THE ROLE OF *ANOPHELES ARABIENSIS* (DIPTERA: CULICIDAE) IN MALARIA TRANSMISSION AND CONTROL IN GOKWE AND BINGA DISTRICTS, ZIMBABWE.

by

HIERONYMO TAKUNDWA MASENDU

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CONTENTS

DEDICATION

This work is dedicated to the rural communities of Zimbabwe, among them the people of Chiefs Chireya and Siabuwa, who bear the brunt of a harsh environment and the scourge of falciparum malaria.

ABSTRACT

Opportunistic feeding behaviour and partial exophily make *An. arabiensis* much more difficult to control by indoor residual spraying than any other vector in the Afro-tropical region. The persistent malaria outbreaks in Zimbabwe despite decades of indoor house spraying prompted this investigation into the role of *An. arabiensis* in malaria transmission and assessment of the possible impact of this control measure. The study was conducted in the malaria endemic districts of Binga and Gokwe.

An. gambiae complex mosquitoes were collected from artificial outdoor resting sites, and from human dwellings by i) daytime hut searches, ii) pyrethrum spray catches and iii) exit window traps. Mosquito components were processed to enable: i) the distinction of *An. arabiensis* from *An. quadriannulatus* and *An. merus* on the basis of the pale band at the junction of the hind leg 3/4 tarsomeres; ii) species identification and scoring of inversion polymorphism on the basis of the X chromosome and autosomes respectively; iii) the determination of blood meal sources using the Ouchterlony precipitin test; and iv) identification of *An. gambiae s.l.* using polymerase chain reaction (PCR) and enzyme electrophoresis techniques. Entomological assessment of residual spraying included determining: the vector resting densities indoors and outdoors, bioassay and insecticides susceptibility tests. Data were also collected on hut profiles, knowledge-attitudes-practices surveys, and household malaria prevalence surveys.

An. arabiensis and *An. quadriannulatus* were found in sympatry in Binga and Gokwe, and in addition, *An. merus* was found in Gokwe. Most species identifications were made using PCR; which was found to have 7.5% and 41.6% levels of error for *An. arabiensis* and *An. quadriannulatus* respectively, using the cytogenetic technique as benchmark. The pale band technique yielded >80% correct identification for *An. arabiensis* but the extent of overlap in the pale band lengths between *An. arabiensis* and *An. quadriannulatus* renders the method unsuitable for distinguishing these two species. Inversions 2Rb and 3Ra were found floating in *An. arabiensis*, with 60% frequency in the former. The Wright's F statistic value of -0.0416 indicated an excess of heterozygotes, and a state of panmixis in the vector population. No significant differences were observed between 2Rb karyotypes in host choice. Human blood indices among indoor (0.82), exit trap (0.98) and outdoor resting (0.30)

specimens suggested exophilic behaviour. This was corroborated by the high fed:gravid ratios of 6.8:1 and 11.6:1 in sprayed and non-sprayed dwellings respectively. This was worsened by a high feeder-survivor index (FSI) of 93% among exit trap specimens. The susceptibility to deltamethrin coupled with residual efficacy nine weeks post-spray indicated the suitability of the insecticide. Rural dwellings were suitably built for spraying but had no mosquito proofing. Personal protective measures are hardly known; sleeping outdoors occurs in Siabuwa.

While *An. arabiensis* bites humans indoors the partial exophily it exhibits is a threat to indoor residual insecticide spraying. An integrated malaria control approach is recommended.

PREFACE

The field work described in this dissertation was carried out in Chireya, Gokwe and Siabuwa, Binga, Zimbabwe, from December 1993 to April 1994, while the laboratory analyses of mosquito specimens was carried out in Durban at the National Malaria Research Programme of the Medical Research Council of South Africa, under the supervision of Dr. Brian L. Sharp and Professor Christopher C. Appleton.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others it is duly acknowledged in the text.

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INTRODUCTION

1.1 THE EXTENT OF THE MALARIA PROBLEM

The World Health Organization (1994) estimates there are 300-500 million clinical cases of malaria per year worldwide, 90 % of them in Africa, and 1.5-3 million deaths, one million of them children under five years of age. The countries in sub-Saharan Africa are particularly affected with an estimated 270-480 million clinical cases per year (including 140-280 million under fives), and 1.4-2.8 million deaths (including one million under fives).

1.2 MALARIA IN ZIMBABWE

In Zimbabwe more than half of the population of 12 million people is at risk of malaria (Taylor and Mutambu, 1986). *Plasmodium falciparum*, the most pathogenic of the human malaria, is responsible for the bulk (over 97%) of the cases in the country, while *Anopheles gambiae* complex mosquitoes are responsible for transmission (Wolfe, 1964; Taylor, 1985; Crees and Mhlanga, 1985). The most important factors affecting malaria epidemiology in the country are altitude and season (Taylor, 1985). Malaria seasonality is related to the effects of temperature and rainfall on the breeding and survival of vector mosquitoes. Peak transmission occurs in March/April and is preceded by a mosquito vector population peak in February/March (Mpofu, 1985). Altitude influences malaria distribution through its effect on temperature, with lower altitudes having higher average temperatures. The central watershed in the country is regarded as a malaria free zone, while the low lying areas of the Zambezi Valley to the north and the Save and Limpopo river basins to the south of the highveld experience considerable malaria transmission. The latitudinal differences between the north and south of the central highveld result in comparatively higher temperatures in the north for the same altitude. Hence the Zambezi Valley experiences more malaria transmission than its southern altitude counterpart (Taylor, 1985).

Leeson (1931) implicated *Anopheles gambiae* and *Anopheles funestus* as the vectors responsible for the blackwater fever outbreak in Zimbabwe (then Southern Rhodesia) in the

1920s. However, present knowledge of the complex nature of both these mosquito taxons has rendered Leeson's work of limited value. It has however formed the basis for the barrier spraying scheme that was practised in the country, which involved the spraying of houses in the middle altitude zones (Alves and Blair, 1955). In Zimbabwe the eradication programme did not get beyond 'pilot' stage (Taylor and Mutambu, 1986) contrary to the report that by the end of 1960 the people of Zimbabwe, along with those in the Republic of South Africa and in Swaziland, were in areas where malaria eradication had reached consolidation or maintenance phase (Kouznetsov, 1977).

1.3 VECTOR CONTROL BY INDOOR RESIDUAL SPRAYING

Vector control by indoor spraying of houses with residual insecticides and the use of chemotherapy are the primary strategies of malaria control in Zimbabwe. Indoor spraying dates back to the late 1940s when BHC (benzene hexa-chloride, commonly known as 'gammexane') was initially used, followed by a long history with DDT (dichlorodiphenyltrichloroethane) until 1990 when the synthetic pyrethroids deltamethrin and lambda-cyhalothrin were introduced, which have now virtually replaced DDT. Alves and Blair (1953) described the initiation of malaria control in the country.

The effectiveness of malaria control based on the use of indoor spraying with residual insecticides is reliant on the feeding and resting habits of the vector mosquito. Its behavioural tendency to rest on walls in human habitations after feeding on the occupants provides an opportunity for the mosquito to come into contact with residual insecticide in appropriately sprayed houses. De Meillon (1934) (cited by Coetzee and Hunt, 1993) provided evidence that mosquitoes spent a considerable time inside houses. Work initiated by Park Ross in the Natal province in 1931 formed the basis for the widely acclaimed house spraying which was later adapted for the malaria eradication programme by the World Health Organization (le Sueur *et al*, 1993). While immediate kills are desirable with residual insecticides, they are not essential, as the main objective is to shorten the mosquito's life expectancy to below what is necessary for the completion of the development cycle of the malaria parasite in the vector (Pampana, 1963). Theoretically interruption of transmission can be achieved by a consistent,

overall, daily mortality of about 50% (Davidson, 1982). It is assumed that the insecticide will produce uniform reduction of longevity within the vector population on condition that the individual vectors have equal probabilities of contact with the sprayed surface. Successes with DDT in Spain, Crete, Italy and other areas led to the launching in 1955 of a Global Malaria Eradication Campaign by the World Health Organization (Pampana, 1963; Coluzzi, 1992). The introduction of DDT was pivotal in shifting malaria control emphasis from larviciding of breeding sites to the treatment of houses to kill adult mosquitoes. New horizons were opened as the control of rural malaria became a practical possibility (Hocking, 1965, p. 553; Schliessmann, 1983). The advent of insecticides, especially DDT, raised hopes and even euphoria as expressed in this excerpt from Jordan (1950, p. 302):

> 'It now seems possible that the systematic use of DDT or 'gammexane' will exterminate certain species of mosquitoes over very wide areas of land, whole islands, whole countries and perhaps one day, over whole continents'.

Despite the disappearance of malaria in Europe and North America being widely attributed to this campaign, it has also been argued that the decline was due (at least in part) to concomitant new agricultural practices and changed social conditions (Kouznetsov, 1977; Coluzzi, 1992; de Zulueta, 1994).

In Africa malaria eradication programmes were implemented on the islands of Pemba and Zanzibar while former control programmes in South Africa, Swaziland, Mauritius and La Réunion were converted into eradication pilot projects (Cullen and De Zulueta, 1964; Kouznetsov, 1977). Malaria was successfully eradicated in Mauritius and La Réunion, while eradication was approached in South Africa and Cape Verde Islands, and a high degree of control was achieved in Zimbabwe, Zanzibar and Pemba but little progress was reported in Ethiopia (Kouznetsov, 1977). The effectiveness of house-spraying with residual insecticides was demonstrated in different epidemiological conditions in Cameroon, Liberia, Nigeria, Senegal, Upper Volta, Benin and Togo where nine million people were protected. Generally, however, much of tropical Africa was not included in the eradication programme on the grounds that the existing infrastructure was not suitable for such an exercise (Davidson,

1982).

Indoor use of residual insecticides was effective in temperate areas but not in tropical Africa because of the genetic versatility of the vector species (Gillett, 1985; Coluzzi, 1992). One of the notorious malaria vectors, *An. funestus*, is assumed to have been eliminated by the indoor use of DDT in southern Africa (Sharp *et al*, 1984; Crees and Mhlanga, 1985). The disappearance of *An. funestus* has been documented from other localities where DDT and dieldrin have been used; the Pare area in Tanzania (Draper and Smith, 1960) and the Antananarivo area in Madagascar (Ralisoa Randrianasolo and Coluzzi, 1987) are examples. Similar insecticide impact has been observed on other vector species elsewhere such as *An. stephensi*, *An. culicifacies* and *An. subpictus* in Pakistan (Reisen, 1986) and *An. hyrcanus* in Afghanistan (Onori *et al*, 1975). Problems were, however, encountered as mosquitoes developed resistance to the insecticides and behavioural deviations meant that some vector species became less amenable to control by indoor spraying of houses.

Behavioural diversity of mosquito vectors was reported as being responsible for the failure to interrupt malaria transmission following house spraying with propoxur in the Garki district of Nigeria (Molineaux and Gramiccia, 1980). *An. gambiae s.l.* in the area consisted of *An. gambiae s.s.* and *An. arabiensis*, with the latter being predominant. The existence of mixtures of endophilic and exophilic mosquitoes in vector populations results in non-uniform exposure to residual insecticide sprayed indoors and therefore the expected impact of vector control will not be achieved (Molineaux *et al*, 1979). Coluzzi *et al* (1977) demonstrated a relationship between behavioural and genetic variations in the *An. gambiae* complex species from the same area. In Venezuela the failure of the spraying programme was attributed to the exophilic habits of *An.* (*Nyssorhynchus*) *nuneztovari* Gabaldón (Rubio-Palis *et al*, 1992). Work by Hii (1985) in the Sabah area of East Malaysia corroborated the existence of such genetic variability with respect to host seeking and indoor resting in the malaria and filariasis vector, *An. balabacensis*. Nutsathapana *et al* (1986) further demonstrated a significantly different level of heterogeneity for host preference in the malaria vector *An. minimus* in Thailand. In contrast, Rawlings and Curtis (1982) were unable to establish the presence of any genetic variability in host choice or indoor resting behaviour in *An. culicifacies* in Sri Lanka. In Papua New Guinea, no heterogeneity was found between indoor and outdoor biters

of any of the three species of the *An. punctulatus* group of mosquitoes (Charlwood *et al*, 1985). Similarly, Lines *et al* (1986) reported the absence of behavioural variation in the predominantly *An. gambiae s.s.* vector population in a coastal area in Tanzania but recommended that the more exophilic *An. arabiensis* should be investigated. In Zimbabwe, Masendu *et al* (1992) used the mark-release-recapture technique described by Lines *et al* (1986) to assess the existence of behavioural polymorphism in a predominantly *An. arabiensis* vector population but could not make incisive conclusions owing to the inadequate mosquito populations available at Mbizha (18^0 0'S 26^0 15' E). Rawlings and Curtis (1982), however, argued that evidence for such genetic variation within populations is still circumstantial and called for further research. Interestingly, Hii *et al* (1991) suggested that mark-release experiments should be conducted with F_1 progeny, following their observations on doubly-marked recaptures which strongly indicated a "learning" component was involved in host preference and choice of resting site in wild caught *An. balabacensis*. An analysis of chromosomal inversions and correlation of these to site of capture and feeding and resting preferences might provide an alternative way of ascertaining the existence of behavioural polymorphism among members of the *Anopheles gambiae* complex as documented by Coluzzi *et al* (1979).

The role played by the human host in the transmission of malaria has been the subject of several studies. Certain behavioural traits in humans predispose them to parasitic disease including malaria (Gillett, 1985). Carnevale *et al* (1978) investigated the agressivity of *An. gambiae* in relation to the age and sex of human subjects. While they concluded that the anophelines bit both males and females indiscriminately, they observed an age-related biting pattern. The success of adult mosquito control by indoor residual spraying is contingent upon three factors, namely, (1) houses being favourably constructed for the application of insecticide; (2) people sleeping indoors consistently; and (3) vector mosquitoes habitually resting indoors (Fontaine, 1983). The peoples' perception, attitude and practices regarding malaria are aspects that come into the realms of the sociology of malaria control (Reuben, 1989). The political and economic issues that come into play in malaria control are well illustrated in the Ethiopian example described by Gish (1992).

1.4 THE *ANOPHELES GAMBIAE* COMPLEX

The occurrence of species complexes in the genus *Anopheles* has been described for a number of medically important vectors of malaria and filariasis: the *An. maculipennis* complex in Europe and the Nearctic which was the first to be resolved, the *An. gambiae* complex in Africa and the *An. sinensis* complex in Asia are examples. It has been proposed that parasites, *Plasmodium* in particular, act as a "wedge" that results in the split of a particular vector species into sibling species (Steiner, 1981). Coluzzi *et al* (1979) in contrast, attribute the evolution of species complexes, *An. gambiae* at least, to human activities.

