the salivary gland sporozoite infections were graded empirically as 1+, 2+ or 3+ to reflect the sporozoite load as in the conventional dissection method.

6.3 RESULTS

Table 6.1 shows that along the transect there was variation in the monthly sporozoite rates of the day-resting members of the *An. gambiae* complex in which the mean sporozoite rates increased from a low level of 0.7% in *An. arabiensis* to as high as 12.5% in *An. gambiae* s.s. with rising altitude. The mean sporozoite rate of 3.7% in the *An. gambiae* s.s. at Oriwo (1524 m) was significantly higher than 0.7% in *An. arabiensis* from Ahero. This trend was also evident with the monthly sporozoite rates, the highest of which, 14.5% in *An. gambiae* s.s. was significantly higher than the 2.1% in *An. arabiensis*. This showed that the sporozoite rates changed both by season and in a clinal fashion. At Kegati, the sporozoite rate for *An. gambiae* s.s. appeared higher, possibly because of low numbers of vectors caught.

Table 6.1: Sporozoite rates in An. arabiensis and An. gambiae s.l. from the transect determined by ELISA for sporozoite infection (Jan 1989 - Dec 1990)

Month	SITE NAME AND ALTITUDE				
	1219 m Ahero *	1350 m Rota **	1524 m Oriwo **	1829 m Kegati **	2134 m Ichuni **
Jan	5/636 (0.8)	25/442 (5.7)	1/2 (50)	0/3 (0)	0 (0)
Feb	2/244 (0.8)	13/240 (5.4)	1/4 (25)	0/6 (0)	0 (0)
March	2/266 (0.8)	32/453 (7.1)	0/3 (0)	0/3 (0)	0 (0)
April	1/286 (0.4)	11/628 (1.8)	4/190 (2.0)	0/18 (0)	0 (0)
May	2/328 (0.6)	23/435 (5.3)	9/62 (14.5)	2/45 (4.4)	0 (0)
June	3/436 (0.7)	8/166 (4.8)	20/435 (4.4)	6/62 (9.7)	1/7 (14.3)
July	1/293 (0.3)	18/119 (15.1)	0/14 (0)	1/10 (10)	0 (0)
August	12/558 (2.1)	6/67 (9.0)	1/15 (6.7)	1/16 (6.3)	0 (0)
Sept	7/925 (0.8)	3/72 (4.2)	0 (0)	0/3 (0)	0 (0)
October	0/397 (0)	4/63 (6.3)	1/82 (1.2)	0/13 (0)	0/1 (0)
Nov	1/234 (0.4)	5/176 (2.8)	1/28 (3.6)	0/4 (0)	0 (0)
Dec	0/139 (0)	15/212 (7.1)	0 (0)	0/6 (0)	0 (0)
Mean	36/4930 (0.7)	163/3073 (5.3)	45/1207 (3.7)	10/189 (5.3)	1/8 (12.5)

Species * = An. arabiensis, ** = An. gambiae s.l.
First Row figures = Number sporozoite positive mosquitoes over number examined.
Figure in paranthesis = Sporozoite rate (%)

Table 6.2 shows that although the mean sporozoite rates in day-resting *An. funestus* rose with altitude, they did not increase in a clinal fashion with rising altitude, but varied rather hapharzadly from as low as 1.9% at 1219 m (Ahero) to as high as 11.1% at 1829 m, with no significant difference between the two altitudes of 1350 m and 1524 m, both of which registered a sporozoite rate of about 4%. The high rate of 11.1% observed at 1829 m might be exagerated because of small sample numbers. It is also possible that the difference in sporozoite rate could be due to mosquito species rather than altitude.

Analysis of Sella's stages showed that sporozoite infection in half-gravid vectors was higher than in the fully gravid. This condition was persistent at all altitudinal levels indicating that this environmental factor did not influence sporozoite development. Generally, the highest infection rate occurred in feds and gravids. At 1219 m none of the unfed *An. arabiensis* was infected. There was no significant tendency of the sporozoite rate to increase with altitude. At 1524 m (Oriwo) the sporozoite rate was highest in half-gravid mosquitoes although it did not significantly differ from that of the feds and gravids. Sporozoite infection in *An. funestus* increased from low altitude (1.9%) to 4.0% at 1524 m. This trend was reflected in most ovarian stages of this mosquito. The sporozoite rate in unfed mosquitoes ranged from 0.7% to 2.4%, indicating that at all altitudes such mosquitoes could be infective at the second feeding session. The apparently much higher sporozoite rate in *An. funestus* from Kegati might be exagerated, because of the small numbers of vectors caught at this altitude.

A more detailed examination of sporozoite distribution with altitude in Fig 6.1 shows that while *An. funestus* at 1219 m is infected with sporozoites throughout the year the second vector, *An. arabiensis* at this site was infected for only 8 months, and with consistently lower sporozoite rates than the former. The significance of this is that in a rice growing

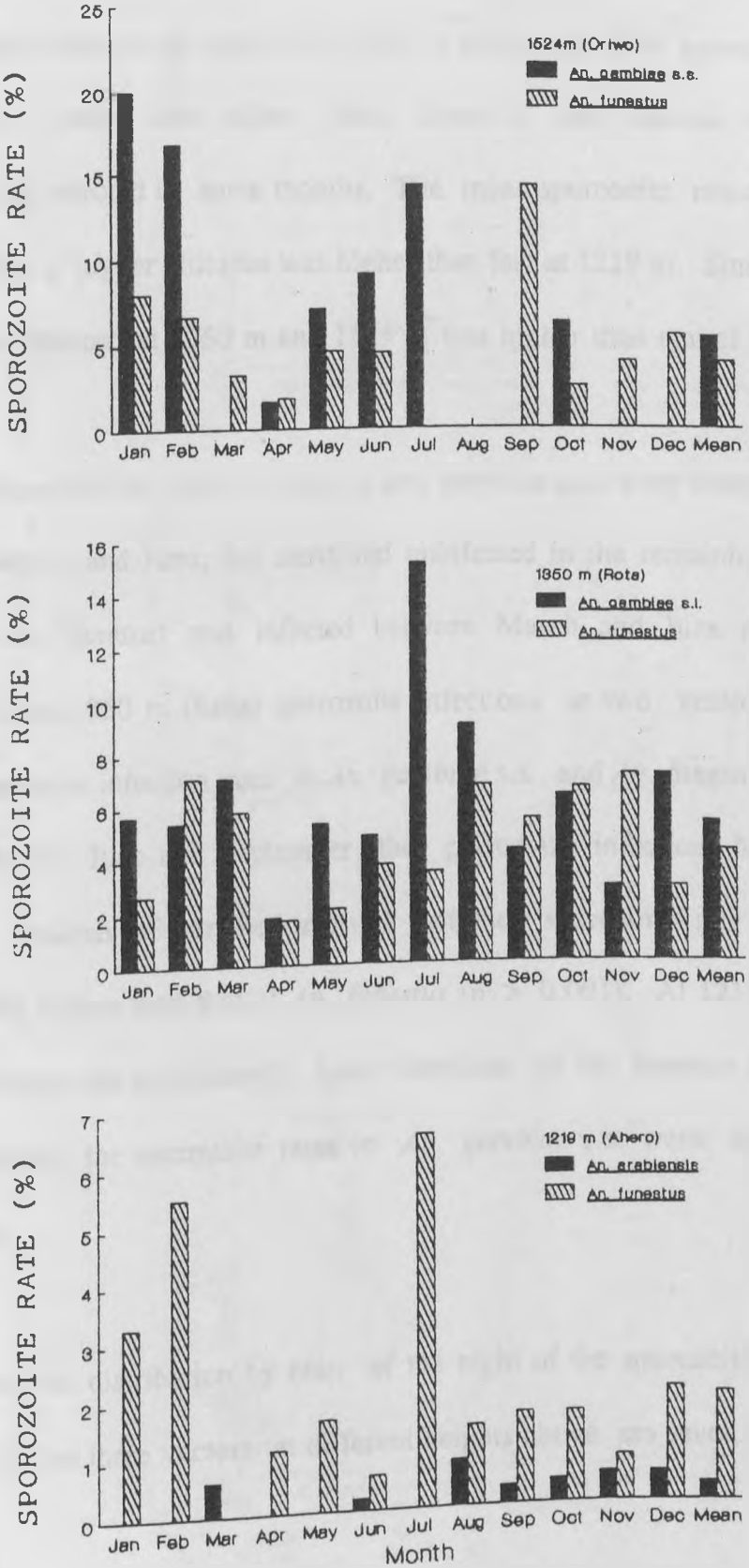
Table 6.2: Sporozoite rates in An. funestus from the transect determined by ELISA for sporozoite infection (Jan 1989 - Dec 1990)

Month	SITE NAME AND ALTITUDE				
	1219 m Ahero	1350 m Rota	1524 m Oriwo	1829 m Kegati	2134 m Ichuni
Jan	2/16 (3.3)	8/296 (2.7)	2/25 (8.0)	0/1 (0)	0 (0)
Feb	5/91 (5.5)	9/184 (4.9)	1/15 (6.7)	0 (0)	0 (0)
March	0/38 (0)	21/364 (5.8)	1/31 (3.2)	0 (0)	0 (0)
April	1/89 (1.1)	4 /236 (1.7)	1/55 (1.8)	0/2 (0)	0 (0)
May	4/249 (1.6)	5/242 (2.1)	3/65 (4.6)	0/3 (0)	0 (0)
June	1/159 (0.6)	16/436 (3.7)	1/22 (4.5)	0/2 (0)	0 (0)
July	3/46 (6.5)	11/321 (3.4)	0/6 (0)	0 (0)	0 (0)
August	2/143 (1.4)	26/389 (6.7)	0/14 (0)	0 (0)	0 (0)
Sept	2/122 (1.6)	6/111 (5.4)	1/7 (14.3)	0 (0)	0 (0)
Oct	1/61 (1.6)	4/61 (6.6)	1/40 (2.5)	0 (0)	0 (0)
Nov	1/133 (0.8)	6/84 (7.1)	1/25 (4.0)	0 (0)	0 (0)
Dec	2/100 (2.0)	2/71 (2.8)	2/36 (5.6)	0 (0)	0 (0)
Mean	24/1292 (1.9)	118/2795 (4.2)	14/351 (4.0)	1/9 (11.1)	0 (0)

First row figures = Number sporozoite positive mosquitoes over number examined.