Following work on *An. maculipennis*, Hackett (1936), cited by Paterson (1963), suggested that *An. gambiae* Giles was a species complex. Paterson (1962, 1964), Davidson and Jackson (1962), and Paterson *et al* (1963) provided much of the evidence on the existence of various members of the *Anopheles gambiae* complex. This resolution of the *An. gambiae* into a number of genetic species explained the behavioural and ecological differences that had been noted about the species, especially in situations where insecticides had been used in malaria control operations (Paterson *et al*, 1963). The sibling species are defined by reproductive barriers (White, 1971) and cytotaxonomic characters (Coluzzi *et al*, 1979). By the late sixties the *An. gambiae* complex was known to consist of the following five species: *merus*, *melas*, A, B, and C (Davidson and Jackson, 1962; Paterson *et al*, 1963; Davidson, 1964; WHO, 1968). Presently, six sibling species have been described for the *Anopheles gambiae* complex, (eg. White, 1974b, 1982; Service, 1985). There are two salt water breeding species, *An. melas* Theobald and *An. merus* Dönitz, which although occupying similar ecological niches, are allopatric, occurring along the West and East African coasts respectively. The recognition of these two species was initially based on ecological evidence and slight morphological distinctions. There are three fresh water breeders, originally referred to as 'groups' (Davidson and Jackson, 1962), and 'forms' A, B and C (Paterson, 1964) and later, informally, as species A, B, and C (Davidson, 1964). They have since assumed the formal names *An. gambiae* Giles *sensu stricto*, *An. arabiensis* Patton and *An. quadriannulatus* Theobald respectively (White, 1975; Mattingly, 1977). These three fresh water breeders share an essentially similar larval ecology although subtle differences have been documented (le Sueur and Sharp, 1988). The sixth member of the complex, *An.*

bwambae White (formerly species D), is only known from the Burung'a hot springs area of the Semliki forest in Bwamba County, Uganda, where it breeds in mineral water, and bites people in the absence of alternative hosts (Davidson and White, 1972; Gillett, 1885; White 1985). An account of the history of the resolution of the *An. gambiae* complex is given by Paterson (1993).

The sibling species of the *An. gambiae* complex have been shown to have different capacities in the transmission of human malaria. *An. gambiae s.s.* exhibits the most anthropophilic and endophagic behaviour, and is thus one of the most efficient malaria vectors known. White (1974b), for example, computed an annual average sporozoite rate of 4.23 $\pm$ 0.71% for *An. gambiae s.s.* compared to only 0.32 $\pm$ 0.16% for *An. arabiensis* at Segera, Tanzania. The latter species tends to show mixed feeding and resting behaviour but is also an important malaria vector at numerous localities (White, 1974b; Dukeen and Omer, 1986; Sharp and le Sueur, 1991). *An. quadriannulatus* is considered a non-vector owing to its strong zoophilic and exophilic behaviour. *An. merus*, *An. melas* and *An. bwambae* are minor malaria vectors in their areas of distribution (White, 1974b, 1985). Sibling species of the *An. gambiae* complex have been found in various combinations of sympatry, the extent of which is limited by the restricted distribution of the halophilous species and the brackish water breeder, and the limited distribution of *An. quadriannulatus*. While all six species have been successfully crossed in the laboratory, the low frequency of hybridization ($<0.1\%$) in nature reflects the existence of mating barriers which, however, appear to break down under certain ecological circumstances (White, 1971).

Paterson (1963) and Gillies and De Meillon (1968) provided distribution maps of the *An. gambiae* complex in the then Ethiopian Zoogeographical Region (now Afro-Tropical), which was revised by Gillies and Coetzee (1987). White (1985) also provided details of the distribution of the sibling species of the complex. An up to date account of the distribution of these in this region is provided by Coetzee *et al* (1993b). In Zimbabwe various combinations of sympatry have been observed: *An. gambiae*, *An. arabiensis* and *An. quadriannulatus* at Chirundu (16^{0}S 28^0 53'E) (Paterson, 1963) and at Chitengu (17^0 13'S 31^0 45'E) (Mpofu, 1985); *An. arabiensis*, *An. merus* and *An. quadriannulatus* at Hippo Valley (21^0 7'S 13^0 38'E), and *An. gambiae* and *An. arabiensis* at Kanyemba (15^0 40'S 30^0 20'E)

(Mahon *et al*, 1976). The sibling species composition in the two present study areas were unknown at the time this study was conducted, but recent studies at Mutimutema (28^0 35' E 17^0 45'S), some 30 km south of Chireya, have indicated a predominance of *An. arabiensis* among adults raised from larval samples collected in artesian well run-off pools (Masendu and Freeman, in press.).

An. quadriannulatus has a distinct preference for feeding on animals and resting outdoors (White 1974b; Service 1985) although it has been reported to rest indoors (Sharp *et al*, 1984; Hunt and Mahon, 1986). *An. gambiae* and *An. arabiensis* show the widest distribution and are closely associated with humans (Coluzzi, *et al*, 1985). The sympatric distribution of *An. gambiae*, *An. arabiensis* and *An. quadriannulatus* produces a complicated situation with regards to disease transmission and impact assessment of vector control. Observations on 'change in habit' after the application of BHC and DDT were reported by Alves and Blair (1953) in Zimbabwe and Mastbaum (1954) in Swaziland. These were eventually explained by the realization that i) *Anopheles gambiae* was not one taxon as previously conceived and ii) that, after observations on species C, the supposed 'behavioural resistance' was due to different habits among sibling species (Paterson *et al*, 1963).

1.5 SPECIES IDENTIFICATION

The existence of sibling species has profound epidemiological implications as it is necessary that only those species involved in disease transmission should be targeted for cost-effective control. A practical and pertinent example is given by Green (1981, 1982) when the use of isoenzyme electrophoresis for species identification in Zimbabwe revealed that in a WHO dieldrin resistance test, almost all the susceptible specimens consisted of the non-vector *An. quadriannulatus* while all survivors were *An. arabiensis*. Green (1981) aptly summarized these observations as follows:

> 'The effective treatment of the specific taxon *An. gambiae s.l.* as a real entity in nature would have had disastrous consequences for disease control.'

There has been limited success in the various attempts to examine morphological divergences among the partially incompatible forms of the *An. gambiae* complex (eg., Coluzzi, 1964; Coetzee, 1986). Because the sibling speciation in *An. gambiae* complex gives little morphological evidence for taxonomic separation of vectors from non-vectors, identification of morphologically identical species has been a priority research topic. The taxonomic distinction of the sibling species has practical implications for the control of malaria. Various approaches (both morphological and biochemical) have been explored for use in the identification of the members of the *An. gambiae* complex, and this has been a subject of several comprehensive reviews (White, 1974; Green, 1985; Zahar, 1993). Collins *et al* (1988) summarize the limitations associated with some of the methods with respect to specificity, simplicity and compatibility with the needs of large scale epidemiological studies. Table 1 summarizes the various methods developed for the identification of members of the *An. gambiae* complex in 'chronological' order. The use of immunoaffinity ELISA for species identification described by Ma *et al* (1990) is rare in the literature and has not been included in this summary.

1.6 SIGNIFICANCE OF CHROMOSOMAL PARACENTRIC INVERSIONS

Differences in polytene chromosomes among members of the *An. gambiae* complex relate to differences in band sequences due to paracentric inversions (Coluzzi *et al*, 1979). Paracentric inversions (see; Ayala and Kiger, 1980, for definitions) have been valuable in mosquito phylogeny investigations. Work on polytene chromosomes from larvae (Coluzzi and Sabatini, 1967, 1968 and 1969) and the nuclei of nurse cells of stage-III and stage IV ovaries from adult female anophelines (Coluzzi, 1968) provided arguably the best method available for species identification. Fixed paracentric inversions on the X chromosome have also been used in species identification in the following complexes: *An. funestus* (Green and Hunt, 1980) *An. culicifacies* (Green and Miles, 1980), *An. willmori* and *An. dirus* (Green *et al*, 1992). There are, however, some limitations owing to the fact that the X chromosomes are homosequential between some species; eg., *merus/gambiae*, *melas/bwambae/quadriannulatus*, and *An. funestus/An. aruni*? Green and Hunt, 1980). These can be distinguished by their different autosomal band sequences, such as the 2R arm for *merus/gambiae*. In the case of

gambiae, *arabiensis* and *quadriannulatus*, which share common larval habitats and are thus sympatric in the strictest sense, differences in the X chromosome are sufficient to separate them.

Cytotaxonomy's greatest asset lies in the unique information on inversions that can be gleaned from the same polytene chromosomal material used for species identification. When polymorphic or 'floating' inversions occur, a mosquito population can have variable proportions of three possible karyotypes; the standard homozygote, the inverted homozygote and the heterozygote. The heterozygote inversions, which arise when standard and inverted gene arrangements interbreed freely in a population, exhibit heterotic vigour but reduce genetic variability (White, 1974a). The intraspecific behavioural differences observed in both *An. arabiensis* and *An. gambiae s.s.* in Garki by Coluzzi *et al* (1977, 1979) have been associated with a complex pattern of polymorphic inversions observed in these species. The majority of inversions in *An. arabiensis* and *An. gambiae s.s.* occur on arm 2R, and the frequencies of certain of these chromosomal arrangements are correlated with clines of climatic conditions and vegetation zones (Coluzzi *et al*, 1979). Significant differences in chromosomal types between samples positive for human and animal blood and between samples collected resting indoor in human huts and biting outdoor on animals have been documented (Coluzzi, 1973). The 2Ra inverted homozygous and/or 2Rc heterozygous karyotypes have been linked with greater exophagy and exophily than carriers of other 2R karyotypes. Variations in endophily and endophagy imply non-uniform exposure to indoor residual spraying. Shelley (1972) cited by White (1974b) also observed the association between 2Rc heterozygotes and exophily and zoophagy. As a corollary, the lack of the above karyotypes in *An. arabiensis* in the Ethiopian highlands was observed to be accompanied by complete endophagic behaviour in this vector (White, 1974b). Although there is no documented evidence, it has been suggested that the time of feeding appears to be another variable linked with chromosomal polymorphism; the proportions of 2Rb and 3Ra heterozygotes have been reported to rise progressively in successive catches during the night (White, 1974b). In respect of host choice, Sharp and le Sueur (1991) reported a homogeneous distribution between human and bovine fed *An. arabiensis* among carriers of different 2Rb inversion karyotypes in KwaZulu\Natal, South Africa.

Table 1. A summary of major techniques used in species identification of members of the Anopheles gambiae complex

Approach	Remarks	Limitations	References
Crossing experiments	initial technique to determine biological species	cumbersome; small numbers can only be used	Paterson (1962); Davidson (1964a);
Salinity tolerance	distinguishes salt water breeders from fresh water breeders	requires rearing F_1 generation	Ribbands (1944); Muirhead-Thomson (1951);
Morphology	ease of use; minimum skills required; indefinite specimen storage possible	limited success; freshwater species inseparable	Ribbands (1944); Coluzzi (1964); Coetzee (1986); Hunt and Coetzee (1986a); Sharp et al (1989).
Cytotaxonomy	considered the benchmark; easy, inexpensive; unique inversion information; permits population studies. large numbers applicable	sex- and stage-specific; slow; shortcomings exposed in homosequential taxons.	Coluzzi & Sabatini (1967, '68, '69) Green (1972); Hunt (1973); Green & Hunt (1980); White (1975).
Zymotaxonomy	simple procedure; not stage specific; large samples applicable; permits population studies.	special and costly preservation, locality verification necessary	Mahon et al (1976); Miles (1978 and 1979); Marchand & Mnzava (1985); Coetzee et al (1993a).
Cuticular hydrocarbons	on-the spot identification; stable and easily obtainable cuticular hydrocarbons;	expensive equipment not developed to permit reliable and routine use	Carlson & Service (1979, 1980); Hamilton and Service (1983); Phillips and Milligan (1986).
DNA probes	applicable to field use, with no expensive equipment neither stage nor sex-specific, ease of storage male-specificity of earlier techniques now overcome	sensitivity, specificity; once inapplicable to virgin females owing to male-specific probe	Panyim et al (1988); Gale and Crampton (1987); Collins et al (1987 , '88); Hill and Crampton (1994).
PCR	neither stage nor sex-specific, ease of storage large numbers applicable, competitive costs per specimen	initial equipment costs high,	Paskewitz and Collins (1990); Paskewitz et al, 1993 Gale and Crampton (1987, '89); Bredenkamp and Sharp (1993).

Analysis of chromosomal polymorphism within *An. gambiae. s.s.* in West Africa (Coluzzi *et al*, 1985) has led to the identification of chromosomal forms provisionally designated by the non-Linnaean nomenclature as: Mopti, Bamako, Bissau and Savanna. While these are not recognized as species as defined by Miles (1981), there is incomplete intergradation between some of these karyotypes and indications of some patterns of spatial and seasonal distribution that may be assumed to be associated with competitive exclusion (Bryan, *et al*, 1982).

1.7 *ANOPHELES ARABIENSIS*

Kitzmiller (1982) has this to say about *arabiensis*:

'After long years of obscurity in synonymy *arabiensis* has renewed importance as the name with taxonomic priority for the taxon which was known as species B of the *Anopheles gambiae* complex'.

Patton (1905), cited by White (1975) and Kitzmiller (1982) is reported to have linked the name *arabiensis* with the great Arabian desert. *An. gambiae* and *An. arabiensis* are widely distributed in Africa, and are medically the most important members of the *Anopheles gambiae* complex. The former is rare in the southern African region (Bransby-Williams, 1979; Petrarca *et al*, 1984; Coetzee *et al*, 1993b), whereas the latter species is the primary malaria vector in this region. There are distinct behavioural differences between the two: *An. gambiae* is comparatively a much more efficient malaria vector as it is highly anthropophilic and endophagic, while *An. arabiensis* tends to be more catholic in its host preference and is comparatively less endophilic (Akiyama, 1973; Bransby-Williams, 1979; White, 1974b; Service, 1985). White (1974b, 1980) notes that the multiple inversion polymorphism associated with *An. arabiensis* endows it with greater ecophenotypic plasticity than any other mosquito. The greater number of polymorphic inversions in *An. arabiensis*, (17 compared with only eight in *An. gambiae*) has been associated with the broader distribution and better adaptability for the former. The greater exophily of *An. arabiensis* led Service (1970) and White (1974b) among others, to predict that this species would be much more difficult to control with indoor residual spraying than *An. gambiae*. Partial exophily was reported to be

responsible for the mediocre results obtained after spraying with propoxur in the Garki Project where a 'high pre-spraying ratio between the man-biting and indoor-resting densities was observed' in the predominantly *An. arabiensis* vector population (Molineaux and Gramiccia, 1980). The use of residual insecticides against *An. arabiensis* as a unilateral approach to malaria control is not recommended unless (genetic) surveys suggest exophilic genotypes are absent in the population (White, 1974b; WHO, 1993). In situations where *An. arabiensis* shows a low genetic diversity, as was observed in Burundi's Rusizi Valley by Coosemans *et al* (1989), a uniform exposure to indoor residual spraying would be expected. In northern KwaZulu\Natal, South Africa, Sharp *et al* (1990) observed a high exodus of *An. arabiensis* from dwellings irrespective of the DDT status of the huts. The presence of DDT appeared to inhibit mosquito feeding indoors, which led Sharp *et al* (1990) to hypothesize that this could result in an increase in mosquito biting of man outdoors. DDT was however found not to be solely responsible for the exodus of mosquitoes as this phenomenon was also occurring in unsprayed houses (Sharp and le Sueur, 1991).