Figures in parenthesis = Sporozoite rate (%)

Fig 6.1: Sporozoite rates of indoor day-resting Anopheles caught by PSC at three altitudinal sites over a two year period (Jan 1989-Dec 1990).



environment, although larger numbers of *An. arabiensis* than *An. funestus* rest indoors the vectorial capacity of the latter may be of more significance. On the other hand, sporozoite infections were higher throughout the year among the population of *An. gambiae* s.s. than in *An. funestus* at 1350 m above sea level. The vectorial capacity of *An. gambiae* s.s. was also likely to be higher. At 1524 m above sea level sporozoite infections in the *An. gambiae* s.s. were also higher than those in *An. funestus* vectors which were only intermitently infected in some months. The mean sporozoite rate for member species of the *An. gambiae* at higher altitudes was higher than that at 1219 m. Similarly, the mean sporozoite rate of *An. funestus* at 1350 m and 1524 m was higher than that at 1219 m.

Fig 6.2 shows that at 1524 m (Oriwo) *An. gambiae* s.s. were infected with sporozoites only between March and June, but remained uninfected in the remaining months of the year. In contrast, *An. funestus* was infected between March and June and between October and December. At 1350 m (Rota) sporozoite infections in two vectors occurred throughout the year. Sporozoite infection rates in *An. gambiae* s.s. and *An. funestus* were compared between the months of June and September, the period the infections had been observed to be highest. Analysis of variance showed that the sporozoite rate in *An. gambiae* s.s. was significantly higher than that of *An. funestus* ($p > 0.001$). At 1219 m the sporozoite rate of *An. arabiensis* was significantly lower than that of *An. funestus* ($p < 0.05$). Between the sibling species, the sporozoite rates in *An. gambiae* s.s. were much higher than in *An. arabiensis*.

Considering the distribution by hour of the night of the sporozoite-positive bites and of all the bites by the three vectors at different heights above sea level, Fig 6.3 shows that at 1524

Fig 6.2: Sporozoite rates of Anopheles caught in night-bite collections by human-bait technique at different altitudes over a two year period (Jan 1989-Dec 1990

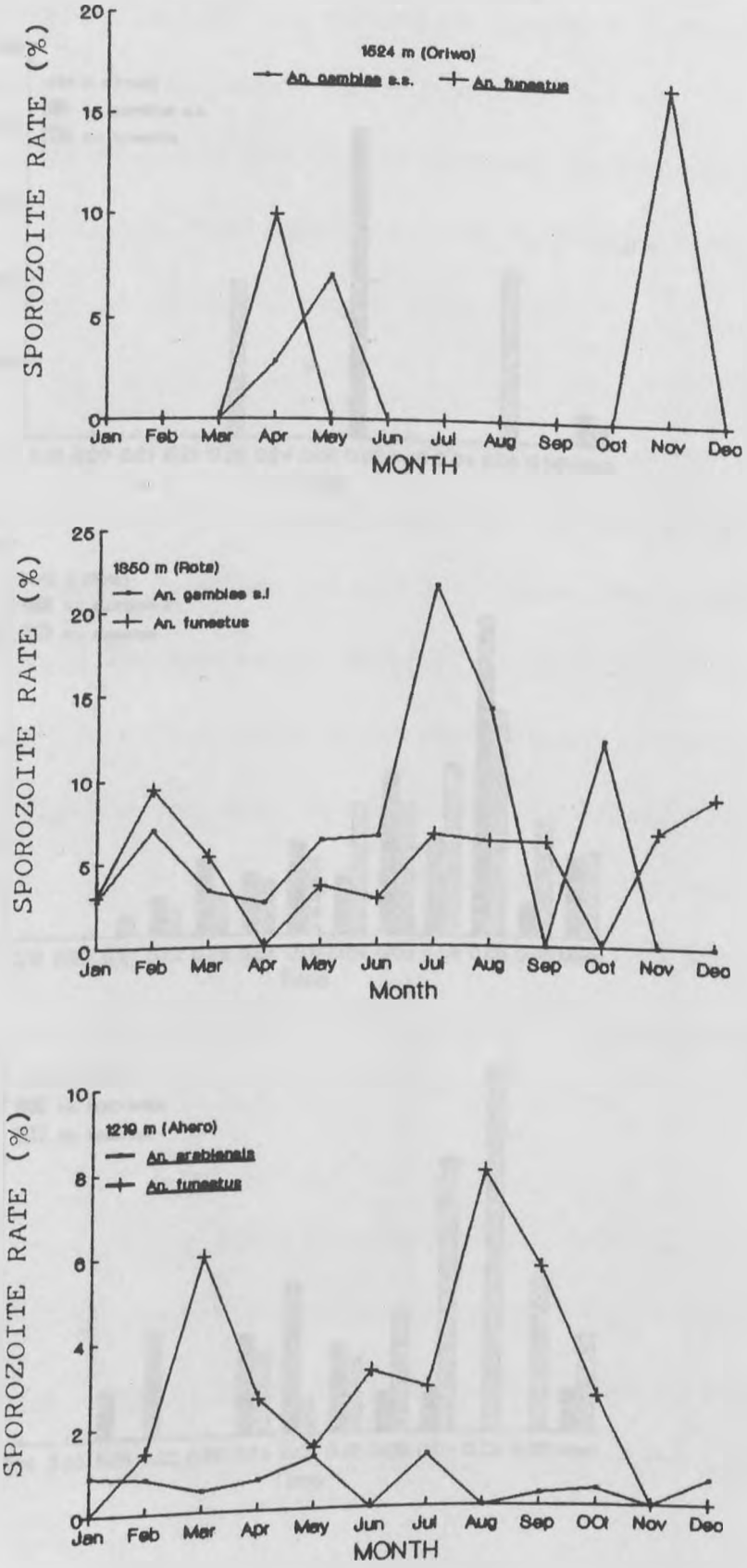
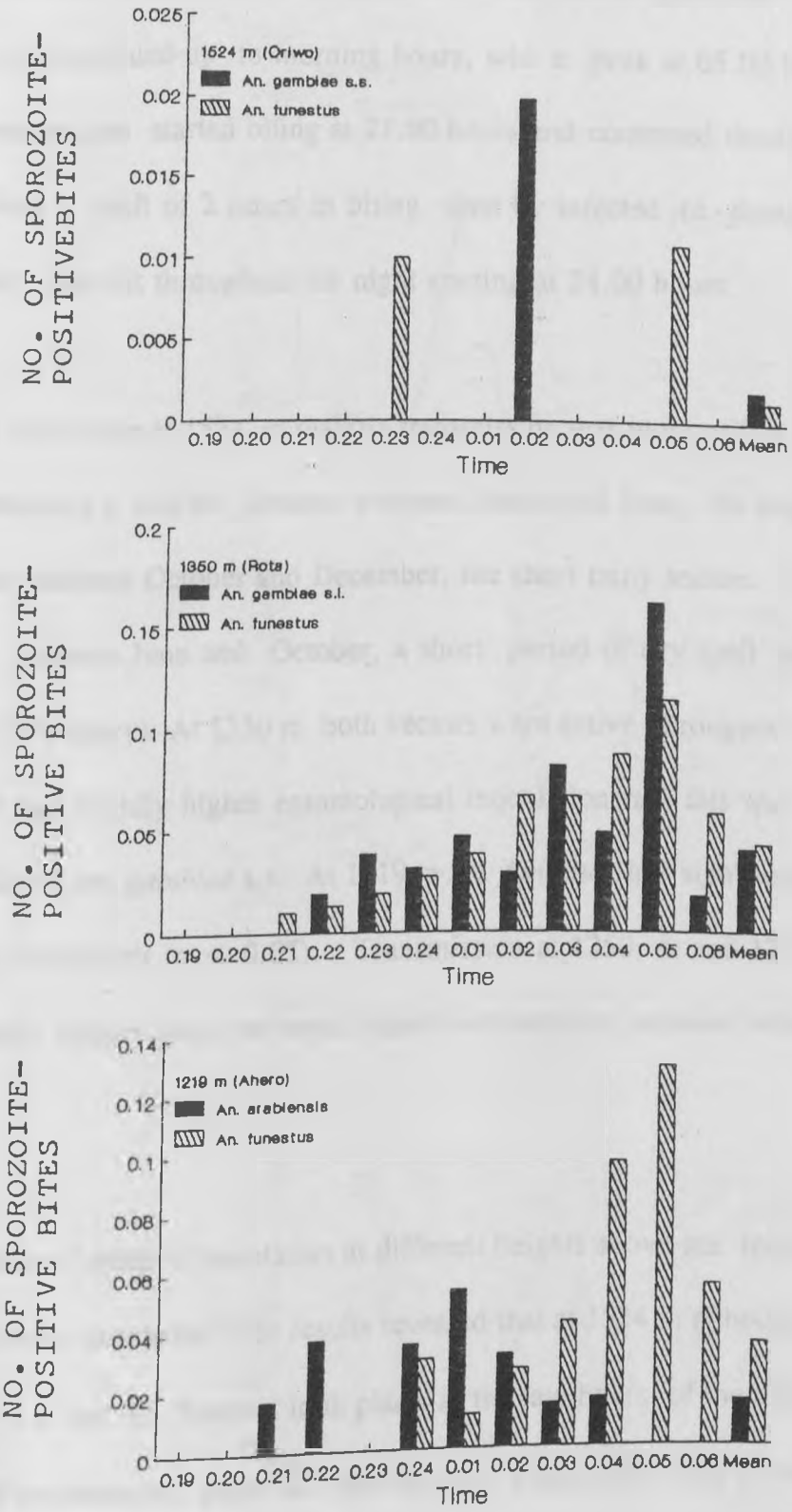


Fig 6.3: Distribution by hour of the night of the sporozoite-positive bites by Anopheles caught by human-bait at different altitudes over a two year period (Jan 1989-Dec 1990).