Given that *An. arabiensis* is the principal malaria vector in Zimbabwe and that the National Malaria Control Programme is presently reliant upon residual insecticide spraying for vector control, it is essential that the man biting and resting behaviour of this vector and the impact of indoor spraying on this species be carefully assessed.

1.8 HOST PREFERENCE

Mammalian blood is essential for complete ovarian development in most anopheline mosquitoes. The vectorial efficiency of a mosquito species is reflected by the potential to feed on humans. The presence of hosts other than man could lead to a diversion of host choice to these alternatives as documented for *An. arabiensis* (White, 1974b). The study of host preferences of mosquitoes requires the analysis of their blood meals and various techniques have been described that enable the identification of the natural hosts of blood sucking insects. The precipitin test (Peetoom, 1963; Southwood, 1978) is the most widely used, and enzyme-linked immunosorbent assay, ELISA, (Service *et al*, 1986) although relatively recent, is gaining in popularity. Depending on the host source, blood meals can either be simple or mixed; the latter can further be categorized as patent or cryptic, depending on whether the

blood meal is obtained from two or more vertebrate species, or two or more individuals of the same species respectively (Boreham and Garrett-Jones, 1973). The polymerase chain reaction (PCR) technique has been used to identify individual human hosts from mosquito blood meals (Coulson *et al*, 1990). It is also essential to analyze mosquito blood meals in order to determine the effect of insecticide spraying on the extent of both feeding and resting indoors.

An (chronological) annotated bibliography of published and unpublished work on Zimbabwean vectors is shown in Appendix 1. From this local literature it is evident that none of the previous studies in Zimbabwe have specifically investigated wild *An. arabiensis*, the principal malaria vector, its behaviour and amenability to control by house-spraying with residual insecticides.

1.9 GENERAL OBJECTIVES

The purpose of the study was to describe and evaluate the role of *An. arabiensis* in malaria transmission at two localities in Zimbabwe within the context of indoor residual spraying and human behavioural patterns.

1.9.1 SPECIFIC OBJECTIVES

i) to determine the indoor and outdoor resting preferences of local vector populations,

ii) to evaluate the various methods of identifying members of the *An. gambiae* complex mosquitoes using morphological, isoenzyme electrophoresis, polymerase chain reaction (PCR) and cytogenetic techniques,

iii) to assess host preference by blood-meal analyses,

iv) to ascertain the susceptibility of the vector population to the insecticide in current use in malaria control in the Zimbabwe National Malaria Control Programme,

vi) to determine the extent to which paracentric inversions are correlated with feeding and resting behaviour, and

vii) to assess human behaviour and housing and their roles in malaria transmission.

Chapter 2
MATERIALS AND METHODS

2.1 STUDY AREAS

The study was conducted in two areas, namely Chireya and Siabuwa, in the north west of Zimbabwe on the Zambezi escarpment (Appendix 3a and 3b). The geology of this region is described by Hursey and Allsopp (1984) as consisting of mainly Karoo grit, coal measures and sandstone. The vegetation consists of *Brachystegia/Julbernadia* spp. woodland interspaced with short mixed *Combretum* and extensive tracts of mopane woodland (*Colophospermum mopane*). Dense riparian vegetation occurs along the larger rivers, which flow northwards towards the Zambezi river. Chireya and Siabuwa were sprayed for malaria vector control with K-Othrine 25 SC (2.5% deltamethrin) at an application rate of 15 mg a.i./m^2 in December 1993. Both Chireya and Siabuwa were in their 4th cycle of annual deltamethrin spraying; DDT had been used erratically prior to this. In addition these areas fall within the region which had been subjected to aerial and ground spraying with endosulfan and DDT during an anti-tsetse campaign launched by the Tsetse and Trypanosomiasis Control Department in the Ministry of Lands, Agriculture and Rural Development. A special formulation of 0.4 % deltamethrin in diesoline applied at 0.25 g/ha was followed by four applications of thiodan 30 % EC (endosulfan) at 20, 18, 14 then 14 g/ha (Hursey and Allsopp, 1984). All aspects of the study activities described hereunder apply to both the study areas of Chireya and Siabuwa unless otherwise stated.

2.1.1 Chireya, Gokwe North

The study was conducted in five villages adjacent to Chireya Mission Hospital, (28^0 36'E 17^0 33'S), namely Taka, Nenhere, Kaparapate, Matashu and Venganayi. Chireya is approximately 450 km from Harare, and lies at 670 m above sea level and is characteristically hot and subject to seasonal malaria episodes. The area falls under Natural Region IV which is characterized by an annual rainfall of 450-650 mm. The rainy season starts in October and ends in March/April (Fig. 1).

The people's main occupation here is subsistence farming which is solely dependent

on rainfed crops. The area of Gokwe is well known for its sordic soils which are suitable for cotton growing. There is limited use of artificial chemical fertilizers, but cotton growing entails the extensive use of the pesticides dimethoate, carbaryl and fenvalerate among others. In the past the land was sparsely populated by the indigenous Shangwe people, but a recent influx of people from malaria free areas seeking land has resulted in markedly high records of malaria cases associated with this essentially non-immune group. A large proportion of inhabitants encountered have been in the area for less than ten years. The population within the hospital catchment area is estimated at 4500 with an average of 6 people per household. The literacy level is fairly high, but per capita income is low (Central Statistical Office, 1992).

The circular or rectangular pole and mud or brick and thatched hut is the dominant form of housing structure in most households (Plate I). Two huts are often encountered in each household; one for cooking purposes, and the other is reserved for sleeping. There is no electricity and wood is used in cooking while candles or kerosine lamps are used for lighting. Other structures are occasionally present and these serve as granaries or chicken pens. Most dwellings have no Blair toilets, and neither are there water wells. Domestic animals commonly found are cattle, goats, donkeys, dogs and chickens. Cattle, sheep and goats are housed in kraals (Plate II), at various distances from homesteads. Goat and sheep pens are invariably thatched but not to the same standard as the human huts. The women are seasonally engaged in traditional salt mining at the Bare salt pan on Katsvanzva stream. The brown salt obtained is mainly for local consumption.

Chireya township is a focal point in the area and is undergoing rapid development. There is a primary and a secondary school, a police station, and a depot for the Cotton Marketing Board. Chireya Mission Hospital caters for almost all the health needs of the community within a 30 km radius. A limited amount of employment is provided by the above establishments. Health facilities are within walking distance for a large proportion of the people, although the ox- or donkey- pulled scotch cart is occasionally used in the event of serious illness. The access gravel road from the tarred Gokwe - Binga highway becomes slippery and impassable when it rains, and buses using this road avoid it during such periods. Most tracts of land in the immediate vicinity of the homes are not cultivated, and are prone

to water logging in susceptible places. While the three rivers Ume, Katsvanzva and Murongwezhi flood during the rainy season, they revert to mere sand beds by March/April. Several excavations locally known as *mifuku* (Plate III) are made on the river bed in an attempt to get water for domestic use when the rains stop. The complete absence of water in the vicinity of the villages means that both man and animals have to trek to the residual pools along the Ume river. Households adjacent to the police camp obtain water from the borehole available there.

Malaria prevalence is high during the wet season and there are indications that chloroquine (the country's first line drug) resistance in the area is on the increase (A. Chihaka, pers. comm.). No previous vector studies have been undertaken in the area, and the present study will provide a much needed insight into the dynamics of malaria transmission and control.

2.1.2 Siabuwa, Binga

Six villages, Zeula, Guteni, Machebuka, Male, Banenge and Siamtenge near Chief Siabuwa's homestead (28^0 3'E 17^0 29'S), were involved in the study. Some of the people living here were moved from the banks of the Zambezi river as a result of the construction of the Kariba Dam in the sixties. The main landmarks of the area include the Tundazi mountain to the south, the Kasumula hills to the north, Siabuwa Rural Clinic, shops, a primary and a secondary school, and the police station on the banks of Nabusenga dam. The area lies below 690 m above sea level, 60 km south of Mujere on the banks of Kariba dam, 80 km west of Chireya and 80 km from the district headquarters in Binga.

The Tonga people are indigenous to this and surrounding areas within the district and beyond. Tonga and to a lesser extent, Ndebele, are the languages of communication. The population of Nabusenga Ward is just over 5 000 (Central Statistical Office, 1992). The community is essentially dependent on subsistence farming, although in the past they were essentially hunter gatherers. Maize, sorghum and to a lesser extent, cotton, are the main rainfed crops grown. The vegetation resembles that of Chireya although baobab (*Adansonia digitata*) is more common here.

Nabusenga dam provides piped water which is available for domestic use at several

points along the Chinhoyi-Binga road. Pipes and cement-lined canals supply water into night storage dams for irrigation. The absence of water puddles in the irrigation scheme makes irrigation malaria most unlikely. The irrigation is mainly for maize, tomatoes, beans and bananas, crops which are not suited for flood irrigation. Nabusenga River flows from the dam and meanders west through the villages. The river floods during the rainy season but is completely dry by March/April of each year.

Traditional pole, mud and thatched huts as found in Chireya are the most common form of house (Plate I). Homes are occasionally vacated during summer as the people move to the fields to guard the crops against wild animals, such as elephants and baboons. Makeshift field shelters, *ngazis* (crop-huts) are used as accommodation on these occasions. Both men and women till the land, but the fields are not as extensive as those found in Chireya. Womenfolk are also engaged in the traditional preparation of maize or sorghum meal at home. Men and boys frequently fish in the Nabusenga dam. Traditional beer is consumed at several unsheltered marketing sites situated away from the homes. Such social gatherings have tendency to run into late hours of the night. Cattle, donkeys, goats and sheep are the common domestic animals found in the area. There are substantially more goats in this area than Chireya but fewer goat enclosures are present. There is no industry in the area although some people are involved in fish marketing at Mujere. Present diamond prospecting in the area promises to create jobs for the locals in the same manner that safari operations have done in the past. Access to the area is facilitated by the gravel road, and buses from Harare, Gokwe, Bulawayo, Hwange and Binga pass through Siabuwa everyday. The area serves as a short-cut route between the tourist resort areas of Victoria Falls and Bumi Hills.

The area experiences malaria epidemics and is under a residual insecticide spraying programme as a means of vector control. Like Chireya, deltamethrin has superseded DDT in vector control operations since the 1990/91 malaria season. The area has also been subjected to endosulfan and DDT in the fight against tsetse. Some of the villages were deliberately left unsprayed during the 1993/94 spraying season and the repellent soap provided as an alternative is under evaluation as a control measure. As in Chireya, vector abundance and species composition in Siabuwa are unknown as there have been no studies in the past. Fig. 2 illustrates the weather patterns characteristic of the area.

2.2 METEOROLOGICAL DATA

Rainfall data were obtained from the local Agriculture Extension Officers in the respective study areas. Temperature and relative humidity were recorded by automatic Casella[R] thermohygrographs placed indoors and outdoors but sheltered from direct sunlight. On one occasion measurements were made for comparison between a thatched house and a corrugated iron roofed house. The thermohygrographs were calibrated once by staff from the Meteorological Department prior to their use in the field. Historical weather data for the respective district centres of Gokwe and Binga were obtained from the Meteorological Services Department, Harare.

2.3 WATER SALINITY SAMPLES

Water samples were collected from the Bare salt pan and Kurima stream, a tributary to Ume river, for the estimation of salinity. Salt content was determined by titration with a solution of silver nitrate and 5% potassium chromate as indicator (WHO, 1975).

2.4 MOSQUITO SAMPLING

There are various techniques available for sampling malaria vector mosquitoes (Service, 1976), and the choice is determined by the entomological investigation under consideration. In this instance, the sources of three groups of adult mosquitoes were being sought: the outdoor resting, the indoor resting and the house-leaving groups. Notes on the physiological status of female mosquitoes were made at the point of collection. All catches were placed in paper cups for later processing at the field camp. Field work was started in December 1993, but mosquitoes were collected from January 1994 to April 1994. Collections were conducted for at least one week per month in each study area.

2.4.1 Outdoor resting

Pit shelters (Plate IV) as developed by Muirhead-Thomson (1958) and further described by WHO (1975) and Service (1976), were constructed under naturally bushy cover

in January 1994. All pits, six in Chireya and four in Siabuwa, were fenced with barbed wire to prevent accidents with both humans and animals. Mosquitoes were caught using a mouth operated sucking tube (WHO, 1975) and, occasionally, a battery operated aspirator and collected from the pits before 0800 hours.

Cattle kraals (Plate II), goat pens, hollows on termite mounds and trees, and bases of tree trunks were also searched but failed to yield any *An. gambiae* complex mosquitoes. CDC light traps (Hausherr's Machine Works, New Jersey) were also used both indoors and outdoors (Plate V) but did not yield mosquitoes. The houses in which the light traps were set had no bed nets.

2.4.2 Indoor resting

Two complementary methods were used to assess the indoor resting component of the available mosquito population.

2.4.2.1 Indoor resting catches

The hand catch method (Service, 1976) was used in a 5-minute search for mosquitoes on wall, thatch, clothing and other household furnishings by five catchers. This amounted to 25 person-minutes/house searches. A torch and a sucking tube were used as in pit shelters.

2.4.2.2 Pyrethrum knockdowns

The pyrethrum spray catch (PSC) (also known by various other terms: pyrethrum spray-sheet collection or knockdown method (WHO, 1975; Service, 1976; Service *et al*, 1978) was used to complement the hand catch method described above. A commercial pyrethroid-based aerosol, Killem^{R} (active ingredients: 0.2 % tetramethrin and 0.05% δ-phenothrin) was sprayed simultaneously indoors on the thatch and walls and outside on the eaves (Plate I) and the room was left undisturbed for eight minutes to permit knockdown action. The knockdowns were conducted on all window trap-bearing houses (most of which were sprayed) at the end of each field visit in order to minimize the possible short term repellent effect of the aerosol interfering with mosquito movement into and out of the houses. Mosquito collections using this method were made between 0630 and 0900 hours.

2.4.3 House-leaving

Exit window traps as originally used by Muirhead-Thomson (1947) and modified by Service (1963) were further modified (Plate VI) and installed, one trap per hut, at the beginning of each field visit. Huts were conveniently selected to allow easy accessibility by car during the wet season. Once installed, the exit traps were left in place and were only removed at the end of each weekly study visit. They were inspected between 0630 and 0800 hours for at least 5 consecutive days. Ants, spiders and other trapped arthropods were cleared from the traps daily. Nineteen exit traps were used on the same huts in February, March and April in each of the two study areas.

2.5 MOSQUITO PROCESSING

An. gambiae complex mosquitoes were separated from other anophelines on the basis of morphological characteristics as described by Gillies and Coetzee (1987). Mosquitoes were immobilized by stunning in the sucking tube. Each specimen was coded with a unique label reflecting the specimen number, capture technique and catch station, and the date of sampling. Coding followed that designed by Sharp (pers. comm.) to facilitate the later correlation of the various specific analysis carried out on each from each specimen, eg. chromosomes, bloodmeals, leg banding and species identification.