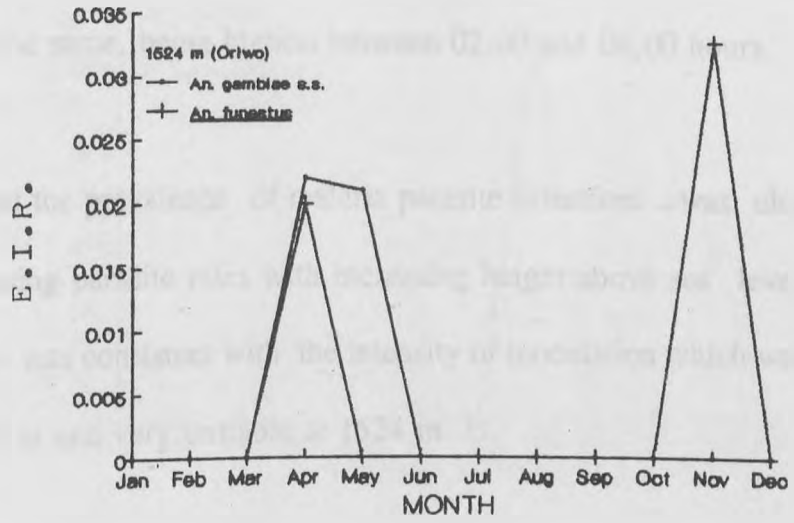


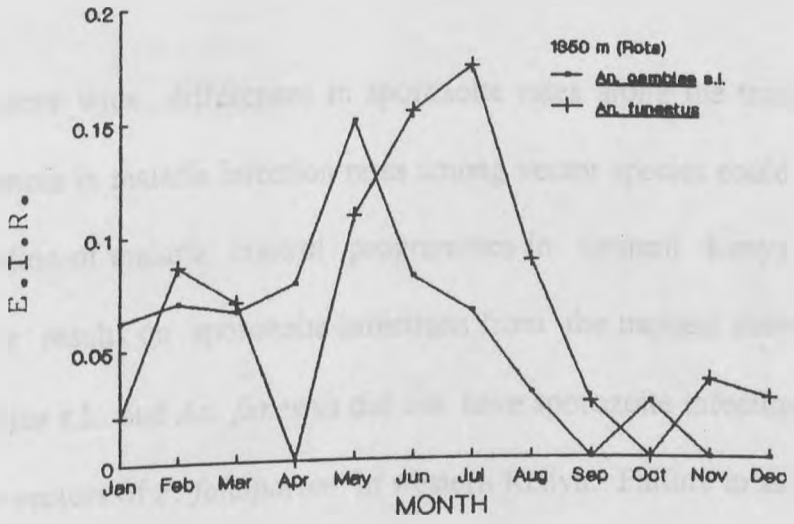
m infected *An. gambiae* s.s. bit only at 02.00 hours in contrast to infected *An. funestus* which bit from 23.00 hours and also at 05.00 hours. On the other hand at 1219 m infected *An. arabiensis* bit from as early as 21.00 hours and ceasesed at 04.00 hours. This contrasted with the bites at 1350 m by infected *An. gambiae* s.s., which started at 22.00 hours and continued up to morning hours, with a peak at 05.00 hours. At Rota, infected *An. funestus* also started biting at 21.00 hours and continued throughout the night. There was a shift of 2 hours in biting time by infected *An. funestus* at this altitude, which however, also bit throughout the night starting at 24.00 hours.

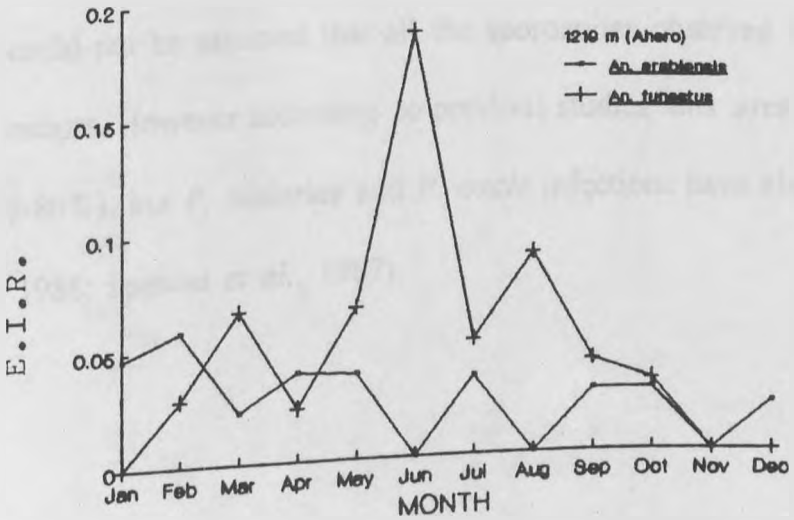
Fig 6.4 shows that at 1524 m malaria transmission was more or less equally carried out by both *An. gambiae* s.s. and *An. funestus* between March and June, the long rainy season but by only the latter between October and December, the short rainy season. The transmission cycle was broken between June and October, a short period of dry spell and during the dry season (January/February). At 1350 m both vectors were active throughout the year, but although *An. funestus* had slightly higher entomological inoculation rate this was not significantly different from that of *An. gambiae* s.s. At 1219 m *An. funestus* had significantly higher infective bites than *An. arabiensis* ($p < 0.05$). Transmission at 1350 m and 1219 m was stable, however the general picture seems to depict higher transmission at lower altitude, decreasing as altitude rose.

The pattern of malaria inoculation at different heights above sea level during different hours of the night were compared. The results revealed that at 1524 m although inoculation by both *An. gambiae* s.s. and *An. funestus* took place in the late hours of the night the times of inoculation were not synchronous, since *An. gambiae* s.s. inoculation wave preceded that of *An. funestus*

Fig 6.4: Entomological inoculation Rate (EIR) by month of Anopheles caught by human-bait in night-bite collections at different altitudes over a two year period (Jan 1989 - Dec 1990)







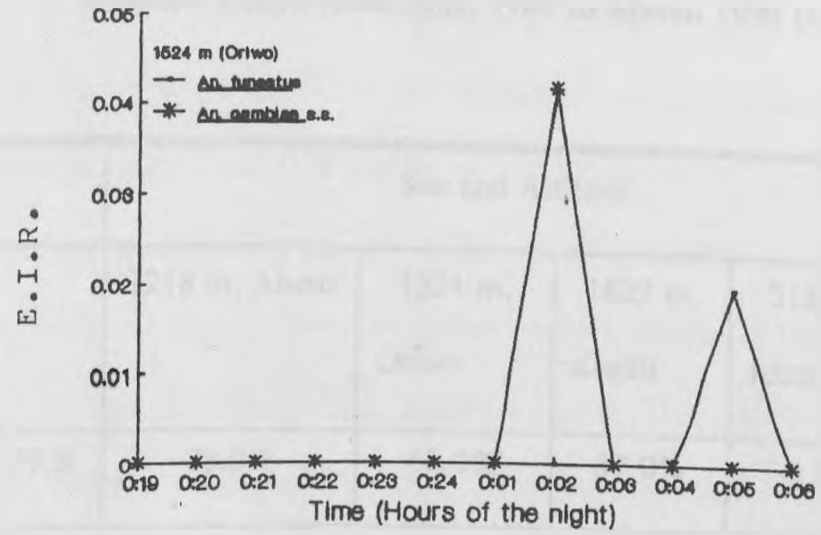
as seen in Fig 6.5. At Ahero (1219 m), transmission by *An. funestus* was significantly higher than that of *An. arabiensis* in the late night hours. At Rota transmissions by *An. gambiae* s.s. and *An. funestus* were not significantly different at all hours of the night. Overall transmission by *An. funestus* at 1350 m was slightly more intense than at 1219 m but the pattern was the same, being highest between 02.00 and 06.00 hours.

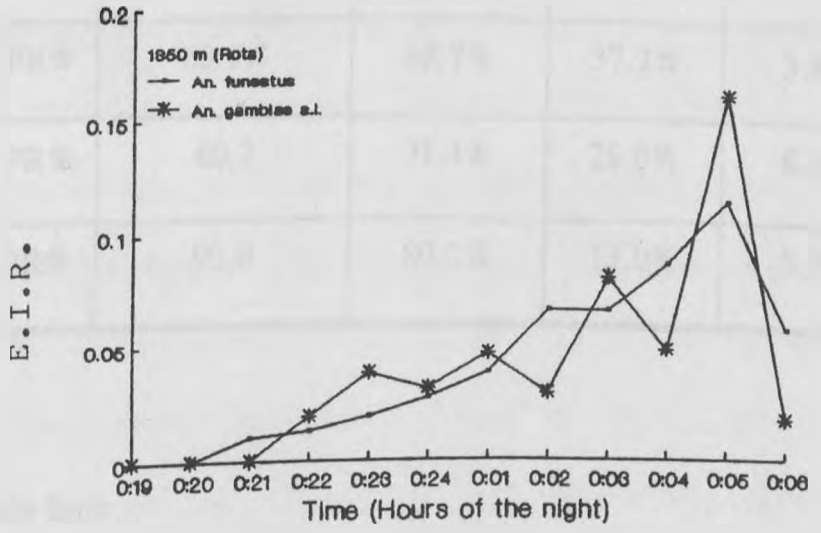
Table 6.3 shows that the prevalence of malaria parasite infections was closely related to altitude, i.e., decreasing parasite rates with increasing height above sea level and also by months. This pattern was consistent with the intensity of inoculation which was highest at 1219 m, medium at 1350 m and very unstable at 1524 m.

6.4 DISCUSSION

The fact that there were wide differences in sporozoite rates along the transect showed that knowledge of differences in malaria infection rates among vector species could be critical to the effective implementation of malaria control programmes in western Kenya, and indeed in any other area. The results on sporozoite infections from the transect showing that species other than *An. gambiae* s.l. and *An. funestus* did not have sporozoite infections confirmed that these were the major vectors of *P. falciparum* in western Kenya. Failure to acquire monoclonal antibodies to species other than *P. falciparum*, precluded detecton of other sporozoite species, and thus it could not be assumed that all the sporozoites observed were those of P. falciparum in these vectors. However according to previous studies this area is holoendemic for *P. falciparum* (79-80%), but *P. malariae* and *P. ovale* infections have also been observed (M.S. Beier *et al.*, 1988; Spencer *et al.*, 1987).

Fig 6.5: Comparison of entomological inoculation rates and pattern of different vectors by hour during the night at different altitudes over a two year period (Jan 1989 -Dec 1990)





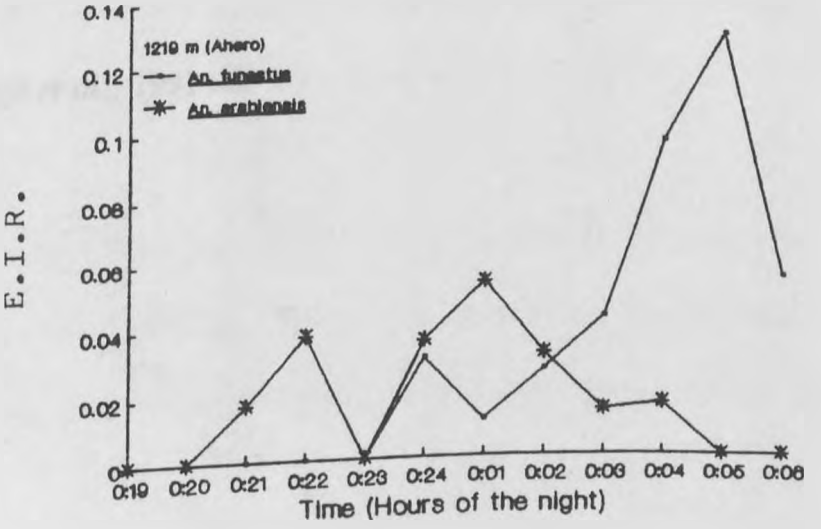


Table 6.3:Prevalence of malaria parasite infectiolns in children at different altitudes in Western Kenya from June, 1988 to March 1990 (2 years)

		Site and Altitude			
Month		1218 m, Ahero	1524 m, Oriwo	1829 m, Kegati	2134 m, Ichuni
June	PR%	78.0%	68.0%	37.0%	6.0%
Sept	PR%	74.0%	67.0%	29.0%	3.0%
Nov	PR%	60.0%	68.7%	37.2%	3.4%
Feb	PR%	60.2	71.4%	28.0%	6.0%
March	PR%	90.0	80.0%	13.0%	5.0%

PR = Parasite Rate

From Adungo *et al.*, 1991

The ability to employ cytotaxonomic methods for distinguishing members of the *An. gambiae* complex afforded an added opportunity to compare sporozoite infections in field collections of *An. gambiae* s.s. and *An. arabiensis* in addition to comparing the two vectors with *An. funestus*. The earlier studies of malaria transmission in the Kisumu area of western Kenya have been markedly divergent. To begin with, prior to fenithrothion trial for malaria control in the Kisumu area, Joshi *et al.* (1975) and Service *et al.* (1978) found no significant difference in sporozoite rates of *An. gambiae* s.s. and *An. arabiensis*. On the other hand, Highton *et al.* (1979) working in western Kenya in the same period found a marked difference in infection rates, with *An. gambiae* having 5.3% and *An. arabiensis* 0.3%. This result is consistent with that observed at the transect which showed that the mean sporozoite rate of 3.7% in a homogeneous population of *An. gambiae* s.s. at Oriwo was significantly higher than that of 0.7% in an equally homogeneous population of *An. arabiensis* at Ahero, with each species having higher or lower rates in some seasons. It has also to be noted that even in a sympatric population species specific sporozoite rates may fluctuate and reflect higher or lower infection rates in a given month because of population changes. The marked differences reported in the comparisons of relative importance of *An. gambiae* s.s. and *An. arabiensis* as malaria vectors in western Kenya should therefore, be seen in the context of geographic differences in mosquito populations sampled e.g., rice growing area *visa vis* savannah-type habitat; and the season. Clearly, in both ecosystems *An. funestus* was a more important vector than *An. arabiensis*, and comparable to *An. gambiae* s.s.

A number of factors have been considered to be responsible for inter species differences in sporozoite infection rates. The greater zoophily of *An. arabiensis* accounts for the intrinsically lower sporozoite rates in this vector than in any other. White (1974) suggested that an

additional factor may be that the longevity of *An. arabiensis* in nature is less than that of *An. gambiae*. White, Magayuka and Boreham (1972) pointed out, the greater availability of antimalarials in some districts than in others may depress the level of infectivity of the human population. However, although difference in the infectivity of gametocyte carriers to *An. gambiae* and *An. arabiensis* was found by Chauvet *et al.* (1972) in Madagascar, Githeko *et al.* (1992) has atributed the variation of sporozoite rates in the same species from place to place to differences in reservoirs of infection, of which he reported children to have been more significant than adults .

Commenting on an analysis of the relationship between inoculation rate and malaria parasite prevalence Birley and Charlwood (1987) speculated on the biological basis for the reported variation in prevalence and suggested that among other factors the HBI, number of persons sleeping under a bed-net, the relative abundance in distribution of humans and animals e.g pigs, dogs, cattle and their relative location to humans and house construction all helped to explain parasitological differences. In Papua New Guinea significant differences in sporozoite rates and human biting rates in villages accounted for the differences in entomological inoculation rates (Burkot *et al.* 1988). Githeko (1992), however has argued that biting rates by themselves may not be crucial determinants of the outcome of the disease process, because immunological profiles in a community at risk such as those between children and adults is not the same. Moreover, because of this immunological profile, malaria epidemics could occur at the highlands as a result of a few bites unlike at the lower lands. Githeko *et al.* (1992) have reported that children acted as a bigger reservoir of infection because of the enhanced presence of infective gametes, and therefore in an area with more children the prevalence of malaria was likely to be higher. In a review of the vectorial importance of anophelines in Africa,

Service (1985) has observed that *An. gambiae* s.s. is likely the main vector in Kenya as in many other parts of Africa. From this study it can be added that *An. funestus* is perhaps becoming a serious rival of *An. gambiae* s.s., and is of particular importance in that its breeding continues more or less unabated even during dry periods when breeding sites are restricted to permanent water bodies such as swamps and lake shores. The significance of this is that human populations in these areas are continually re-infected throughout their lives, thus effectively maintaining uninterrupted malaria reservoirs.

CHAPTER SEVEN

7 LOCAL VARIATION IN BLOOD FEEDING HABITS OF *Anopheles* spp. (DIPTERA:CULICIDAE) IN SOME AREAS OF WESTERN KENYA

7.1 INTRODUCTION

The innate host-seeking capabilities of a mosquito species involves many host types to be successively detected, located, and fed upon. However, Tempelis (1975) has reported that the availability of acceptable hosts in a locality influences the feeding patterns of many mosquito populations . This may lead to various degrees of host preference and opportunistic feeding to be experienced in different localities (Tempelis, 1975; Washino & Tempelis, 1983). These local variations in blood feeding by insect species is believed to be capable of influencing the timing and distribution, or even the occurence of epidemics of mosquito borne malaria pathogens. This is especially evident in areas which experience an outburst of mosquito populations as a result of exceptionally heavy rainy seasons. Therefore, knowledge of the causes and extent of the feeding habits of blood-feeding vectors is essential to understanding the epidemiology of malaria in a given locality. In this study, using the ELISA technique for bloodmeal determination which has been described by Service *et al.* (1986), the feeding behaviour and pattern of several species of *Anopheles* at different altitudinal localties was examined by determining host bloodmeal which had been fed upon.

7.2 MATERIALS AND METHODS

Mosquito material for bloodmeal studies were obtained from a random sample of the monthly PSC collections of mosquitoes captured from the entire transect and containing blood. The majority were from the three altitudinal sites of 1219m (Ahero),1350m (Rota) and 1524m (Oriwo), where there was a large abundance of blood fed mosquitoes in the collections. Members of the *An. gambiae* complex species from Ahero and Rota and Oriwo were identified cytogenetically, unlike those from the rest of the transect which were very few and usually not at a stage suitable for chromosome preparation. Livestock, especially bovids, sheep, goats and chicken were commonly kept at the proximity of the study homes Bloodmeals were prepared from specimens of dried *An. arabiensis* from Ahero mosquitoes or filter paper bloodmeal smears which had been collected as described above in Chapter 3. The abdomens of the dry blood-engorged mosquitoes were ground in 1.0 ml phosphate buffered saline containing 0.1% Tween 20 (PBS-Tween 20) and tested for bloodmeal types using the direct ELISA procedure (Service *et al.*, 1986). In the case of bloodmeal smears the filter paper segments were cut out and the smears eluted with 1.0 ml PBS-Tween 20 solution for one hour at room temperature and ELISA performed. Each mosquito was initially screened for human and bovine bloodmeals. Those not positive for either or both of these hosts were further tested against sheep, goat or avian hosts. The ELISA procedure used for bloodmeal identification was that described by Service *et al.* (1986). The test was carried out by performing a series of incubations of 100 *ul* of eluate of individual mosquitoes per well using pre-coated 96-well plates which were supplied with reagents in kit form by Dr M. W. Service of the Liverpool School of Tropical Medicine, United Kingdom. The final result was read visually (positive sample: yellow or orange; negative sample: colourless to very pale yellow).

7.3 RESULTS.

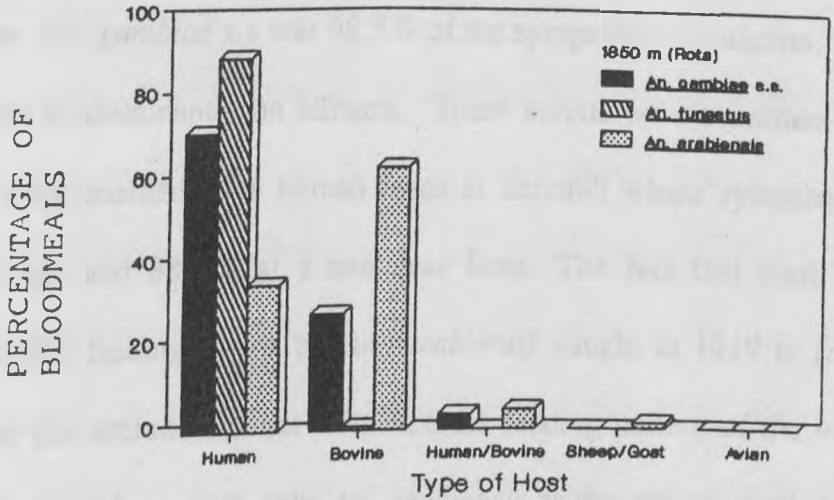
During testing it appeared that 90% of the bloodmeals were positive for either or both human and bovid hosts. The remaining 10% not reacting were tested further for other common domestic hosts such as avian and sheep, and or goats.

Table 7.1 shows that very few blood fed *Anopheles* were caught at sites more than 1829m above sea level. In general,human feeding was predominant among *An. gambiae* s.s. and *An. funestus* caught at all altitudes. However, at Ahero *An. arabiensis* fed mainly on bovids. There was very little or no tendency of vector mosquitoes to feed on sheep, goats or avian hosts, although these were also available.

Fig 7.1 shows that of the 400 bloodmeals tested in *An.arabiensis* from 1219 m (Ahero), only 28.8% had fed on humans, although other animals like cattle abound. This compared with 35.3% (n=85) from 1350m (Rota) that had fed on humans. Bovid feeding by *An. arabiensis* at 1350m and 1219m above sea level did not appear to differ significantly by altitude. The feeding pattern of *An. gambiae* s.s. at 1350m (Rota) was 71.9% human and only 28.1% bovid. In general *An. gambiae* s.s. feeding pattern was over 90% human irrespective of the area of collection. The feeding pattern of *An. funestus,* varied from 88.9% to 100% human. Bovid feeds at Ahero where this vector is the only one co-existing with *An. arabiensis* was only 9.2%, indicative of the high anthropophagic nature of this vector even in the presence of other hosts. All the three vectors hardly fed on sheep/goat or avian hosts.

Table: 7.1: Blood identification at sites along the transect.