2.5.1 Morphological slide

One hind leg, (in some instances both hind legs), was removed with forceps and mounted under a coverslip on a slide as described by Hunt and Coetzee (1986a), in order to determine the pale band width at the junction of the third and fourth hind tarsomeres (Coetzee, 1986). The slides were appropriately coded. Measurements were carried out with a Zeiss compound microscope using a 10X eyepiece and 10x objective with an eyepiece micrometer (Sharp *et al*, 1989). The maximum and minimum lengths of the pale band at the junction of the third and fourth hind tarsomeres were measured and the mean value multiplied by 0.078 to convert the pale band width to millimetres (Sharp *et al*, 1989). The cutoff point was determined by the following dichotomy as used by Coetzee (1986):

<0.099 mm *An. gambiae/An. arabiensis*

>0.1 mm *An. merus/An. quadriannulatus*

A comparison of the genetic species identification and a model of Coetzee (1986) was undertaken in order to determine the validity of using hind-leg banding patterns for the identification of members of the *An. gambiae* complex in Gokwe and Binga.

2.5.2 Ovary preservation and analyses

Ovaries in Christophers' Stage III-IV of gonadal development (commonly termed the half-gravid stage) (WHO, 1975) were pulled out with forceps and placed in freshly-prepared Carnoy's fixative (3:1, absolute alcohol: glacial acetic acid). Some of the freshly blood fed mosquitoes had to be kept in warm humid conditions for approximately 8 hours to allow the ovaries to develop to this critical stage. Each set of ovaries was separately preserved in Carnoy's fixative and appropriately coded. In the laboratory ovaries were prepared by the method outlined by Green (1972), and scored according to the nomenclature of Coluzzi *et al* (1979). The Wright's F statistic (Brown, 1970) cited by Bryan *et al* (1987), was used to test for deviation from the Hardy-Weinberg equilibrium:

$$F = (4ac - b^2)/(2a + b)(2c + b)$$

where a and c are absolute frequencies of the homozygous classes and b the absolute frequency of the heterozygotes. Significant ($P<0.05$) deviation from the Hardy-Weinberg predicted value is obtained when the absolute value of $F > 1.96/\sqrt{N}$, where N is the sample size. An F value greater than zero indicates deficiency of heterozygotes, while a value lower than zero indicates excess.

2.5.3 Blood-smears

Blood meals from freshly fed females captured in pyrethrum spray catches and from specimens whose ovaries had been cropped were smeared onto filter paper (Schleicher and Schnell^R N° 595), and allowed to dry. Appropriately coded filter papers were stored in polythene bags away from heat and ants. In the laboratory each blood smear was eluted in

70 μl distilled water and the Ouchterlony double diffusion precipitin technique (Peetom, 1963; Southwood, 1978) was used to determine the mammalian source of the blood meal, in particular the human host component, in order to determine the human blood indices (Garrett-Jones, 1964). Precipitin tests were carried out in 0.75% agar dissolved in phosphate buffer (pH 7.0) containing 0.02% sodium azide. Two millilitres of molten agar was poured onto a glass plate (6 x 6 cm) over which a perspex template with six rosettes, each rosette with one central and four peripheral wells, was laid. In each rosette three of the wells were filled with unknown eluted blood, and the fourth outer well was filled with known blood as positive control. Anti-serum to the latter was added to the central well and the gel reagents were allowed to run in humid conditions for 24 hours at ambient temperature. Nine blood eluates could be run per plate with one column of 3 X 4 well rosettes for human and the other for bovine anti-serum. Donkey anti-serum was tested on a second plate. A total of eighteen samples was the optimum that could be run on three gel plates. Blood smears were tested against human, bovine and donkey anti-sera. Precipitates were visualized on a light box after 24 hours which was extended to 48 hours when necessary at which point the template was removed before final reading. Positive results were seen as one or more distinct white lines against a pale background. Estimates of feeding preferences and probability of interrupted feeding were calculated using the methods described by Burkot *et al* (1988). Results were recorded on work sheets before being transferred to the computer data base.

2.5.4 Head-thorax segments

The head-thorax carcass was stored individually in either a gelatin capsule and preserved in liquid nitrogen, or in a vial and soaked in isopropanol. The aim was to obtain approximately equal quantities by both methods of preservation. Each specimen was appropriately coded. The specimens preserved in liquid nitrogen were available for species identification by either one or a combination of the following methods: electrophoresis (Mahon *et al*, 1976; Miles, 1980), polymerase chain reaction (PCR) (Paskewitz and Collins, 1990; Paskewitz *et al*, 1993; Bredenkamp and Sharp, 1993), DNA probes (eg., Gale and Crampton, 1987). In addition, sporozoite assays could be performed by either ELISA (eg., Wirtz *et al*, 1987; Robert *et al*, 1988; Beier *et al*, 1988), DNA probes (Barker *et al*, 1986) or by the

polymerase chain reaction (Tassanakajon *et al*, 1993).

2.5.5 WHO deltamethrin susceptibility test

The standard WHO insecticide susceptibility time-mortality test was conducted on deltamethrin papers with wild, freshly blood fed mosquitoes obtained from an exit trap from one of the control huts in Chireya and a human baited tent (Mpofu and Masendu, 1986) (Plate VII). The criteria of Davidson and Zahar (1973) were used to assess the levels of susceptibility. Survivors and casualties were appropriately coded and preserved for species identification.

2.5.6 24-hour holding period

Mosquitoes from exit traps were held in a cooler box with a moist environment for 24 hours. At the end of the holding period, mortalities were recorded according to the physiological condition of the females at the time of capture and the spray status of the exit trap-bearing hut. Survivors and casualties were appropriately coded and preserved for species identification.

2.5.7 Bioassay test

Twenty freshly blood fed, wild-caught *An. gambiae* complex mosquitoes from the exit trap on a control hut were released into bioassay cones mounted on the walls and thatch of sprayed and control huts. Mortalities were recorded after 30 minutes (WHO, 1975), while survivors were observed over a 24-hour holding period in order to determine the delayed effect of the insecticide. This exercise was carried out on two successive days using different sets of test houses on each occasion. Survivors and casualties were appropriately coded and preserved for species identification.

2.6 HUT PROFILES

Information on all huts involved in window trap and pyrethrum spray catches was obtained by a combination of measurement, observation and interview. Measurement were

carried out to get information on surface area of the wall and thatch, alternative resting surfaces, and various apertures available as entry and exit ports for mosquitoes. Direct questioning was used to obtain information on the spray status of the huts and the number, age and sex of the occupants. Observation on the manner the verges of the homestead were utilized was also made. All the physical aspects of the huts were obtained by measurement after getting the consent of the owners. Information on spray status and occupancy was, where applicable, cross checked with that obtained from the KAP exercise (see following page). The object of the hut study was to determine any relationship between house structure, location, occupants and spray status with mosquito catches and malaria cases associated with each house.

In addition, the temperature and relative humidity regimes in one representative house in each study site were monitored over the duration of the study period in order to make comparisons with those obtained outdoors. Automatic casella (T9420) (battery-powered) thermohygrographs were used for these measurements.

2.7 KNOWLEDGE, ATTITUDES AND PRACTICES (KAP) STUDY

One person from each of the window trap-bearing households was interviewed in order to assess the knowledge, attitudes, practices and nocturnal behaviour of the people relative to malaria transmission, treatment, prevention and control. This represented approximately one sixth of the average household population. The information was obtained using a questionnaire designed for the study (Appendix 2). The questionnaire was divided into three sections as follows: (1) background information, with emphasis on demographic data; (2) nocturnal distribution and activities by age- and sex-group of the household occupants, and (3) residual spraying and other antimalarial practices as previously investigated by Elliott (1968), Gardiner *et al* (1984) and Aikins *et al* (1994), but modified to suit this study. The interviewer recorded the appropriate responses using a pre-coded checklist suitable for data entry and analysis by SPSS/PC+. Interviews were conducted in *Shona* language by the author in Chireya, while a local health officer conducted the interviews in *Tonga* language in Siabuwa.

2.8 HOUSEHOLD MALARIA PREVALENCE SURVEYS

After obtaining consent from the head of the household, all individuals resident at the homesteads with a window trap-bearing hut were finger-pricked and thick blood smears were prepared. The slides were examined in the field by an experienced microscopist for the presence of *Plasmodium* parasites. The degree of parasitaemia was not specifically quantified. A random sample of slides was cross checked by another equally experienced microscopist. The prevalence surveys were conducted on the same households in February, March and April, in order to monitor the change in slide positivity rate in relation to mosquito densities, spray status of the huts and other characteristics of the occupants. The recorded ages of the occupants were categorized as follows: "infants" - for all children five years and below irrespective of sex, "boys", and "girls" - children six to fifteen years of age, while "men" and "women" referred to adults above fifteen years of age.

2.9 ANTHROPOMETRIC SURVEYS

The following examinations were performed on all residents sharing the same homestead with a window trap-bearing house: height, weight and triceps skinfolds. Details of the methods and findings will be reported elsewhere otherwise only pertinent issues will be discussed in this report.

2.10 DATA ANALYSIS

A data base was created with SPSS/DE at Blair Research Laboratory, Harare, but subsequent entries and management were made using DBase IV at the National Malaria Research Programme in Congella, Durban. Data were checked using SPSS/DE and analyzed using SAS (hut profiles) and SPSS/PC+ (Mascie-Taylor and Madsen, 1992). Graphics were done using Harvard Graphics 3.0.

Chapter 3
RESULTS

3.1 CLIMATE AND METEOROLOGICAL DATA

Rainfall at Chireya and Siabuwa during the 1993-94 rain season totalled 559 and 616 mm respectively. There was very little rain by March in either of the two study sites in 1994, consequently water availability became restricted to the main rivers: Ume in Chireya and Nyabusenga in Siabuwa. The average rainfall and relative humidity data for Chireya for the period 1990-1994 are shown below (Fig. 1). Maximum daily temperatures are frequently above 30°C during the wet season. The temperature profile for Chireya is not very different from that for Siabuwa shown in Fig. 2.

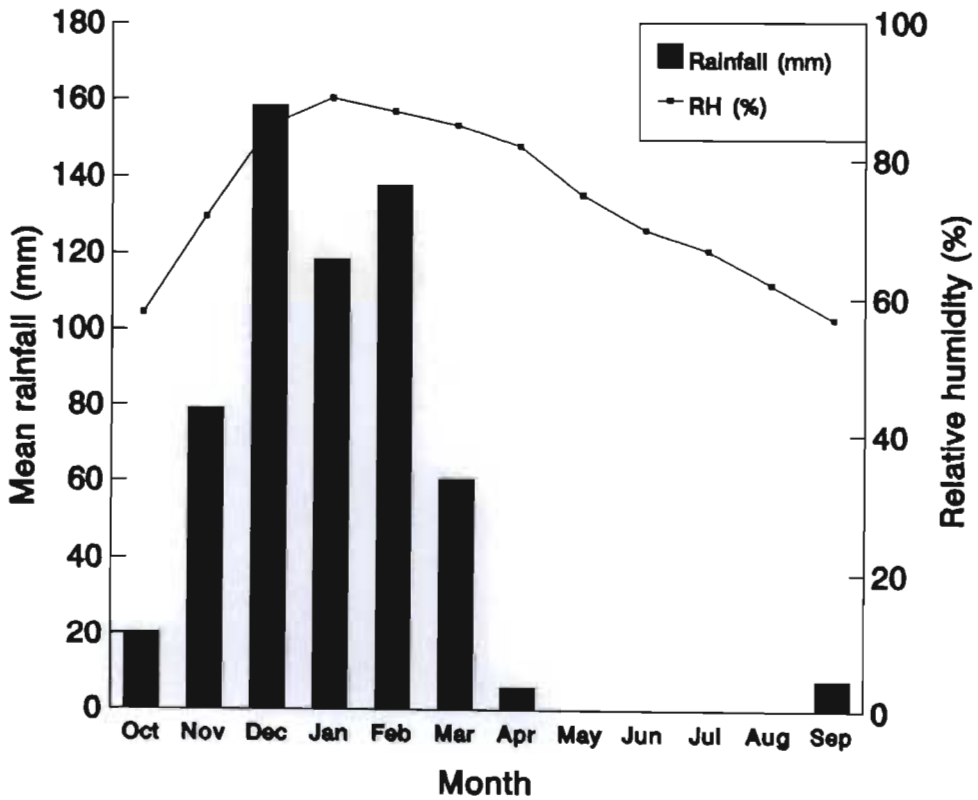

Fig. 1. Mean monthly rainfall and relative humidity for Chireya area for the period 1990-1994.

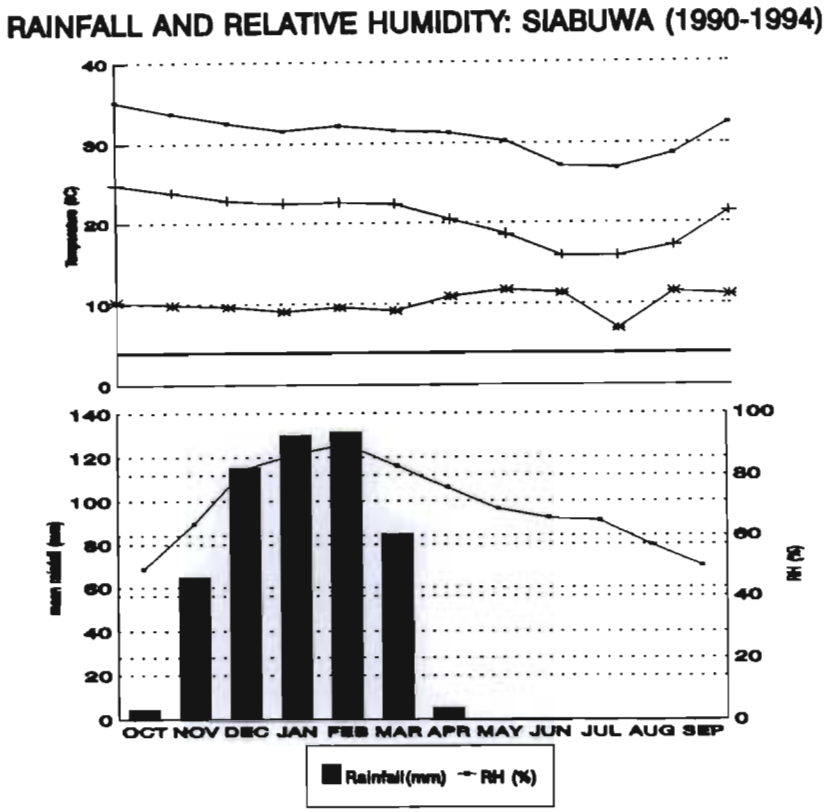

Fig. 2. Climatology in Siabuwa area, showing top) mean maximum, minimum and range temperatures, bottom) mean monthly rainfall and relative humidity

3.2 SALINITY OF WATER FROM BREEDING SITES

Water samples from Kurima stream and Bare salt pan were found to have 1.0 and 4.0 g chloride/l, equivalent to 5.26% and 21.05% sea water, respectively. Only the chloride concentration expressed as percent equivalent of seawater was used as a measure of the levels of salinity (WHO, 1975). Seawater is considered as approximately 3.5% or 35 g/l dissolved salts (chloride, carbonate, sulphate, bicarbonate, calcium and magnesium) (Rogo *et al*, 1985). Alternatively, sea water is defined as containing 31.7 g/l sodium chloride (NaCl), that is 100% sea water, while fresh water is considered as less than 0.05% NaCl (Njogu and Kinoti, 1971). The observed salinities of the water samples fall within the tolerance ranges of 0 - 5.7% and 14.25 - 108% sea water reported for *An. quadriannulatus* and *An. merus*, respectively (Le Sueur and Sharp, 1988).

3.3 ANOPHELINE FAUNA RECORDED

Seven anopheline species were identified: *An. arabiensis, An. quadriannulatus, An. merus, An. pretoriensis, An. funestus, An. rufipes* and *An. squamosus*. Only the first two species and *An. pretoriensis* were found in large numbers, and these were more abundant in Chireya than in Siabuwa. *An. funestus* was not identified to sibling species level but it is unlikely that the species is of epidemiological significance. *An. arabiensis* is the principal vector in all the southern African countries that have a residual spraying programme and this will be the focus of discussion in this report.

3.4 SPECIES IDENTIFICATION

3.4.1 Species identification techniques

Results of the PCR (polymerase chain reaction) and polytene chromosome identifications are shown in Table 2. The bulk of the specimens were processed using the PCR (n = 807), while only 64 ovaries and 21 specimens were identified by the polytene chromosome and the enzyme electrophoresis method respectively. Typical PCR results are shown in Fig. 3. The majority of the ovaries were under-developed, consequently limited correlation between PCR and the polytene chromosome techniques could be performed.

Table 2. Identification of the *Anopheles gambiae* complex specimens using chromosome and PCR techniques

Identification	**Chromosome**	**PCR**
An. arabiensis	49	499
An. quadriannulatus	14	186
An. merus	1	13
Negative	60	109
Not done	232	234

Table 3. Correlation between PCR and chromosome techniques for positively identified members of the *Anopheles gambiae* complex

		PCR		
		An. arabiensis	*An. quadriannulatus*	*An. merus*
Chromosome	*An. arabiensis*	37	5	-
	An. quadriannulatus	3	7	1
	An. merus	-	-	1

Typical electrophoresis results of PCR had additional bands to those used for diagnosis (Fig. 3). The level of error with PCR identifications was 7.5% and 41.6% for *An. arabiensis* and *An. quadriannulatus* respectively (Table 3). Out of 40 specimens identified as *An. arabiensis*, 37 (92.5%) were confirmed by the polytene chromosome technique while the remainder was identified as *An. quadriannulatus*. A disturbingly poor correlation was obtained for the specimens identified as *An. quadriannulatus* by PCR; only 58% correct identification was recorded (Table 3). There was also a mis-identification among the *An. merus*; one specimen identified by PCR as *An. merus* was confirmed by cytotaxonomy while the other was identified as *An. quadriannulatus* by the polytene chromosomes technique. Twenty two specimens were processed for identification using enzyme electrophoresis and 21 of these had the *An. arabiensis* R_f values: 100/100 for aspartate amino transferase (AAT, E.C.2.6.1.1.), 100/100 for superoxidase dismutase (SOD, E.C. 1.15.1.1.) and 95/95 for octanol dehydrogenase (ODH, E.C. 1.1.1.73). Only one specimen had an R_f value of 95/95 for AAT which is characteristic of *An. quadriannulatus*. No provision was made for these specimens to be cross-checked with PCR, and the ovaries for these were among the under-developed or those not done.

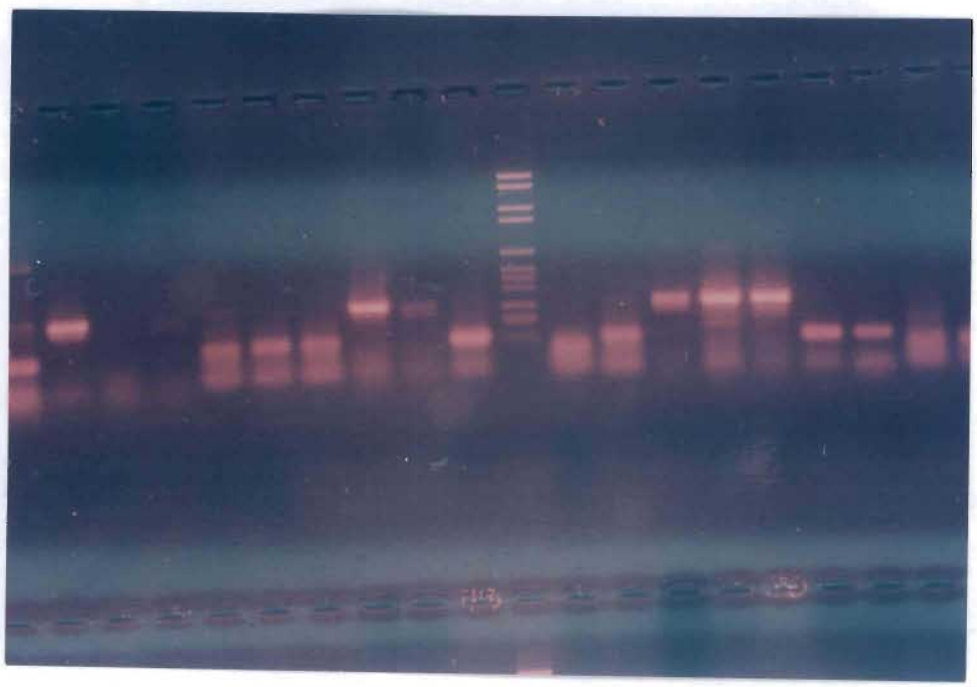

Fig. 3. Identification of *An. arabiensis*, and *An. quadriannulatus* by polymerase chain reaction from an ethidium-stained agarose gel. From left to right, lanes 11 and 20 contain the DNA standard marker and negative control, respectively. Lanes 2, 4, 8, 9, 14, 15, and 16: *An. arabiensis*. Lanes 1, 5, 6, 7, 10, 17 and 18: *An. quadriannulatus*. Lanes 3, 12 and 19: negative.

3.4.2 Leg-band measurements

The pale bands at the junction of tarsomere 3/4 were measured for 220 specimens and these were identified by PCR as *An. arabiensis* (n = 143), *An. quadriannulatus* (n = 73) and *An. merus* (n = 4). The pale leg-band widths were categorized using the Coetzee (1986) dichotomy thus: $\leq$ 0.099 mm for *An. gambiae/An. arabiensis* and $>$ 0.1 mm for *An. merus/An. quadriannulatus*. The percentage correct identifications obtained are summarized in Table 4. More than 80% of *An. arabiensis* from Chireya were correctly identified, regardless of trapping technique, while in Siabuwa 80% correct identification was obtained among exit trap specimens compared with only 50% among pit shelter specimens. The percentage correct identification of *An. quadriannulatus* from Chireya was 66% and 73% among exit trap and pit shelter collected specimens respectively. Seventy eight percent of *An. quadriannulatus* collected from pit shelters from Siabuwa were correctly identified. Two of the four *An. merus* specimens which had leg-bands measured were correctly identified, while one each from the pits and indoor resting were wrongly identified using Coetzee's (1986) criteria. Mosquitoes obtained by the pyrethrum spray technique were generally not well represented in the samples as most had missing hind legs. Two of the four *An. merus* specimens which had leg-bands measured were correctly identified, while one each from the pits and indoor resting collections were wrongly identified using the Coetzee (1986) criteria.

The distribution of the leg-band measurements for the species common to both study localities peaked at 0.09 mm and 0.11 mm for *An. arabiensis* and *An. quadriannulatus* respectively (Figs. 4 and 5). The frequency distribution of *An. arabiensis* from window traps and pit shelters, the two main sources at Chireya, showed different peaks of 0.08 mm and 0.09 mm respectively. The leg band measurements for these two mosquito sources were, however, similar in spread. Differences in the leg band measurements were observed among *An. arabiensis* and among *An. quadriannulatus* from the two areas. This area difference was highly significant for *An. quadriannulatus* ($P < 0.000$ by a Mann-Whitney test), but was not for *An. arabiensis* ($P > 0.5$).

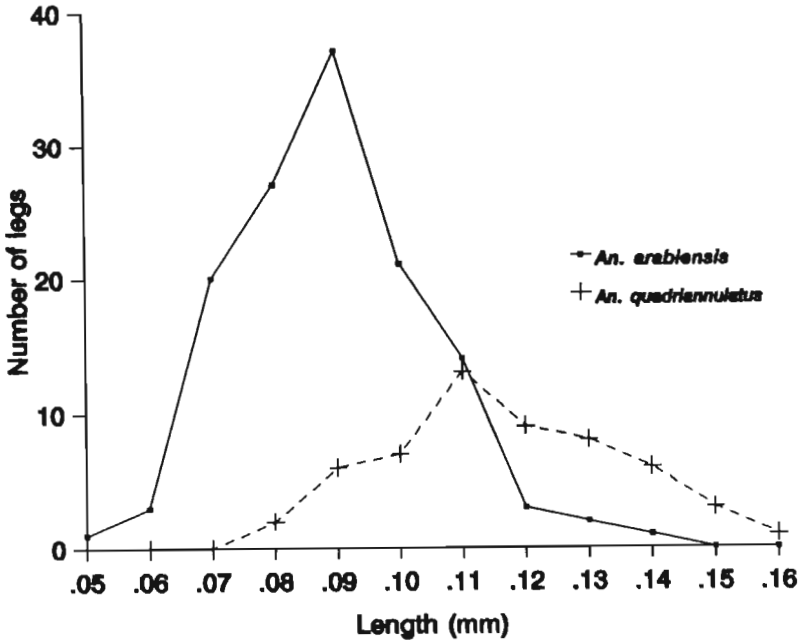

Fig. 4. Frequency distribution of the leg-banding measurements of *An. arabiensis* and *An. quadriannulatus* in Chireya

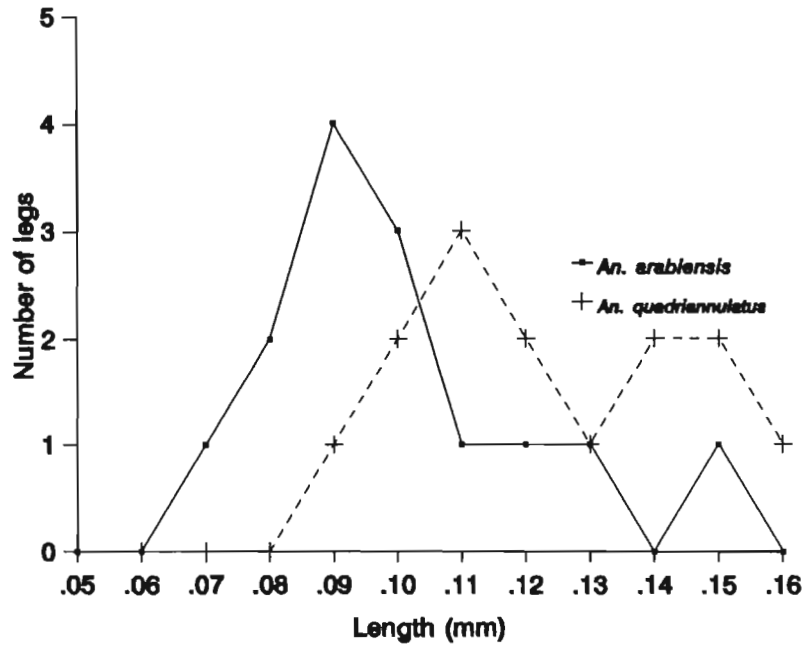

Fig. 5. Frequency distribution of the leg-banding measurements of *An. arabiensis* and *An. quadriannulatus* in Siabuwa

Table 4. Percentage correct identification of *Anopheles gambiae* complex species using the Coetzee (1986) method

Species	Area	Trapping technique	N°. of adults	% correct identification
An. arabiensis	Chireya	Exit trap	67	83.5
		Pit shelter	59	84.7
		Knockdown	3	100.0
		Total	129	84.5
	Siabuwa	Exit trap	10	80.0
		Pit shelter	4	50.0
		Total	14	71.4
An. quadriannulatus	Chireya	Exit trap	3	66.6
		Pit shelter	52	73.1
		Knockdown	1	100.0
		Total	56	73.2
	Siabuwa	Pit shelter	17	82.4
An. merus	Chireya	Exit trap	1	0.0
		Pit shelter	2	100.0
		Knockdown	1	0.0
		Total	4	50.0

3.5 SPECIES COMPOSITION

3.5.1 *An. gambiae* complex

Table 5 shows the numbers of species caught by the different sampling techniques in the two study areas. Three members of the *An. gambiae* Giles complex were found in Chireya, viz, *An. arabiensis* Patton, *An. quadriannulatus* Theobald and *An. merus* Dönitz. In Siabuwa only *An. arabiensis* and *An. quadriannulatus* were found. *An. arabiensis* was the predominant species irrespective of the sampling technique used in Chireya.

Table 5. Percentages of *Anopheles arabiensis*, *An. quadriannulatus* and *An. merus* collected by different sampling techniques identified by PCR from Chireya and Siabuwa

Sampling method	Study area	n	*An. arabiensis*	*An. quadriannulatus*	*An. merus*
ET	Siabuwa	10	100	0	0
	Chireya	245	95.1	3.3	1.6
PSC	Siabuwa	15	60.0	40.0	0
	Chireya	40	80.0	17.5	2.5
PT	Siabuwa	25	20.0	80.0	0
	Chireya	359	57.4	40.4	2.2

Where ET = exit trap; PSC = pyrethrum spray catches; and PT = pit shelter

The overall mean number of indoor resting female *An. arabiensis*/hut/day and leaving per exit trap/day in Chireya were greater than those in Siabuwa (Table 6). In Chireya mosquitoes were caught resting in and leaving houses irrespective of the presence or absence of deltamethrin. In both indoor resting and exit window trap collections the mean number of mosquitoes was greater in control houses than in the sprayed ones. One window trap (on hut 19) dominated the exit trap collections from Chireya as it accounted for 89.7% of the total *An. gambiae s.l.* collected by this method. Eighty percent of the mosquito specimens from this trap were identified as *An. arabiensis* using PCR. *An. arabiensis* was the dominant member of the *An. gambiae* complex from the day-time indoor resting catches. In the pit shelter collections the proportion of *An. arabiensis* maintained its dominance, although the

proportion of *An. quadriannulatus* was higher, accounting for 40.4% (n = 359) of the total (Table 5).

Only two members of the *An. gambiae* complex were identified in Siabuwa, viz. *An. arabiensis* and *An. quadriannulatus* (Table 5). *An. arabiensis* was found among indoor resting and in exit window trap collections as well as in pit shelter collections. It was the only species from exit window trap catches, and formed 60% of the indoor resting catch and 20% of the pit shelter collections. The overall mean number of *An. arabiensis* caught resting indoors in Siabuwa was higher than that caught in exit window traps (Table 6) but the number caught overall was extremely low. In contrast, the overall exit trap density in Chireya was greater than the overall indoor resting density. Day-time indoor resting *An. arabiensis* from Siabuwa were only recorded from unsprayed and repellent soap-equipped houses whereas none were recorded from the sprayed houses (n = 7) and from the one sprayed-but-replastered house. *An. arabiensis* were found in exit traps only from control and repellent soap-equipped houses; none were recorded from the sprayed houses.