Site Altitude	Species	HOST					Total Examined
		Human	Bovine	Human Bovine	Sheep / Goat	Avian	
Rota 1350 m	*An.gambiae* *An.arabiensis* *An.funestus*	41 71.9% 30 35.3% 117 90.1%	16 28.1% 54 4.7% 1 0.7%	2 3.5% 4 4.7% 0	0 1 1.2% 0	0 0 0	57 85 130
Ahero 1219 m	*An.arabiensis* *An.funestus*	107 28.8% 186 89.9%	288 72% 19 9.2%	3 0.8% 3 1.4%	5 1.3% 1 0.5%	0 0	400 206
Oriwo 1524 m	*An.gambiae* *An.funestus*	126 92.0% 8 100%	11 8.0% 0	0 0	0 0	0 0	138 8
Kegati 1829 m	*An.gambiae*	11 100%	0	0	0	0	11
Ichuni 2134 m	*An.gambiae*	1 50%	1 50%	0	0	0	2


PERCENTAGE OF BLOODMEALS
100
80
60
40
20
0
1850 m (Rota)
An. gambiae s.s.
An. funestus
An. arabiensis
Human
Bovine
Human/Bovine
Sheep/Goat
Avian
Type of Host

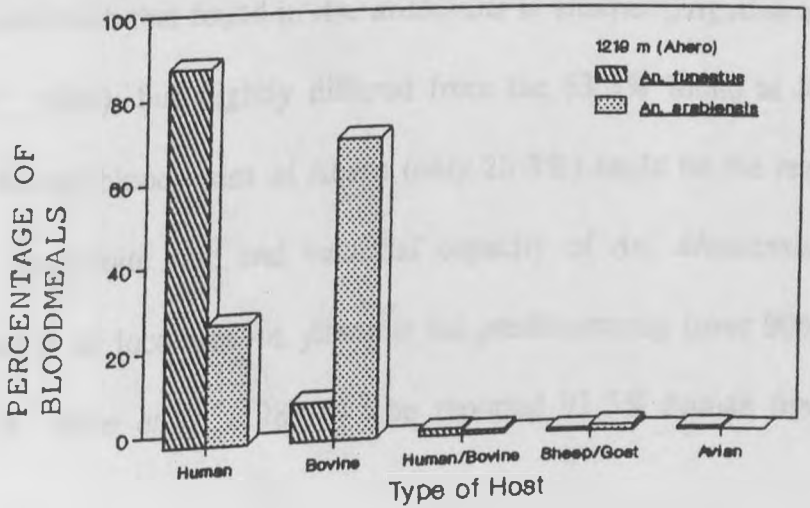
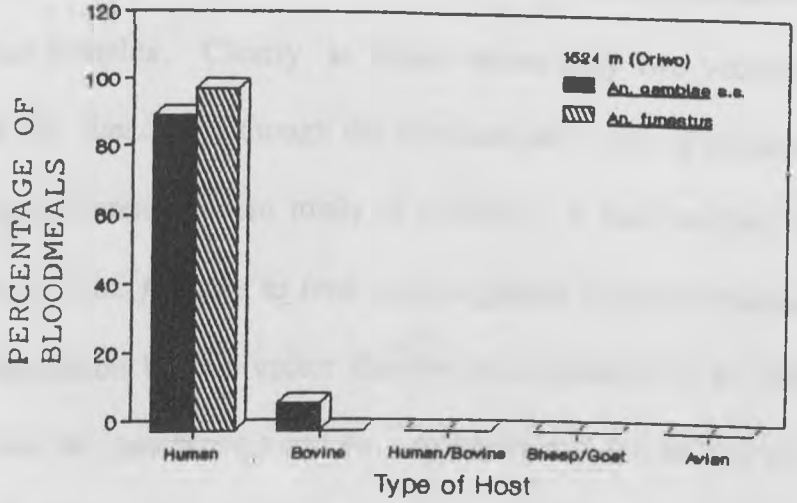

PERCENTAGE OF BLOODMEALS
100
80
60
40
20
0
1219 m (Ahero)
An. funestus
An. arabiensis
Human
Bovine
Human/Bovine
Sheep/Goat
Avian
Type of Host

Fig 7.1: Percentage bloodmeals found positive for various hosts by the ELISA test in vectors collected by PSC at different altitudes over a two year period (Jan 1989 -Dec 1990)



7.4 DISCUSSION

Blood-feeding habits of arthropod vectors such as mosquitoes is an important component in the epidemiology of malaria. Probing alone is sufficient to transmit malaria besides actual blood sucking. In this study the data demonstrated several differences between the two sibling species of *An. gambiae* complex. Clearly, at Ahero where only two vectors occured, namely *An. arabiensis* and *An. funestus*, although the numbers and types of available hosts was constant, relative feeding preferences were totaly in contrast. It was evident that at this altitude the greater tendency of *An. funestus* to feed on man paused a higher epidemiological significance in malaria transmission by this vector than by *An. arabiensis*. At 1350 m (Rota), although sympatry between *An. gambiae* s.s and *An. arabiensis* was at a ratio of 1.3:1 preference was for human feeds (71.9%) by *An. gambiae* s.s. and for bovine feeds by *An. arabienis*. At 1524 m (Oriwo), where *An. gambiae* s.s was 98.5% of the sympantric population, both this vector and *An. funestus* fed predominantly on humans. These results were consistent with those of Beier *et al.* (1988) who recorded 82% human feeds at Saradidi where sympatry of the two sibling species also exists and 88.2% at a site near Rota. The fact that there was no significant difference in bovid feeding trends by *An. arabiensis* caught at 1219 m (Ahero) and 1350 m (Rota) showed that altitude did not influence the feeding pattern of the vectors. The feeding pattern exhibited at Ahero with only *An. arabiensis* as the sibling species with bovid feeding of 72% concured with that found in *An. arabiensis* in another irrigation area of Kenya, Karima (Ijumba *et al.*, 1990), but slightly differed from the 63.5% found at Rota where sympantry exists. Low human blood index at Ahero (only 28.8%) could be the major factor responsible for the low sporozoite rate and vectorial capacity of *An. arabiensis* in this area. These observation that in all localities *An. funestus* fed predominantly (over 90%) on humans was in agreement with Beier *et al.* (1988 b) who reported 91.3% human feeds by this vector in

western Kenya. Other feeds, especially bovid accounted for about 10%. Since there was no significant difference in the percentage of human feeding in different altitudes by this vector ($p = 0.056$); the results suggested that this was probably an innate characteristic of this vector rather than opportunistic feeding since in all localties of the study both the human host and domestic animals (especially bovine) were always available. This however, contrasted with the observation of Ijumba *et al.* (1990) which indicated the existence of some *An. funestus* sibling species in Mwea-Tebere which had 100% non-human feeds. Moreover, existence of strongly zoophilic *An. funestus* that cannot be distinguished from *An. funestus* s.s. has been documented (Gillies & Coetzee, 1987). These results may be suggestive of differential scattering or selective distribution of strongly anthropophilic and strongly zoophilic sibling species of *An. funestus*.

The high frequencies of human feeding, and secondarilly of cattle feeding by member species of the *An. gambiae* complex and *An. funestus* were expected based on previous studies in the Kisumu area (Joshi *et al*, 1975; Service *et al.*, 1978, Highton *et al.*, 1979). Few bloodmeals of other hosts were detected, indicating the high anthropophagic nature of these vectors. Although mixed blood feeding by insect vectors, particularly anophelines is not an uncommon phenomenon the relatively small occurrence of this at all altitudes among these species may be another major factor responsible for their high sporozoite rates.

CHAPTER EIGHT

8 GENERAL DISCUSSION AND CONCLUSIONS

8.1 Species composition and seasonal abundance of malaria vectors in relation to altitude

The first conclusion from the field observations in this study was the confirmation that in western Kenya, *An. gambiae* s.s. *An. arabiensis* and *An. funestus* are the major vectors of *P. falciparum*. The presence and distribution of these vectors in the varios localties appeared to be determined by a number of ecological variables. While *An. funestus* was found both in the rice-growing area and in the savannah, invariably in association with member species of the *An. gambiae* complex, its absence above 2100 m suggested an apparent failure by this vector to colonise such high altitudes. Gillies & de Meillon (1968) have suggested the exixtence of at least 3 sub-groups within the *funestus* group. It was difficult to deduce whether the apparent selective distribution of this species was a selective adaptation to the environment. It is important to confirm this by conducting larval surveys in water pools to determine if indeed there is total absence of breeding of *An. funestus* in high altitudinal sites and also to conduct dispersal experiments.

In the study areas, two species of the *An. gambiae* complex, namely *An. gambiae* s.s. and *An. arabiensis* were found to be present. While at one extreme, in the rice-growing area of Ahero, *An. arabiensis* was existing as the only sibling species of the *An. gambiae* complex throughout the year, at the high altitude of Oriwo, *An. gambiae* s.s. was existing almost as a homogeneous

population despite the savannah type of environment. The existence of *An. arabiensis* sympatrically with *An. gambiae* s.s. at intermediate altitude of Rota, in contrast to the two extremes could be interpreted by invoking behavioural diversities which probably are related to different karyotypes affecting breeding, feeding and resting behaviour. This calls for investigation. Since very few other anophelines notably *An. coustani* and *An. pharoensis* were captured in PSC catches, and none of these on dissection had sporozoite infection it is concluded that there was no evidence from these results to incriminate these mosquitoes in malaria transmission in western Kenya, despite *An. pharoensis* being a vector in Egypt (Gillies & de Meillon, 1968; Gillies & Coetzee, 1987), and Senegal (Carrara *et al.*, 1990). However, that Ijumba *et al.*, 1990 found a sporozoite rate of 0.1% in *An. pharoensis* from Mwea-Tebere suggests that one should be wary of this mosquito in Kenya.

The reduction in house densities with rising altitude may be attributable to several factors one of which could be the reduction in breeding sites, which affected the whole population density. Outdoor resting is a common feature of both *An. funestus* and *An. gambiae* s.l. It is possible that some females preferred to rest outdoors after feeding. Grain stores have been found to be a more productive source of females resting outside houses than pit shelters (Clark *et al.*, 1980). The succession sequence between the populations of the two species may be a feature of seasonal differrential breeding and larval development. Perhaps one of the most significant phenomenon from the results of population densities is the differential degree of sympantry between *An. gambiae* s.s. and *An. ararabiensis*. While the two species may co-exist in some areas, two trends are apparent. The first was the evident absence of *An. gambiae* s.s. in rice irrigation schemes and the second is the general absence of *An. arabiensis* at high altitude. Both these anomalies would seem to rule out simple climate-based explanation. It appears more

plausible to view altitude as acting as a segregating factor between the species and associated with clinal chromosomal differentiation, while climatic factors, especially season acts on polymorphism. In respect of malaria control it is important to understand these phenomena in order to apply correct control strategies.

8.2 Man-biting rates and biting cycles

At all the three altitudes the well known late biting cycle of *An. gambiae* s.l. and *An. funestus* was evident, and appeared not to be altered by altitude. However, the biting densities were clearly dependent on altitude. For example, at Oriwo the reduction in man-biting rates of *An. funestus* were more than ten-fold those at Ahero and Rota. It was paradoxical that the highest biting rates and transmission of malaria should occur in Ahero, an agricultural scheme which is meant to enhance food resources in the country and, thus to improve the socio-economic status of the communities. For while indeed hunger and poverty are being alleviated morbidity and mortality due to malaria are being enhanced. Besides this, irrigation projects involve extensive use of agricultural pesticides for crop protection, thereby resulting in introduction of insecticide resistance in disease vector species.

From the point of view of malaria control biting cycles are important determinants of the timing strategies. Perhaps one of the commonest methods used by communities for protection against mosquitoes and other biting insects is the use of bednets (Lindsay & Gibson, 1988). Ross (1910) in particular advocated the use of bednets against malaria, and since then several studies have confirmed the protection against malaria by the use of bednets (Bradley, 1986; Snow *et al.*, 1988). Although protection was noted to be greatlly enhanced by the use of insecticide impregnated bednets (Gouck *et al.*, 1967), many field trials which have been

reviewed in detail by Rozendaal (1989), have shown from the results of inoculation studies that succesful use of bednets in malaria control was also time dependent.

8.3 Age-composition and parity rates

As already stated in earlier chapters the extrinsic cycle of the parasite must be outgrown by survival of the mosquitoes in order for transmission to occur. The higher parity rates observed at the higher altitude of Rota would suggest higher survivorship by both *An. funestus* and *An. gambiae* s.s. at the higher altitude than that of *An. arabiensis* at Ahero. This view may be supported by the fact that a higher percentage of mosquitoes at Rota lived through more gonotrophic cycles than those at Ahero. However, because of the high rate of recruitment of nulliparous mosquitoes at Ahero due to unfettered breeding in rice fields this comparison may not be tenable. Considering that at Rota, despite the sympantric existence the dominant species is *An. gambiae* s.s., a comparison of survivorship of *An. arabiensis* and *An. gambiae* s.s. suggests the latter species may have a higher survivorship because of altitude. Although White (1974), has discussed the important question of whether *An. gambiae* s.s. shows greater inherent longevity than *An. arabiensis*, and using the parous rate concluded that this was the case, the evidence for this has been conflicting. For example, Garret-Jones and Shridawi (1969) in Nigeria reported that *An. arabiensis* had higher survival rates than *An. gambiae* s.s.

8.4 Sporozoite rates and inoculation rates in malaria vectors in relation to malaria prevalence

The malaria parasite prevalence observed at altitudes of over 2000 m is an indication that malaria transmission was also taking place at such high altitudes despite the very low temperatures sometimes attained which would

affect sporogony. However, it would appear that this transmission was caused by only *An. gambiae* s.s. because of the absence of *An. funestus*. Not withstanding the inherent difference in infection rates between species it was evident altitude appeared to exert a limiting influence on sporozoite infectivity among mosquito species which in turn reduced entomological inoculation rates. This may be due to the fact that sporogony tends to be slowed by lower temperatures, which are obtaining at high altitude. White (1974), however, has suggested that the longevity of *An. arabiensis* in nature is less than that of *An. gambiae* s.s., which factor could also account for higher sporozoite rates at higher altitude.

8.5 Feeding habits of malaria vectors

Particular attention has been paid to the existence of differences in feeding behaviour between *An. gambiae* s.s. and *An. arabiensis*. The absence of *An. gambiae* s.s. in rice irrigation schemes, and of *An. arabiensis* at high altitude precluded a comparison of the feeding behaviour of these two species in relation to altitude. Where the two species were sympatric, however, and as such exposed to the same host types it was evident, *An. arabiensis* fed on bovids as much as *An. gambiae* s.s. fed on humans and vice versa. This confirmed that if cattle are present in any numbers a high proportion of feeds by An. arabiensis are taken on cattle. However, the observation that about 30% of *An. gambiae* s.s. in the circumstances fed on cattle, indicated that this species might not be very catholic on human feeds unlike *An. arabiensis* which would prefer cattle. In contrast *An. funestus* appeared to be consistent (over 90%) on human feeding throughout all altitudes. These results were in conformity with those of Chandler *et al.* (1975) and Pull and Grab (1974) for this area of western Kenya.

8.6 Relation of this study to malaria control

The ultimate goal in studying epidemiological parameters or transmission agents of any disease is to apply the knowledge acquired for innovation of implements or technologies to control that disease. This thesis would, therefore, hardly be complete without an insight into the implications of the observations made in this study on the possible control strategies directed at reducing morbidity and mortality due to malaria, especially among the most vulnerable rural communities. It is acknowledged that due to the high level of malaria transmission and the heterogeneity of the vectorial systems in Africa, no single method to date has succeeded in interruption of the disease. Malaria control approaches therefore, must of necessity be multi-faceted, and include chemoprophylaxis to control the human stage of the parasite, insecticide treatments, and use of bio-control agents to control both the adult and immature stages of the vector; environmental management, and above all, community participation in reducing or preventing altogether man-vector contacts.

Malaria control activities may be viewed at two levels:
the national control programme and the supplementary programme. The national control programme would need extensive government involvement in two fronts, namely, (i) chemotherapy, and (ii) vector control. Given the high endemicity of malaria in most areas of Kenya, however, it can at once be seen that chemotherapy is prohibitively expensive, because of the large population at risk. However, prompt diagnosis and adequate treatment of malaria cases goes a long way to reducing mortality and morbidity, albeit in only accessible areas, such as urban centres. The major drawback in chemothetapy programmes is that only government has the resources to detect and prempt drug resistance.

Vector control therefore, appears to be the easier option as it requires relatively un sophiscated infra structure or highly trained personnel. Vector control can be implemented at both national and domicilliary level. At national level government contribution would be the provision of affordable insecticides and supervision of use of the same by extension officers in rural areas. Vector control by residual house spraying at domicilliary level might perhaps be the easiest and most highly applied method for control of malaria transmission. But it remains miniscule and unsustainable, because of the probihitive cost of commercial insecticide sprays to individual families. Moreover, the observations from this study showing vector diversity according to locality imply that no universal insecticide might be applicable either at national or domicilliary level. However, in all likelihood, the pattern of vector distribution may be extrapolated to other rural areas or savanna zones. The main vectors *An. gambiae*, *An. arabiensis* and *An. funestus* are the same throughout the zone and so are the ecological and climatic factors. This then would suggest that control strategies could be fairly uniform, provided at given altitudes a particular vector species is targeted with the understanding of its behavioural traits, such as exophily or endophily.

The final conclusion from this study is the need for community awareness before involvent in malaria control in a given locality, which is necessitated by the ecological and vector diversity. Although domicilliary spraying could be an effective method of tackling the vectors, it is obvious that insecticide based control strategies that may be successful in an irrigation area, such as Ahero, may not be equally applicable at Oriwo, and vice versa. Moreover, since the balance of the ecosystem must be maintained in insecticide usage, environmental management to improve drainage and filling-in excavation fields used by the vector to breed, coupled with chemotherapy and selective insecticide application seem to be more attractive control options.

REFERENCES

Adungo, N.I., Mokaya, P.O., Oloo, A.J., Ondijo, S.O. & Mahadevan, S. (1991 a). Sporozoite rate, vector density and malaria prevalence in relation to altitude. *Proceedings of the 12th Annual Medical Scientific Conference of the Kenya Medical Research Institute & Kenya Trypanosomiasis Research Institute*, Nairobi.

Adungo, N.I., Mahadevan, S., Situbi, A.P., Mulaya, N.L., & Githure, J.I. (1991 b). Comparative determination of *Plasmodium falciparum* sporozoite rates in Afrotropical *Anopheles* (Diptera:Culicidae) from Kenya by dissection and Enzyme linked immunosorbent assay (ELISA). *Annals of Tropical Medicine and Parasitology*, 85 (4), 387-394.

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

Beier, J.C., Perkins, P.V., Wirtz, R.A., Whitmire, R.E., Mugambi, M. & Hockmeyer, W. (1987). Field evaluation of an enzyme-linked immunosorbent assay (Elisa) for *Plasmodium falciparum* sporozoite detection in Anopheline mosquitoes from Kenya. *American Journal of Tropical Medicine & Hygiene*, 36(3), 459-468.

Beier, J.C., Asiago, C.M., Onyango, F.K. & Koros, J.K. (1988 a). ELISA absorbance cut off method affects malaria sporozoite rate determination in wild Afrotropical *Anopheles*. *Medical and Veterinary Entomology*, 2, 259-264.

Beier, J.C., Perkins, P.V., Wirtz, R.A., Koros, J., Diggs, Diana, Gargan, T.P. & Koech, Davy, K. (1988 b). Bloodmeal identification by direct enzyme-linked immunosorbent assay (Elisa), tested on *Anopheles* (Diptera:Culicidae) in Kenya. *Journal of Medical Entomology*, 25 (1), 9-16.

Beier, M. S., Schwartz, I.K., Beier, J.C., Perkins, P.V., Onyango, F., Koros, J.K., Campbell, G.H., Andrysiak, P.M. and Brandling-Bennett, A.D. (1988). Identification of malaria species by ELISA in sporozoite and oocyst infected *Anopheles* from Western Kenya. *American Journal of Tropical Medicine & Hygiene*, 39(4), 323-327.

Birley, M.H.(1984). Estimation, tactics and disease transmission. In: *Pest and Pathogen Control: Stategy, Tactics and Policy Models*. Ed: Conway G.R., Wiley-IISA, International Series on Applied Systems Analysis, A Wiley Interscience Publ., Chichester. pp 488.

Birley, M.H. & Charlwoood, J.D. (1987). Sporozoite rate and malaria prevalence. *Parasitology Today*, 3, 8, 231-232.

Boreham, P.F.L., Chandler, J.A. & Highton, R.B. (1975). Studies on the feeding patterns of mosquitoes of the genera *Ficalbia, Mimomyia* and *Uranotaemia* in the Kisumu area of Kenya. *Bulletin of Entomological Research*. 65, 69-74.

Boreham, P F L, Chandler, J A & Jolly, J (1978). The incidence of mosquitoes feeding on mothers and babies at Kisumu, Kenya. *Journal of Tropical Medicine & Hygiene*, 81, 63-67.

Bradley, A.K., Greenwood, B.M., Greenwood, A.M., Mash, K., Byas, P., Tulloch, S. & Hayes, R. (1986). Bednets (mosquito nets) and morbidity from malaria. *Lancet* ii, 204-207.

Bruce-Chwatt, L.J., Garret-Jones, & Weitz, B.(1966). Ten years' study (1955-64) of host selection by Anopheline mosquitoes. *Bulletin of the World Health Organisation*, 35, 405-39.

Burkot, T.R., Williams, J.L., & Schneider, I. (1984). Identification of *Plasmodium falciparum* infected mosquitoes by double antibody Enzyme-linked Immunosorbent Assay. *American Journal of Tropical Medicine & Hygiene*, 33, 7, 83-88.

Burkot, T.R., Graves, P.M., Cattani, J.A., Wirtz, R.A. & Gibson, F.D. (1987). The efficiency of transmission of sporozoites of the human malarias, *Plasmodium falciparum* and *P. vivax*. *Bulletin of the World Health Organisation*. 3, 375-382.