3.5.2 Temporal variation

Differences in *An. arabiensis* densities over the sampling period are illustrated in Fig. 6. Rainfall patterns from October 1993 to April 1994 appear to have been the factor determining low densities but apparently had a diametrically opposite effect on the two most common members of the *An. gambiae* complex, viz, *An. arabiensis* and *An. quadriannulatus* in Chireya. While *An. arabiensis* densities declined following the cessation of the rains in March, *An. quadriannulatus* densities actually rose from February to March.

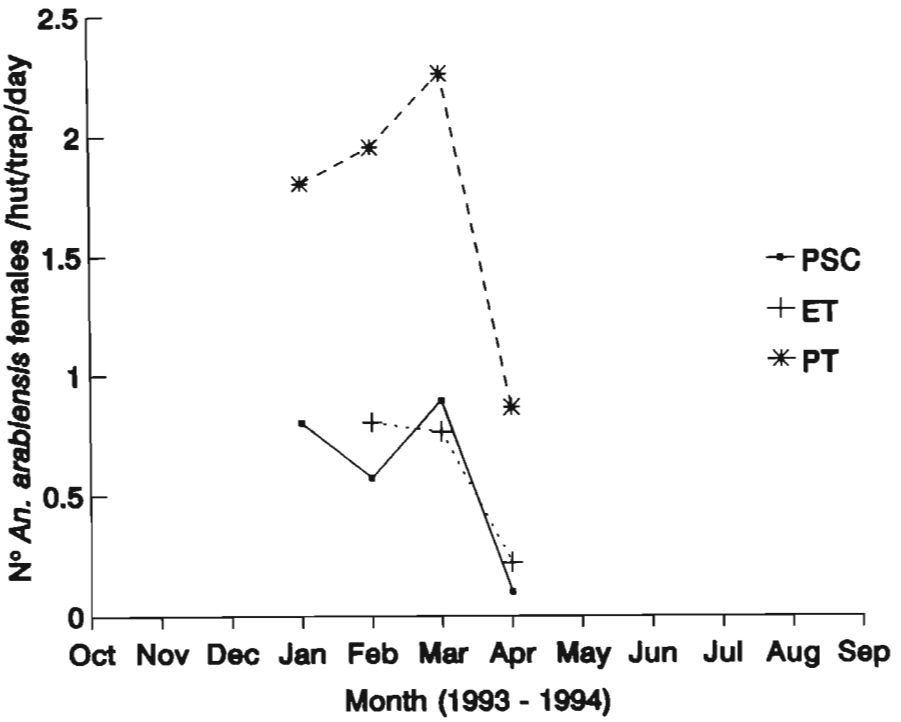

Fig. 6. Mean monthly female *Anopheles arabiensis* catch per trapping technique in Chireya. PSC = pyrethrum spray catches; ET = exit window traps; PT = pit shelters.

3.5.3 Spatial variation

The location of the catch stations in Chireya and Siabuwa are shown in the sketch maps of the study areas (Appendix 2a and 2b). Variation in density indices was observed between houses on the one hand and pit shelters on the other in both study areas (Tables 6 and 7). The highest daytime indoor resting density index was 2.33 female *An. arabiensis* per hut per day which was obtained from two houses in Taka village; one of these (house 6) was sprayed and the other (house 19) was not (Appendix 2a). The limited sample size of unsprayed houses (n = 4) precludes any statistical comparison of yields between sprayed and control houses. An outstanding exit trap density index of 9.15 female *An. arabiensis* per hut per day was obtained from house 19, otherwise the rest of the houses yielded indices ranging from 0 to 0.4 females *An. arabiensis* per hut per day. As was the case with indoor resting density indices, exit trap densities did not appear to be affected by the spray status of the houses concerned. A swamp 30m from house 19 was identified as the breeding site from

which mosquitoes found in houses 3 and 19, and pit 1 most probably originated.

Pit 1 was only functional in January; it collapsed following heavy rains in February and could not be rehabilitated. Outdoor daytime resting indices ranged from 0.16 to 3.28 female *An. arabiensis* per pit per day (Table 7). Pit shelters located where cattle were not present yielded poor catches. Pits 2 and 5, which were adjacent to cattle kraals, were the most productive in Chireya. In Siabuwa pit shelters were generally not very productive. The proximity and numbers of cattle and goats associated with these two pits also contributed to the yield (see also Table 15). Water availability was, however, an overriding factor; there were hardly any breeding sites near the least productive pits, N^0 3 and 4 in Chireya.

Table 6. Distribution density indices of female *Anopheles arabiensis* from indoor resting and exit window trap collections in Chireya and Siabuwa

	Area					
	Chireya			Siabuwa		
Hut N°	**Spray status**	**N°/hut/day (PSC)**	**N°/trap/day (ET)**	**Spray status**	**N°/hut/day (PSC)**	**N°/trap/day (ET)**
1	yes	0.33	0.5	no	0	0
2	yes	0	0	s/r*	0	0.06
3	no	0	0.4	no	0	0
4	yes	1	0.1	yes	0	0
5	yes	0.33	0.15	Mosbar	0	0
6	yes	2.33	0.05	Mosbar	0.33	0.06
7	yes	0	0.2	Mosbar	0	0
8	yes	0	0	Mosbar	0	0.06
9	yes	0.33	0	Mosbar	2	0.19
10	yes	0.33	0	no	0	0.25
11	yes	0.66	0	yes	0	0
12	no	1	0.05	yes	0	0
13	yes	0	0.2	yes	0	0
14	yes	0	0.35	yes	0	0
15	yes	0	0.1	no	0.67	0
16	no	0.66	0	no	0	0
17	yes	0	0	yes	0	0
18	yes	0	0.1	Mosbar	0	0
19	no	2.33	9.15	no	0	0
20	-	-	-	yes	0	0
Mean ± SD	Sprayed	0.35±0.62	0.12±0.15	Sprayed	nil	nil
	Control	0.99±0.98	2.40±4.50	Control	0.11±0.27	0.04±0.10
	Mosbar	n/a	n/a	Mosbar	0.39±0.80	0.05±0.07
	Overall	**0.49±0.49**	**0.60±2.08**	**Overall**	**0.15±0.46**	**0.03±0.07**

* s/r = sprayed but replastered

Table 7. Distribution of density indices of female *Anopheles arabiensis*/pit/day from Chireya and Siabuwa

	Pit N°					
Area	1	2	3	4	5	6
Chireya	2	2.76	0.16	0.64	3.28	1.12
Siabuwa	0	0.07	0.2	0.07	-	-

3.6 CHROMOSOMAL POLYMORPHISM

Two inversions, 2Rb and 3Ra, were found floating in *An. arabiensis*. Fifty percent of the twenty specimens scored for the 2Rb polymorphism were heterozygotes while the standard and inverted homozygotes constituted 15% and 35% respectively. The twenty specimens which were scored were all from Chireya area. The frequency of the inverted arrangement in the 2R chromosome was 60%. There were no significant differences in the distribution of the above karyotypes between the pit and exit trap collections. Two of the three standard homozygotes specimens were from the pit shelter collections, and one was from the exit trap collection. Sixty percent of the heterozygotes (n = 10) were from pit shelter collections with the remainder coming from exit trap collections. Three and four of the seven inverted homozygotes were from the exit trap and pit shelter collections respectively. Half of the eight exit trap specimens were heterozygotes, with the standard and inverted homozygotes constituting 12.5% and 37.5% respectively. Heterozygotes also dominated the pit shelter collections (50%) while the standard and inverted homozygotes were 16.7% and 33.3% respectively. There were no differences between the three karyotypes in their host choice (Table 8a). The human fed component had 25%, 37.5% and 37.5% standard homozygote, heterozygote and inverted homozygote, respectively. However, none of the standard homozygotes were found to have fed on animals other than humans. There were no differences between exit trap collections and pit shelter resting with respect to the 2Rb polymorphism (Table 8b). All the nine *An. arabiensis* specimens which were scored for 2Rb were found to have the pale leg band between tarsomere 3 and 4 measuring <0.099 mm. Specimens scored for 3Ra polymorphism were too few to permit an analysis of the association of karyotypes with catch station or host preferences. A considerable number of specimens could not be scored as they were under developed.

The Wright's F statistic was -0.0416 which indicates an excess of heterozygotes. However, since the absolute value of F is less than $1.96/\sqrt{N}$, (N = 20), this indicates no

significant departure from what would be expected in a Hardy-Weinberg equilibrium. Thus a panmictic population with random mating between carriers of the different 2Rb karyotypes exists in Chireya. Ideally the Wright's F statistic should have been determined separately for the mosquitoes from different sampling methods. The above F value reflects the situation for specimens pooled from exit traps and pit traps. Xf/+ polymorphism was observed in the *An. quadriannulatus* from both Chireya and Siabuwa.

Table 8a. Homogeneity test for subsamples of *Anopheles arabiensis* found with human versus animal blood meals

	2b/b	2b/+	2+/+
Human	4 (3.3)	4 (4.4)	2 (2.3)
Animal	1 (1.3)	2 (1.3)	2 (2.4)

$X^2 = 7.7$, P = 0.652

Table 8b. Homogeneity test for subsamples of *Anopheles arabiensis* caught in exit trap and pit shelters

	2b/b	2b/+	2+/+
Exit trap	4 (3.7)	6 (4.9)	1 (2.4)
Pit shelter	5 (5.3)	6 (7.1)	5 (3.6)

$X^2 = 1.9$, P = 0.383

3.7 FEEDING BEHAVIOUR

3.7.1. Blood meal sources

The precipitin tests managed to identify 86.1 % of the blood after less than 24 hours of digestion. Three hundred and nine blood fed *An. gambiae s.l.* were analyzed from the two study areas; 45 of these were from daytime indoor resting sites, 130 from outdoor resting sites and 134 from exit window trap collections. Most (92%) of the blood fed specimens were from Chireya area. The 309 blood fed *An. gambiae s.l.* consisted of 72.5% *An. arabiensis*, with the remainder being *An. quadriannulatus* (25.5%) and *An. merus* (1.9%), respectively. The *An. arabiensis* component from outdoor resting collections was low (44.6%) compared with 75.6% from indoor resting and 98.5% from exit trap collections. Over 87% (n=181) of *An. arabiensis* from Chireya responded to at least one antiserum while 11.6% had mixed blood meals (Table 9). The principal hosts among indoor resting and exit window trap collections in both Chireya and Siabuwa were humans, whereas cattle were the preferred hosts among outdoor resting collections. In contrast *An. arabiensis* from Siabuwa had 37.5%

responding to at least one antiserum and 62.5% had mixed blood meals. A third of the blood meals from Siabuwa could not be identified as either human, cattle or donkey. The proportion of unassigned blood meals from Chireya was only 14.6%.

3.7.2 Mixed blood meals

Only a few mixed feeds among *An. arabiensis* were observed. These involved two outdoor resting specimens which had mixed human/bovine blood. Most blood meals among *An. quadriannulatus* were single host type, with only one human/bovine case from the pit shelters. Interestingly there were no mixed bovine/donkey cases among the mixed blood meals. This suggests animals exhibit little avoidance behaviour when they are bitten, and this would result in mosquitoes feeding to repletion on any one animal. The observed mixed human/donkey blood meals shown in Table 9 are suspected to have been the result of cross-reactivity between human anti-serum to whole donkey serum. In other words it is unlikely that there were true mixed human/donkey blood meals.

3.7.3 Feeding preferences

High human blood indices (HBI) were observed among *An. arabiensis* from indoor resting (HBI = 0.82) and exit trap (HBI = 0.98) collections in Chireya (Table 11). The limited sample size of the *An. arabiensis* from Siabuwa precludes any comparison, even though a high HBI of 0.71 was recorded for the seven vector mosquitoes from indoor resting collections. The observed 0.30 HBI in outdoor resting *An. arabiensis* from Chireya suggests either outdoor biting followed by outdoor resting or indoor biting followed by outdoor resting. Gillies (1956) has termed the latter situation of postprandial exophily 'type A' deliberate exophily. The absence of any human feds among both outdoor and indoor resting *An. quadriannulatus* supports the generally accepted principle that this is a zoophilic species even though some do bite humans (Gillies and Coetzee, 1987). Humans outnumbered domestic animals in Chireya (Table 12). Besides the major hosts listed in Table 12, other animals that were encountered included two pigs (near pit 3), dogs at various homesteads, six ducks (Hut 2), over a dozen turkeys (Hut 16), chickens and the ubiquitous rodents and the inevitable avian and reptilian fauna. No animals shared dwellings with humans as a matter of course.

Feeding indices (FI) were estimated from the formula:

$$FI = (Ne/Ne')/(Ef/Ef')$$

where Ne and Ne' are the observed number of mosquito blood meals on hosts 1 and 2; Ef and Ef' are the number of hosts 1 and 2 present in the village (Burkot *et al*, 1988). Overall, *An. arabiensis* preferred humans to cattle, and cattle to donkeys whereas *An. quadriannulatus* preferred cattle to donkeys to humans (Table 10). The order of host preference among *An. arabiensis* from outdoor-resting collections was cattle > donkey > humans (Table 13).

The fact that there was a greater number of "other" blood meals among the zoophilic and exophilic *An. quadriannulatus* and not among *An. arabiensis* suggests an animal such as a goat or sheep was being fed on. Goats and a limited number of sheep were part of the domestic animals kept in the two study areas, and these were more abundant in Siabuwa than in Chireya (Table 12).

3.7.4 Probability of interrupted feeding

Mixed meals containing human blood were used in estimating the probability of interruption of a blood meal, I_H (Burkot *et al*, 1988). It was assumed the probability of interruption for a human and a nonhuman was the same ($I_H = I_N$). The estimated probability of an *An. arabiensis* feed being interrupted ranged from 0.17 among indoor resting to 0.86 in exit trap collections (Table 14). The host selection factor, Q (Burkot *et al*, 1988), was high (0.92) among exit trap specimens and comparatively low (0.27) in outdoor resting collections (Table 14). The host selection factor is derived from the proportion of human only meals plus half the proportion of mixed human meals (Burkot *et al*, 1988).

3.7.5 Effect of presence of animals on feeding on humans

An. arabiensis collected from those pit shelters close to animal shelters tended to have more cattle blood meals than from any other host (Table 15). In the absence of animals, there was a shift to human blood meals. More human-fed *An. arabiensis* were collected from those houses with no animals, especially cattle, in proximity to homesteads (Table 16). This supports the concept of zooprophylaxis, and further suggests that not all animals are suitable for this control option.