Burkot, T.R., Graves, P.M., Paru, R, J.A., Wirtz, R.A. & Heywood, P. F. (1988). Human malaria transmission studies in the *Anopheles punctulatus* complex in Papua New Guinea: sporozoite rates, inoculation rates, and sporozoite densities. *American Journal of Tropical Medicine & Hygiene*, 39, 135-144.

Carnevale, P. & Robert, V. (1987). Introduction of irrigation in Burkina Faso and its effect on malaria transmission. *WHO/FAO/UNEP expert report on Environmental management for vector control*; document no. PEEM/7/WP/87.9A, 18 PP, unpublished.

Chandler, J.A., Boreham, P.F.L., Highton, R.B. & Hill, M. N. (1976). A study of the host selection patterns ofthe mosquitoes of the Kisumu area of Kenya. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 9, 415-25.

Chandler, J.A., Parsons, J., Boreham, P.F.L. & Gill, G.S. (1977). Seasonal variation in the proportions of mosquitoes feeding on mammals and birds at a heronry in Western Kenya. *Journal of Medical Entomology*, 14, 2, 233-240.

Chauvet, G.; Davidson, G. & Dejardin, J. (1969). Validite d'une methode chemotaxique de distinction des larves de especes A & B du complexe *An. gambiae* Giles a Madagascar. *Cah. ORSTOM Ent. med.* 7, 51-60.

Clark, J.L., Pradhan, C.D., Joshi, G.P. & Fontaine, R.E. (1980). Assessment of the grain store as an unbaited outdoor shelter for mosquitoes of the *An. gambiae* complex, and *An. funestus* (Diptera:Culicidae) at Kisumu, Kenya. *Journal of Medical Entomology*, 17, 100-102.

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

Collins, F.H., Petrarca, V., Mpofu, S., Brandling-Bennet, A.D., Were, J.B.O., Rasmussen, M.O. & Finnerty, V. (1988). Comparison of DNA probe and cytogenetic method for identifying field collected *Anopheles gambiae* complex mosquitoes. *American Journal of Tropical Medicine & Hygiene*, 33(4), 549-555.

Coluzzi, M. (1968). Cromosomi politenici delle cellule nutrichi ovariche nel complesso *gambiae* del genre *Anopheles*. *Parassitologia* 10, 179-183.

Coluzzi, M. & Sabatini, A. (1967). Cytogenetic observation on species A and B of the *Anopheles gambiae* complex. *Parasitologia*, 9, 73-88.

Coluzzi, M., Sabatini, A., Petrarca, V., & Deco, M.A.Di (1979). Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 73(5), 483-497.

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

Corbet, P. S. (1960). Recognition of nulliparous mosquitoes without dissection. *Nature*, London, 187, 525-526.

Corbet, P. S. (1962). The use of external characters to age-grade adult mosquitoes (Diptera : Culicidae). *Proceedings of International Congress of Entomology XIth* 11, 387-390.

Corbet, P.S. (1963). The reliability of parassitic water-mites (Hydracarina) as indicators of physiological age in mosquitoes (Diptera:Culicidae). *Entomologia exper. appl.* 6, 215-233.

Crowsskey, R. W. (1958). The body weight in unfed *Simulium damnosum* Theobald, and its relation to the time of biting, the fat body and the age. *Annals of Tropical Medicine and Parasitology*, 52, 149-157.

Davidson, G. & Hunt, R.H. (1973). The crossing and chromosomal characteristics of a new, sixth, species of the *Anopheles gambiae* complex. *Parassitologia* 15, 121-128.

Davidson, G., Paterson, H.E., Coluzzi, M., Mason, G.F. & Micks, D.W. (1967). The *Anopheles gambiae* complex. pp. 211-250. In: Wright, J. W. & Pal, R. (*Eds.*). *Genetics of insect vectors of disease.*, 794 pp. Amsterdam, Elsevier.

Detinova, T.S. (1945). The determination of the physiological age of females of *Anopheles* by changes in the tracheoal system of the ovaries. *Medzkaya Parazit.* 14, 45-49.

Detinova, T.S. (1962). Age-grading methods in Diptera of medical importance. *World Health Organisation.* Monograph series no. 47, 216 pp.

Diesfield, H.J. (1978). *Kenya: A Geomedical Monograph.* Springer-Verlag Berlin Heidelberg, vii+ 134 pp.

Fontaine, R.E. & Pull, J.H. (1977). Malaria control: field testing of a new insecticide. *WHO Chronicle,* 31(3), 102-105.

Gardos, J. & Smek, I. (1976). Influence of of blood meal source on egg production in mosquitoes (*Aedes cantans* Meigen). *Biologia Czeechoslovakia* 31, 631-39 (in German, English abstract)

Garnham, P.C.C. (1929). Malaria in Kisumu, Kenya Colony. *Journal of Tropical Medicine & Hygiene.* 32, 207-21.

Garnham, P.C.C. (1945). Malaria epidemics at exceptionally high altitudes in Kenya. *British Medical Journal*, ii, 45-47.

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

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

Garret-Jones, C. & Shidrawi, G.R., (1969). Malaria vectorial capacity of a population of *Anopheles gambiae*. An exercise in epidemiological entomology. *Bulletin of the World Health Organisation*, 40, 531-545.

Gillet, J.D. (1957). Age analysis in the biting cycle of the mosquito *Taeniorynchus (Mansonioides) africanus* Theobald, based on the presence of parasitic mites. *Annals of Tropical Medicine and Parasitology*, 51(4), 151-158.

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

Gillies, M.T. (1954). Studies of house leaving and outside resting of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in East Africa. 1. The outside resting population. *Bulletin of Entomological Research*. 45, 361-373.

Gillies, M.T. (1956). The problem of exophily in *Anopheles gambiae*. *Bulletin of the World Health Organisation,* 15, 437-449.

Gillies, M.T & Coetzee, M. (1987). *A Supplement to the Anophelinae of Africa South of The Sahara (Afrotropical Region)*. South African Institute for Medical Research, Publication No. 55. 143 pp.

Gillies, M.T. & de Meillon, B. (1968). *The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region)*. South African Institute for Medical Research, Publication No. 54, 2nd edn). 343 pp.

Gillies, M.T. & Wilkes, T.J. (1965). A study of the age-composition of populations of *Anopheles gambiae* Giles and *An. funestus* Giles in northeastern Tanzania. *Bulletin of Entomological Research*. 56, 237-262.

Gillies, M.T. & Wilkes, T.J. (1974). The range of attraction as baits for some West African mosquitoes (Diptera : Culicidae). *Bulletin of Entomological Research*. 63, 373-581.

Githeko, A.K. (1992). *The behaviour and ecology of malaria vectors and malaria transmission in Kisumu District of western Kenya*. PhD thesis, University of Liverpool.

Githeko, A.K., Brandling-Bennet, A.D., Beier, M., Atieli, F.K., Owaga, M., Juma, F & Collins, F.H. (1992). The reservior of *P. falciparum* malaria in a holoendemic area of western Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 86, 355-358.

Goock, H.K., Godwin, D.R., Schreck, C.E. and Smith, N. (1967). Field tests with repellent-treated netting against black salt-marsh mosquitoes. *Journal of Economical Entomology* 60, 1451-1452.

Graham, P. (1969). Age-grading of mosquitoes from parasitic mites. *Mosquito News*. 29, 259-260.

Green, C.A. (1972). Cytological maps for the practical identification of females of three freshwater species of the *Anopheles gambiae* complex. *Annals of Tropical Medicine & Parasitology*, 66, 143-147.

Haddow, A.J. (1942). The mosquito fauna and climate of native huts at Kisumu, Kenya. *Bulletin of Entomologiccal Research*, 33, 91-142.

Haddow, A.J. (1945). The mosquitoes of Bwamba County, Uganda. II. Biting activity with special reference to the influence of microclimate. *Bulletin of Entomological Research.* 36, 33-73.

Haddow, A.J. (1960). Studies on the biting habits and medical importance of East African mosquitoes in the genus *Aedes*. 1 - Subgenera *Aedimorphus, Banksinella* and *Dunnius*. *Bulletin of Entomological Research.* 51, 759-779.

Haddow, A.J., Corbet, P.S. and Gillet, J.D. (1961). Entomological studies from a high tower in Mpanga forest, Uganda. *Transactions of the Royal Entomological Society of London*, 113 (2), 249-368.

Haddow, A.J., & Ssenkubuge, Y. (1965). Entomological studies from a steel tower in Zika forest, Uganda. Part 1. The biting activity of mosquitoes and tabanids as shown by twenty-four-catches. *Transactions of the Royal Entomological Society of London*, 117, 215-243.

Hess, A.D., Hays, R.O., Tempelis, C.H. (1968). The use of the forage ratio technique in mosquito host preference studies. *Mosquito News* 28, 386-89.

Highton, R.B., Bryan, J.H., Boreham, P.F.L., & Chandler, J.A. (1979). Studies on the sibling species of *Anopheles gambiae* Giles and *Anopheles arabiensis* Patton (Diptera:Culicidae) in the Kisumu area, Kenya. *Bulletin of Entomological Research*, 69, 43-53.

Holstein, M. (1952). Biologie d' *Anopheles gambiae*. Monograph Series, World Health Organisation, No. 9, 176pp.

Ijumba, J.N., Mwangi, R.W. & Beier, J.C. (1990). Malaria transmission potential of *Anopheles* mosquitoes in the Mwea-Tebere irrigation scheme, Kenya. *Medical and Veterinary Entomology*, 4, 425-432.

Joshi, G.P., Service, M.W. & Pradhan, G.D. (1975). A survey of species A and B of the *Anopheles gambiae* Giles complex in the Kisumu area of Kenya prior to insecticidal spraying with CMS 43 (fenithrothion). *Annals of Tropical Medicine & Parasitology*, 69, 91-104.

Khamala, C.P.M. (1971). The biting flies of the Kano Plains, Kenya: Part II. Larval habitats of common mosquito species (Diptera:Culicidae). *Bulletin of Entomological Research*, 61 (2), 299-307.

Kitzmiller, J.B., Kreutzer, R.D. & Tallaferro, E. (1973). Chromosomal differences in populations of *Anopheles nunetzovari*. *Bulletin of the World Health Organisation*, 48, 435-440.

Koinange, W. (1980). *Strategies for Health in Kenya*. Ministry of Health, Kenya, (unpublished document).

Lewis, D.J. (1958). Observations on *Simulium damnosum Theobald* at Lokoja in northern Nigeria. *Annals of Tropical Medicine and Parasitology*, 52, 216-231

Lindsay, S.W. & Gibson, M. E. (1988). Bednets, revisited - old idea, new angle. *Parasitology Today*. 4, 270-272.

Lumsden, W.H.R. (1952). The crepuscular biting-activity of insects in the forest canopy in Bwamba, Uganda; a study in relation to the sylvan epidemiology of yellow fever. *Bulletin of Entomological Research*, 42, 721-760.