Table 9. Blood meal sources of *Anopheles arabiensis* from indoor- and outdoor-resting and exit window trap collections in Chireya and Siabuwa

Host	Area					
	Chireya			Siabuwa		
	PSC[a]	ET[b]	PT[c]	PSC	ET	PT
Single host						
Human	15	95	11	1	0	0
Donkey	1	2	5	2	0	0
Bovine	3	0	27	0	0	0
Mixed blood meals						
Human/bovine	0	0	2	0	0	0
Human/donkey	4*	14*	1*	4*	1*	0
"Other"	4	16	11	0	3	1
Total identified	23	112	46	7	1	0
Total tested	27	128	57	7	4	1

[a] = pyrethrum spray catches; [b] = exit window trap; [c] = pit shelter

* Cross-reactivity is strongly suspected between human anti-serum to whole donkey serum, see Section 3.7.2

Table 10. Blood meal sources of *Anopheles quadriannulatus* from indoor- and outdoor-resting and exit window trap collections in Chireya and Siabuwa

Host	Area					
	Chireya			Siabuwa		
	PSC[a]	ET[b]	PT[c]	PSC	ET	PT
Single host						
Human	0	2[#]	0	0	0	0
Donkey	0	0	6	0	0	0
Bovine	4	0	24	3	0	2
Mixed blood meals						
Human/bovine	0	0	1	0	0	0
Human/donkey	0	0	0	0	0	0
"Other"	1	0	29	1	0	6
Total identified	4	2	31	3	0	2
Total tested	5	2	60	4	0	8

[a] = pyrethrum spray catches; [b] = exit window trap; [c] = pit shelter

[#] these two specimens were identified as *An. arabiensis* using chromosomes

Table 11. Human blood indices (HBI) for members of the *Anopheles gambiae* complex for indoor and outdoor resting and exit window trap collections

Area	Mosquito source	*An. arabiensis* HBI (N)	*An. quadriannulatus* HBI (N)	*An. merus* HBI (N)
Chireya	Indoor	0.82 (23)	-	-
	Outdoor	0.30 (46)	0.03 (31)	-
	Exit trap	0.98 (112)	1.0 (2*)	1.0 (1)
Siabuwa	Indoor	0.71 (7)	-	-
	Outdoor	-	-	-
	Exit trap	1 (1)	-	-

HBI = total N° of blood meals containing human blood/total N° of blood meals positively identified.
N = total N° of blood meals identified.
* these two specimens were identified as *An. arabiensis* by chromosomes.

Table 12. Relative proportions of human and domestic animals in Chireya and Siabuwa

Area	N° of households surveyed	N° of hosts			
		Humans	cattle	donkeys	goats
Chireya	19	132	40	14	42
Siabuwa	18	95	34	8	171

Table 13. Feeding indices (FI) for *Anopheles arabiensis* from Chireya

Mosquito source	Host 1/host 2	FI*	Comment
Indoor resting	Human/cattle	1.92	humans preferred to cattle
	Human/donkey	1.57	humans preferred to donkeys
	Donkey/cattle	0.95	cattle preferred to donkeys
Outdoor resting	Human/cattle	0.14	cattle preferred to humans
	Human/donkey	0.28	donkeys preferred to humans
	Donkey/cattle	0.49	cattle preferred to donkeys
Exit window trap	Human/donkey	5.04	humans preferred to donkeys
Overall	Human/cattle	1.16	humans preferred to cattle
	Human/donkey	1.63	humans preferred to donkeys
	Donkey/cattle	0.71	cattle preferred to donkeys

*FI = feeding index = (Ne/Ne')/(Ef/Ef'), where Ne and Ne' are the observed number of mosquito blood meals on hosts 1 and 2, and Ef and Ef' are the number of hosts 1 and 2 present in the area (After Burkot *et al*, 1988).

Table 14. Calculations of the probability of *Anopheles arabiensis* being interrupted during blood feeding

Mosquito source	N° of human meals	N° of mixed human meals	Total meals	Predicted Proportion mixed	Q*	I_H**
Indoor	15	4	23	0.1739	0.739	0.451
Exit trap	95	15	112	0.1339	0.915	0.861
Outdoor	11	3	46	0.0652	0.272	0.165

* Q = host selection factor; ** $I_H = I_N$ = the predicted probability of a feeding being interrupted.

Table 15. Pit shelter environment and sources of blood meals among *Anopheles arabiensis* in Chireya

Pit N°	N° of available hosts (Pit-shelter distance in m)				Blood meal source					
	Humans	Cattle	Donkeys	Goats	Hum	Bov	Don	Hum/bov	Hum/don	other
1	5(40)	-	-	-	1	-	-	-	-	2
2	5(63)	7(18)	-	16(18)	2	10	1	-	1	25
3	3(38)	-	3(22)		0	-	1	-	-	-
4	3(39)	-	3(32)	7(32)	1	-	-	1	-	3
5	7(65)	2(94)+5(116)	-	-	4	8	2	1	-	27
6	5(90)	23(124)	-	-	3	9	1	-	-	7

Table 16. Influence of animals near homesteads on host choice in *Anopheles arabiensis* collected in exit traps in Chireya

Hut N°	N° of occupants	N° of animals and distance from hut			Blood meal source					Human:animal fed ratio*
		<25m	25-50m	>50m	Human	Bovine	Donkey	Human/donkey	Other	
1	6	1	3	-	2	-	-	1	-	3:1
2	3	5	-	-	-	-	-	-	-	-
3	2	-	-	-	2	-	-	-	1	2:1
4	4	-	-	-	2	-	-	2	1	4:3
5	3	5	-	-	1	-	-	-	1	1:1
6	4	7	-	-	2	2	-	1	2	3:5
7	2	1	-	23	-	-	-	-	-	-
8	1	-	8	-	-	-	-	-	-	-
9	1	-	-	-	-	-	1	-	-	0:1
10	7	2	8	-	-	1	-	-	-	0:1
11	8	-	6	-	-	-	-	-	2	0:2
12	3	5	-	-	1	-	-	1	1	2:2
13	3	2	-	-	-	-	-	-	1	0:1
14	4	-	-	23	3	-	-	-	1	3:1
15	3	1	59	-	-	-	-	-	-	-
16	4	20	9	-	1	-	-	-	-	1:0
17	4	-	-	-	-	-	-	-	-	-
18	5	-	-	-	-	-	-	-	-	-
19	2	9	-	-	93	-	2	14	10	107:26

3.8 RESTING BEHAVIOUR

Daytime resting densities indoors were estimated from spray catches (PSC) as the hand catch method proved unproductive given the low vector numbers. Assuming a gonotrophic cycle every 2 to 3 days, a 1:1 fed:gravid ratio would be expected, yet at least six times more feds than gravids were observed in the present study. Table 17 illustrates the proportions of the average abdominal conditions observed in Chireya and Siabuwa.

Table 17. The densities of daytime house resting female mosquitoes (number/house) showing the predominance of blood-feds in all houses

		Abdominal appearance		
Area	**Spray status**	**unfed**	**freshly fed**	**gravid**
Chireya	sprayed (*n*=15)	0	2.7	0.4
	unsprayed (*n*=4)	0.8	5.8	0.5
Siabuwa	sprayed* (*n*=8)	0	0.1	0
	repellant (*n*=6)	0.2	1.0	
	unsprayed (*n*=6)	0.2	1.3	0.2

* The one re-plastered house is included in this group. (n = number of houses)

The presence of unfed females in unsprayed houses and not in sprayed houses probably indicates their inability to survive the toxic effects of the residual insecticide in sprayed houses. The paucity of gravid females can partly be attributed to exophily and also to mortalities suffered by mosquitoes after contact with insecticides, both in houses and for agricultural pest control as previously observed by Dukeen and Omer (1986).

Mosquitoes which leave houses seek natural outdoor resting places. It is widely accepted that these mosquitoes are difficult to find because they are widely distributed (Service, 1976). Grass of the savannah was found to be the diurnal resting place of anophelines in Eastern Colombia (Muirhead-Thomson, 1968). Resting places consisting of vegetation provide less protection from wind, sunlight and desiccation than tree and rodent holes. The mosquitoes collected from pit shelters constitute only part of the outdoor resting component as these artificial resting places compete with natural resting sites.

3.9 SURVIVAL OF EXIT TRAP CATCHES AFTER 24-HOUR HOLDING PERIOD

The delayed mortalities obtained from mosquitoes which were kept for a 24-hour holding period are listed in Table 18 according to abdominal appearance. The density of catches was low in exit traps on sprayed houses, where only 26% of the females collected were blood-fed as compared to 96%

blood-fed females collected from unsprayed houses. Hundred percent mortalities were observed for unfed females collected from both sprayed and unsprayed houses. There was greater survival among freshly blood-fed females especially among those from unsprayed houses. A crude feeder-survivor index (FSI) of 93% was estimated from the expression given by Hudson and Esozid (1971):

$$\text{FSI in period } n \text{ after spraying} = 100\ (1-T_nC_0/T_0C_n)$$

where: T_0 = number of feeder-survivors in treated hut before spraying;

C_0 = number of feeder-survivors in control hut before treatment of sprayed hut;

T_n = number of feeder-survivors in treated hut in period *n*;

C_n = number of feeder-survivors in control hut in period *n*.

T_0 and C_0 were not ascertained but were assumed equal in the above estimate.

The number of mosquitoes found dead in the exit traps was low and this was observed only on sprayed houses.

Table 18. Exit window trap collections and survival of *Anopheles gambiae s.l.* from deltamethrin-sprayed and control huts in Chireya over four observation days (20-23 February, 1994)

Hut spray status	Abdominal appearance of females held			% mortalities of females held		
	unfed	blood fed	gravid	unfed	blood fed	gravid
Sprayed (8)	24	9	1	100	33.3	0
Not sprayed (2)	4	105	0	100	15.2	0

Number in parenthesis denotes the number of huts from which mosquitoes were obtained.

3.10 INSECTICIDE SUSCEPTIBILITY TEST

A 100% mortality was obtained after 1-hour contact exposure to 0.025% deltamethrin impregnated filter papers and since there was less than 5% mortality in controls, it was not necessary to correct these results as suggested by Abbott (1987). The *An. gambiae* complex population assessed was therefore susceptible to deltamethrin following the criteria of Davidson and Zahar (1973). The LT_{50} and LT_{90} values were estimated from exposure time - mortality log probit regression as 30 and 40 minutes respectively (Murahwa *et al*, 1994).

3.11 BIOASSAY TEST

Results from bioassay tests on walls and thatch of sprayed and control houses in Chireya were pooled, and the corrected mortalities after 24 hours holding period were 88.9% (n = 20) and 88.5% (n = 23) for wall and thatch respectively. There were no mortalities after 30 minutes' exposure on the walls of both sprayed and control house, but at least 43.5% (n = 23) mortality was recorded from the thatch in the sprayed houses. The bioassays were conducted 9 weeks after spraying. This shows that an active deposit of deltamethrin sufficient to kill mosquitoes was available for at least two months after spraying.

3.12 HUT PROFILES

In both Chireya and Siabuwa pole-and-mud and grass thatch were the most common materials (71.8%) used in the construction of the houses. Brick and thatch or brick and corrugated iron or asbestos were used to a small extent. Most of the houses were rectangular (76.9%), with the remainder retaining the traditional circular shape. The average wall height was 1.8m (range: 1.3 - 2.5m). Average openings on the houses included window gaps of 0.19m^2 (range: 0 - 0.6m), eave gaps of 0.2m^2 (0 - 0.4m) and door gaps of 0.05m^2 (range: 0 - 0.4m). The average sprayable surface in the rectangular and round houses was 53.3 and 34.3m^2, respectively; the wall and roof components for the two study areas are shown in Table 19. Sprayable surfaces in the form of undersurface of furniture, eaves of huts are not included in the summaries in the table, although these were also sprayed. Others structures

like sheds and granaries had both their interiors and undersurfaces sprayed.

Table 19. Sprayable surface area (m^2) in Chireya and Siabuwa

Area	Round huts			Rectangular houses		
	roof	wall	total	roof	wall	total
Chireya	18.1	23.7	41.8	21.1	25.6	46.7
Siabuwa	12.1	16.7	28.8	23.3	37.5	60.8
All areas	14.6	19.7	34.3	22.2	31.1	53.3

The spraying coverage as defined by Molineaux and Gramiccia (1980) thus: '...percentage of houses completely sprayed among those existing at the time of spraying...', was estimated to be 78.9% and 53.3% for Chireya and Siabuwa respectively. The houses which were deliberately left unsprayed in Siabuwa because of the planned use of repellent soap were not included in this analysis.

Numbers of *An. gambiae* complex caught from exit window traps ranged from 0 to 764 (mean: 22), while daytime resting catches ranged from 0 to 9 (mean: 2). The average occupancy was 6 people/house with women being the most common group for each house. Three quarters of the verges were classified as wasteland to distinguish them from cultivated verges that were fields with either maize or cotton.

The number of *An. arabiensis* from indoor-resting collections was not affected by house attributes such as shape, material, size, verge-use, the number or sex of occupants, and window-, eave- and door-gaps. The spray status of the house did not appear to affect the indoor resting densities in Chireya. However, in Siabuwa indoor resting collections were only associated with houses that were not sprayed, with or without mosquito repellant soap. This suggests that insecticide either irritated or had a lethal effect on mosquitoes in sprayed houses. This is supported by exit trap densities that are higher in sprayed than in unsprayed houses. It was also observed that exit window traps on the western side of houses yielded significantly higher densities than those on the eastern side ($X^2 = 49.12$; $P > 0.001$) after pooling data from the two study sites.

3.13 KNOWLEDGE, ATTITUDES AND PRACTICES (KAP) STUDY

The adult males and females interviewed revealed that the majority of the respondents were self-employed subsistence farmers (84%) while 13% were employed in the public sector, either in the health, education or agricultural departments. Most respondents (70%) had attained at least primary level of education. All households had at least one woman (range: 1-8) unlike the other sex-age groups whose occurrence ranged from 0 to 5.

Fetching of water, and bathing occurred most frequently at dusk. The majority (53%) of the people went indoors around 20:00 hours, 2-3 hours after sunset. The majority (87%) of the people indicated that they had slept indoors the night preceding the interview and 95% sleep indoors as a habit. Those inclined to sleep outdoors mentioned unbearable heat indoors as a reason for sleeping outdoors. Most people (89%) are up and about by 05:00 or 06:00 hours attending to daily chores outdoors to avoid the unbearable midday heat.

The majority of the respondents (76%) correctly considered mosquitoes to be a cause of malaria. Ninety seven percent of the respondents indicated that mosquitoes were most noticeable during the night, and 71.1% indicated that the problem associated with mosquito bites and nuisance was severe, with 65.8% saying the problem was evident for all age groups. Most estimates of the number of bites per night fell into the nil-to-low category (57.9%). Fever and headache alone or in combination with weak joints and vomiting were the most common symptoms associated with malaria. Sixty six percent of the respondents had experienced these febrile conditions at least once during the two months prior to the interviews. Just over half the respondents mentioned Norolon^{R} (chloroquine) as the drug used in the treatment of malaria, a quarter did not know what drug they were given at the clinic, while one respondent mentioned prayer as a remedy. The majority of the respondents felt the local health facility was quite near to their homes. Only one respondent (2.6%) used malaria prophylactic drugs.

Enthusiasm and attitude towards residual spraying ranged from moderate (10.5%) to positive (52.6%), with the remainder being negative (36.8%). Over sixty percent of the respondents had their homes sprayed and 76% could correctly explain the reason behind the spraying exercise; 57,9% believed they were benefiting from the spraying. There were mixed feelings regarding suggestions that they should pay for the residual spraying; only 52.6% indicated willingness to do so. There was a significant relationship between the answer to 'willingness to pay for spray' and

enthusiasm (X^2 = 16.4; P>0.05). Another, perhaps natural, observation was the relationship between those who said they derived benefit from house spraying and a positive attitude (X^2 = 30.15; P>0.05). Almost all respondents (92.1%) indicated willingness to participate in community based environmental mosquito source reduction control measures. There was no significant relationship between the various other permutations of other attributes characterizing the respondents.