MacCormack, C.P. (1984). Human ecology and behaviour in malaria control in tropical Africa. *Bulletin of the World Health Organisation (supp)* 62, 81-87.

Macdonald, G. (1952). The analysis of the sporozoite rate. *Tropical Diseases Bulletin*, 49, 569-85.

Macdonald, G. (1957). *The Epidemiology & Control of malaria*. Oxford University Press, London, xiv + 201 + x1 pp.

Mattingly, P.F. (1977). Names of the *Anopheles gambiae* complex. *Mosquito Sytematics*, 9, 323-328.

Minitab, (1982). *Minitab Project*. Statistics Department. The Penyslvania State University.

Molineaux, L. & Gramiccia, G. (1980). The Garki Project. Research on the epidemiology and control of malaria in the Sudan savanna of West Africa. *Bulletin of the World Health Organisation*. Geneva. 311 pp.

Morris, C.D. & De Foliart, G.R. (1970). The physiological age of Wisconsin *Aedes* mosquitoes parasitized by water-mites. *Journal of Medical Entomology* 7: 628-637.

Mosha, F.W. & Mutero, C.M. (1982). The influence of salinity on larval development and population dynamics of *Anopheles merus* Donitz (Diptera:Culicidae). *Bulletin of Entomological Research*, 72, 119-128.

Mosha, F.W. & Petrarca, V. (1983). Ecological studies on *Anopheles gambiae* complex sibling species on the Kenya coast. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 77, 3, 344-45.

Mosha, F.W.& Subra, R. (1982). Ecological studies in *Anopheles gambiae* complex sibling species in Kenya. 1. Preliminary observations on their geographical distribution and chromosomal polymorphic inversions. *WHO 1982, 9 pp. WHO/VBC/82.* 867, unpublished.

Muirhead-Thomson, R.C. (1951). Studies on salt-water and fresh-water *Anopheles gambiae* on the East African coast. *Bulletin of Entomological Research*, 41, 487-502.

Mutero, C.M. (1985). *Estimation of the survival rates of field populations of malaria vectors in Kenya*. PhD Thesis, University of Nairobi.

Mutero, C.M. & Birley, M.H. (1987). Estimation of the survival rate and oviposition cycle of field populations of malaria vectors in Kenya. *Journal of Applied Ecology*, 24,853-863.

Onori, E. & Grab, B. (1980). Indicators for the forecasting of malaria epidemics. *Bulletin of the World Health Organisation*, 56, 91-98.

Oster, C.N., Githeko, A.K., Adungo, N.I. Owaga, M.L., Kamunvi, F., Perkins, P.V., Hendricks, L.D., & Whitmire, R.E. (1986). Attack rate of malaria in school children in Siaya and Kisumu Districts, Nyanza Province, Kenya. *Proceedings of the 7th Annual Medical Scientific Conference of KEMRI/KETRI*, Nairobi.

Paterson, H.E., (1962). Status of East African salt-water-breeding variant of *Anopheles gambiae* Giles. *Nature*, 195, 469-470.

Paterson, H.E., (1963). The species, species control and antimalarial spraying campaigns. Implications of recent work on the *Anopheles gambiae* complex. *South African Journal of Medical Science*, 28, 33-34.

Paterson, H.E., Paterson, J.S. & van Eeden, G.J. (1963). A new member of the *Anopheles gambiae* complex. A preliminary report. *Med. Proc. (Med. Bydraes)*, 9, 414-418.

Perry, E.L. (1912). Malaria in the Jeypore Hill tract and adjoining coast land. *Paludism*, 5, 32-40.

Polovodova, V. P. (1949). The determination of the physiological age of female *Anopheles* by the number of gonotrophic cycles completed. *Medskaya Parazit.*, 18, 352-355.

Rishikesh, N., Di Deco, M.A., Pertrarca, V & Colluzi, M. (1985). Seasonal variations in indoor resting *Anopheles gambiae* and *An. arabiensis* in Kaduna, Nigeria. *Acta Tropica*, 42, 165-170.

Roberts, J.M.D.(1974). Malaria. In: L.C.Vogel *et al.*, (Eds). *Health and Disease in Kenya*, East Africa Literature Bureau, Nairobi, pp 305-317.

Ross, R. (1910). *The prevention of malaria*. John Marray, London. 660 pp.

Senior-White, R. A. (1954). Adult anophelines behavior patterns: suggested classification. *Nature*, 173, 730

Severini, C., Fontenille, D., Ramakajato, M.R. (1990). The importance of *An. funestus* in the transmission of malaria in the hamlet of Mahitsy, in Tananarive, Madagascar. *Bull. Soc. Path. Exot. Filiales*, 83(1), 114-116.

Service, M.W. (1970). Ecological notes on species A and B of *Anopheles gambiae* complex in the Kisumu area of Kenya. *Bulletin of Entomological Research*, 60, 105-108.

Service, M. W. (1976). *Mosquito ecology. Field sampling methods*. Applied Science Publishers Ltd., London, 583 pp.

Service, M. W. (1985). *Anopheles gambiae* species: Africa's principal malaria vector, 1902-1984. *Bulletin of Entomological Society of America*, 31(3), 8-12.

Service, M. W., Joshi, G.P. & Pradhan, G.D. (1978). A survey of *Anopheles gambiae* (species A) and *An. arabiensis* (species B) of the *Anopheles gambiae* Giles complex in the Kisumu area of Kenya following insecticidal spraying with OMS-43 (fenithrothion). *Annals of Tropical Medicine & Parasitology*, 72, 377-386.

Service, M. W., Voller, A. & Bidwell, D. (1986). The Enyme-linked Immunosorbent Assay (ELISA) test for the identification of bloodmeals of haematophagous insects. *Bulletin of Entomological Research*, 76, 321-330.

Snow, R.W., Rowan, K.M., Lindsay, S.W. & Greenwood, B.M. (1988). A trial of bednets (mosquito-nets) as a malaria control strategy in a rural area of the Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine & Hygiene* 82, 212-215.

Spencer, H.C., Kaseje, D.C., Collins, W.E., Shehuta, M.G. Turner, A., Stanfill, P.S., Huong, A.Y., Roberts, J.M., Villinski, M. and Koech, D.K. (1987). Community-based malaria control in Saradidi, Kenya: description of the programme and impact on parasitaemia rates and antimalarial antibodies. *Annals of Tropical Medicine and Parasitology* 81 (Supp. 1): 13-23.

Surtees, G. (1970). Large-scale irrigation and arbovirus epidemiology, Kano Plain, Kenya. 1. Description of the area and preliminary studies on mosquitoes. *Journal of Medical Entomology* 7: 509-517.

Symes, C.B. (1927). Notes on Anophelines and malaria in Kenya. *Kenya and East African Medical Journal,* 138-183.

Symphony, (1985). Lotus Development Corporation. Cambridge.

Tempelis, C.H.(1975). Host feeding patterns of mosquitoes, with a review of advances in analysis of bloodmeals by serology. *Journal of Medical Entomology*, 11, 6, 635-33.

Washino, R.A. & Tempelis, C.H. (1983). Mosquito host bloodmeal identification: Methodology and data analysis. *American Review of Entomology*, 28, 179-200.

Wekesa, J.W; Copeland, R.S. and Mwangi, R.W. (1992). Effect of *Plasmodium falciparum* on blood feeding behaviour of naturally infected *Anopheles* mosquitoes in western Kenya. *American Journal of Tropical Medicine & Hygiene,* 47, 484-488.

Wharton, R.H. (1959). Age determination in *Mansonioides* mosquitoes. *Nature*, London, 184, 830-831.

White, G.B. (1972). The *Anopheles gambiae* complex and malaria transmission around Kisumu, Kenya. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 66, 4, 578-585.

White, G.B. (1974). Biological effects of intraspecific chromosomal polymophism in malaria vector populations. *Bulletin of the World Health Organisation,* 50, 299-306.

WHO (1963). In: *Terminology of malaria and of malaria eradication*; Monograph series no. 13, Geneva.

WHO (1975). *Manual on practical entomology in malaria.* Part II. WHO, Geneva.

WHO (1983). Document VBC/84.3. Vector bionomics. In: Zahar, A.R. (ed). *The epidemiology and control of malaria.*

WHO Technical Report Series No.735, 1986, *WHO Expert Committee on malaria: eighteenth report.*

van Someren, E.C.C. & Furlong. M. (1964). The biting habits of *Aedes (Skusea) pembaesis* Theobald and some other mosquitoes of Faza, Pate Island, East Africa. *Bulletin of Entomological Research*, 55, 97-124.

Williams, C.B. (1937). The use of logarithms in the interpretation of certain entomological problems. *Annals of Applied Biology*, 24, 404-408.

Wirtz, A.R., Burkot, T.R., Andre, R.G., Collins, W.E. & Roberts, D.R. (1985). Identification of *Plasmodium vivax* sporozoites in mosquitoes using an Enzyme-linked Immunosorbent Assay. *American Journal of Tropical Medicine & Hygiene*, 34, 1048-54.

Wirtz, F., Gwardz, R.W., Collins , F.H., Nussenzweig, R.S. & Nussenzwig, V. (1982). Monoclonal antibodies to circusporozoite proteins identify the species of malaria parasites in infected mosquitoes. *Nature*, 299, 737-41.

White, G.B. (1974). Biological effects of intraspecific chromosomal polymophism in malaria vector populations. *Bulletin of the World Health Organisation*, 50, 299-306.

WHO (1963). In: *Terminology of malaria and of malaria eradication*; Monograph series no. 13, Geneva.

WHO (1975). *Manual on practical entomology in malaria*. Part II. WHO, Geneva.

WHO (1983). Document VBC/84.3. Vector bionomics. In: Zahar, A.R. (ed). *The epidemiology and control of malaria*.

WHO Technical Report Series No.735, 1986, *WHO Expert Committee on malaria: eighteenth report*.

van Someren, E.C.C. & Furlong, M. (1964). The biting habits of *Aedes (Skusea) pembaesis* Theobald and some other mosquitoes of Faza, Pate Island, East Africa. *Bulletin of Entomological Research*, 55, 97-124.

Williams, C.B. (1937). The use of logarithms in the interpretation of certain entomological problems. *Annals of Applied Biology*, 24, 404-408.

Wirtz, A.R., Burkot, T.R., Andre, R.G., Collins, W.E. & Roberts, D.R. (1985). Identification of *Plasmodium vivax* sporozoites in mosquitoes using an Enzyme-linked Immunosorbent Assay. *American Journal of Tropical Medicine & Hygiene*, 34, 1048-54.

Zavala, F., Gwardz, R.W., Collins , F.H., Nussenzweig, R.S. & Nussenzwig, V. (1982). Monoclonal antibodies to circusporozoite proteins identify the species of malaria parasites in infected mosquitoes. *Nature*, 299, 737-41.