The personal protective measure that was used to any extent was repellent soap (Mosbar^R), with 21.1% of the respondents indicating daily use, while 23.7% mentioned its use on an occasional basis. Low use of other protective measures was indicated; the majority of the people (81.6%) had never used repellent lotions, 76.3% had never used bed nets and 86.8% had never used mosquito coils. Traditional herbal remedies for either malaria treatment or for the prevention of mosquito bites were not mentioned.

3.14 HOUSEHOLD MALARIA PREVALENCE SURVEYS

There were twice as many malaria positive slides from Siabuwa as from Chireya, and four times as many gametocytes in the population of the former area (Appendix 3a and b). Malaria prevalence in Chireya and Siabuwa was 12% (n = 432) and 25% (n = 224) respectively. All age-sex categories were gametocyte carriers in Siabuwa whereas only the infants, girls and women had gametocytes in Chireya. In Siabuwa 30% of the slide positive cases were gametocyte carriers as compared to only 6.1% in Chireya. Malaria slide positivity was not related to either age or sex in the two study areas although men were less affected in Chireya (Appendix 3c and d). In Chireya, slide positivity was greatest in February and tended to decrease progressively in March and April. In contrast, the cases started at a low level and were highest in April in Siabuwa.

Chapter 4
DISCUSSION

4.1 CLIMATE

The warm weather conditions in the two study areas are characteristic of the low-lying Zambezi Escarpment region. The seasonal rainfall is responsible for the malaria vector population build-up in February/March, which in turn precedes the peak disease transmission in March/April. This seasonality has been previously documented by Harwin and Goldsmid (1972), Taylor and Mutambu (1985), Mpofu (1985). While high temperatures favour the development of immature stages of mosquitoes, high saturation deficits are inimical to the survival of the adult stages (Pampana, 1963) and also the parasite in the vector (Lindsay *et al*, 1991). Some apparently prolific mosquito breeding sites are, however, ephemeral owing to rapid evaporation imposed by high temperatures (Plate VIII). The weather conditions, especially relative humidity, at Siabuwa are probably not affected much by the proximity of Kariba Dam.

4.2 LARVAL BREEDING REQUIREMENTS

That *An. merus* was found in Chireya is not surprising considering that inland salt-waters were available as breeding sites. Generally, fresh water is not suitable for *An. merus* breeding. Coetzee and le Sueur (1988) found *An. merus* to have the better survival rate of 25% in sea water 46.4% than 15.5% in fresh water in laboratory-based observations. The 21.05% sea water assayed in water samples from the vicinity of the Bare salt pan falls within the salinity tolerance ranges for *An. merus* documented by Rogo *et al* (1985), and le Sueur and Sharp (1988). Water salinity in semi-permanent breeding sites was found to be subject to seasonal fluctuation, but *An. gambiae* complex mosquitoes tolerated salinities ranging from 13 to 54 % sea water (Rogo *et al,* 1985). Work in KwaZulu\Natal has shown that *An. arabiensis* is strictly associated with fresh water, whereas *An. quadriannulatus* could tolerate 5.7% sea water and *An. merus*, in contrast, was found in water with a salinity ranging from 14.2 to 108% (le Sueur and Sharp, 1988). The mean salinity for *An. merus* was 42% sea water. There were some sunlit, semi-permanent water bodies which were devoid of any form of immature stages of mosquitoes. The reasons some water bodies are

shunned by ovipositing females calls for further investigation. A high iron content has been found inimical to fresh water species (le Sueur and Sharp, 1988). Eggs or larvae are alleged to provide pheromone cues luring gravid females wanting to oviposit to suitable water bodies (Service, 1993, p. 182) even though this altruism could lead to competition for resources in the chosen pool.

4.3 MALARIA VECTORS

The predominance of *An. arabiensis* in all collection sources confirms this species as the principal malaria vector in the area. The zoophilic and exophilic habits of *An. quadriannulatus* are further confirmed in the present study. The observation of *An. merus* in Chireya is a new record of the occurrence of this halophilic species over 800 km inland. There is need to monitor for *An. funestus* complex even though it is generally accepted that it was eradicated by indoor insecticide spraying. This species was reported to have re-established itself 35 years after 'disappearing' in Madagascar (Fontenille and Rakotoarivony, 1988).

4.4 SPECIES IDENTIFICATION

4.4.1 Species identification techniques

At least four species identification techniques were applied side by side during this study. The need for proper species identification is well summarized in this phrase from Garrett-Jones (1964): '... entomological measurements usually lose most of their value if recorded without distinction of species'. This is particularly necessary in situations like that found in sub-Saharan Africa where sympatry of members of the *An. gambiae* complex occurs. The malaria entomologist must be versed in the available techniques for species identification in order to operate effectively. Sharp *et al* (1984) highlighted the need for species identification following observations of large numbers of indoor resting *An. gambiae* complex in the Mamfene area of KwaZulu-Natal. These turned out to be *An. quadriannulatus* and thus of no medical importance. The consequences of inadequate species identification are well illustrated by Coetzee and Hunt (1985) and Hunt and Mahon (1986).

4.4.2 Correlation of species identification techniques

The disparities between polytene chromosome and PCR identification techniques are most probably due to operational and personal errors. Such errors are not connected with the method or procedure *per se*, but are associated with mis-scores of PCR products after electrophoresis (B. Bredenkamp, 1994, pers. comm.*). Paskewitz *et al* (1993) recorded impressively high concordance between the polytene chromosome and PCR identification methods for members of the *An. gambiae* complex sampled from established colonies and from the wild. Only 0.1% of their sample was misidentified. Despite the high accuracy, they encountered specimens with bands over and above the diagnostic ones for both *An. arabiensis*, *An, quadriannulatus* and *An. merus*. The observed additional bands, which persisted even after re-amplification, were attributed to 'contamination, interspecific hybridization and population variability' (Paskewitz *et al,* 1993). It should be noted that in this previous work none of the wild caught species were found coexisting in one locality, unlike the sympatry observed for the *An. gambiae* complex in both Chireya and Siabuwa areas. Most of the negative results reported here were obtained during the initial PCR runs, and the situation improved as the author mastered the intricacies of the technique. No doubt much higher concordance between PCR and the chromosomal technique would have been achieved had the initial PCR results been excluded from the analysis but this underlines the need to get the technique right the first time. Utmost care is required during DNA extraction and the subsequent pipetting of the delicate 1 μl extract into the master mix. In instances where isopropanol had leaked, the affected specimens were invariably negative with PCR. The preservative features of isopropanol are associated with inactivating the enzyme responsible for DNA denaturation. It is also assumed that the polytene chromosome results used as the benchmark in the determination of the levels of error for PCR were indeed impeccable. This is likely, considering the vast experience of the four mentors who assisted the author, a novice, in the cytotaxonomy work.

The polytene chromosome technique was marred by the numerous under-developed ovaries. Some of the ovaries did not unravel well for adequate diagnosis to be made. With the PCR technique specimens giving negative results can be re-amplified and re-run. In this regard PCR has the edge on the chromosome technique since ovaries once fixed at the wrong stage, they are irreversibly lost - there is nothing like re-processing the material. This highlights the high level of expertise required during the collection of chromosome material, emphasized by Hunt and Coetzee

(1986) and Paskewitz *et al*, (1993) (See also Table 1). On a positive note, however Green (pers. comm.*) demonstrated how well-fixed half gravid ovaries can be re-read by serially squashing a few ovarioles at a time. Shelley (1973) found a correlation between animal-fed mosquitoes and ravelled chromosomes. Those of human-fed mosquitoes unravelled better than those of animal-fed ones. Too few ovaries were processed to permit such an analysis in the present study.

The cost of consumables per mosquito using PCR was estimated at R5.22 (US$ 1.45) compared with R0.36 (US$0.10) and R3.60-5.69 (US$1.00-1.58) using chromosomes and electrophoresis, respectively (Paskewitz *et al,* 1993). This is in contrast to the cheaper R3.09/specimen for PCR and R3.24/specimen for electrophoresis estimated by scientists at The National Malaria Research Programme, Durban. Considering capital equipment requirements, the polytene chromosome method remains the cheapest when compared to the two popular identification techniques, viz, electrophoresis and PCR (Paskewitz *et al,* 1993). The polytene chromosome technique remains the only method that can yield results in the field. However PCR will most probably supersede the earlier techniques despite the cost as it is possible to process up to 72 specimens/day with slightly extended working hours. The need for liquid nitrogen for the preservation of specimens for subsequent identification by the electrophoretic technique is a distinct disadvantage for the method; liquid nitrogen is not only expensive and cumbersome to handle in the hot field conditions but it is a specialist commodity which is available from major cities.

4.4.3 Distribution of leg-band measurements

Leg-band frequency distributions conformed with what would be expected in the Coetzee (1986) dichotomy for the two predominant members of the *An. gambiae* complex found in the two study areas. Correct identifications >83% (n = 129) were observed for *An. arabiensis* from Chireya while 80% was achieved in exit trap specimens from Siabuwa although the sample was small (n = 10). In Chireya over 66% correct identification was achieved among the pooled *An. quadriannulatus*, while 82% correct identification was achieved among pit shelter members of this species. The observed level of accuracy obtained was lower than the 94% reported by Coetzee (1986) and the 89% obtained from the non-sprayed areas by Sharp *et al* (1989). The distribution peaks for both *An. arabiensis* and *An. quadriannulatus* were similar at the two localities, but were different from those observed in the two South African studies. The *An. arabiensis* peaks reported

by Coetzee (1986) and Sharp *et al* (1989) from unsprayed areas were 0.06mm and 0.07mm, respectively, in contrast to the 0.09mm reported here. The peak of 0.9mm found for *An. arabiensis* at Chireya compares more favourably with that of 0.1mm for *An. arabiensis* from sprayed areas observed by Sharp *et al* (1989). A peak of 0.12 mm was obtained by Coetzee (1986) for *An. quadriannulatus* and compares with 0.11 mm observed in the present study.

Sharp *et al* (1989) attributed the disparities between the observations in their work and those of Coetzee (1986) by slight differences in the techniques used but further revealed variability between *An. arabiensis* from sprayed and unsprayed areas. Only *An. arabiensis* from areas where DDT had not been used could be identified with a high degree of accuracy. The *An. arabiensis* from Zimbabwe were all from sprayed areas, albeit with deltamethrin (DDT was last used in 1991) and yet the species generally conformed with the Coetzee (1986) criterion, but the distribution peak fell midway between that from the sprayed and unsprayed areas obtained by Sharp *et al* (1989). It is as if the Zimbabwean *An. arabiensis* populations were in a state of transition from being 'unsprayed' to 'sprayed'. While Sharp *et al* (1989) speculated that DDT caused the variability in the leg-band measurements in *An. arabiensis,* the modalities and duration of this effect were not discussed and neither was the possible effect of insecticides other than DDT. It is possible that deltamethrin does not affect this variability to the same extent as DDT or that it does not affect it at all, implying that the observed distribution reflects the fading effects of DDT used prior to the introduction of deltamethrin.

In their preliminary work Coetzee *et al* (1982) conceded that while the leg banding pattern could not separate the major malaria vectors *An. gambiae* and *An. arabiensis*, the technique could at least distinguish these vectors from the less important *An. merus* and *An. quadriannulatus*. *An. gambiae* is rare in southern Africa (Coetzee *et al*, 1993b), and although *An. merus* has been reported inland, the species' role in malaria transmission in such situations has yet to be determined. Leg-band measurements overlapped from 0.07 to 0.15mm, which accounted for the greater part of the distribution curves of both *An. arabiensis* and *An. quadriannulatus* from the two study areas. The level of accuracy is reflected by the area under the curves that falls outside the region of overlap; this amounted to only 18.6% for *An. arabiensis* and 1.8% for *An. quadriannulatus* at Chireya (Fig. 4). At Siabuwa the area between the 0.06 and 0.08mm accounted for only 21.4%

of the total area under the *An. arabiensis* curve, indicating a maximum probability of correctly identifying this species of only 0.2. Thus, while this technique could be suitable in field situations where crude distinctions between vector and non-vector are required, the effort that goes with the technique suggests it is not a suitable epidemiological tool. The effect of possible insecticide selection pressure on the variability of the size of the pale leg band width in *An. arabiensis* is a confounding factor requiring further investigation.

4.5 SPECIES COMPOSITION

4.5.1 *Anopheles arabiensis* and other anopheline mosquitoes

The observation of *An. arabiensis* and *An. quadriannulatus* in sympatry in the two study localities is not unusual in the country. This has been documented in a number of previous studies in other malarious areas of Zimbabwe (Mahon *et al*, 1976; Green, 1970, 1972, 1982; Mpofu, 1985; Taylor *et al*, 1986; Masendu *et al*, 1992). Of particular interest is the predominance of the vector species in the sympatry. Most previous studies have shown the non-vector *An. quadriannulatus* as the predominant species. Despite the credence of the results being marred by high levels of error in the PCR identifications, indoor resting *An. gambiae s.l.* consisted almost entirely of the vector *An. arabiensis*. The finding of *An. merus* in Chireya is a new record 800km inland. This halophilic species was associated with the abundant saline breeding sites in the area.

The abundance of *An. arabiensis*, in Chireya at least, is further evidence of its status as the principal malaria vector in the country. The recording of *An. arabiensis* in day-time resting catches is clear indication of the association of this vector with human habitations. The indoor resting density observed was 0.49 in Chireya and 0.15 in Siabuwa (Table 6) compared to a range of 0.06 - 0.78/hut (mean = 0.27/hut) recorded by Wolfe (1964). This density varied between houses of different spray status; in Chireya it was 0.35 and 0.99 in sprayed and control houses respectively. In Siabuwa is was 0.11 and 0.39 in control and repellent soap-dependent houses respectively, while it was nil in sprayed houses. The relative paucity of indoor resting mosquitoes in deltamethrin sprayed houses as compared to unsprayed ones is most probably attributable to mortalities occurring after contact with insecticide residues. Dead mosquitoes are generally overlooked and trampled on if they do not fall prey to ants and other indoor scavengers. Recently the author observed both

unfed and freshly fed mosquitoes on white cloth left spread overnight on sprayed hut floors in Gokwe. Some insecticides (such as DDT and dieldrin) have also been observed to discourage (anophelines) mosquito entry in treated huts (Cullen and De Zulueta, 1964; Dartigues, 1987).

The finding of *An. quadriannulatus* and *An. merus* in the midst of *An.arabiensis* in day-time indoor-resting and exit trap catches is of interest as this underlines the need to perform species identifications as this can result in '... confusion and wastage of funds on unnecessary mosquito control...' as noted by Hunt and Mahon (1986). The value of separating *An. arabiensis* from *An. quadriannulatus* in epidemiological entomology is emphasized by Coluzzi (1984) as follows:

> '... the pooling of *An. arabiensis* and *An. funestus*, two species that both show a high vectorial capacity resulting from convergence in biting and resting behaviour, would be in many respects less misleading to the malariologist than the pooling of *An. arabiensis* and *An. quadriannulatus*'.

Despite the recording of *An. quadriannulatus* indoors, this species is essentially zoophilic and much more inclined to rest outdoors than indoors as found in this study. It is therefore unlikely to play a role as a malaria vector (Coetzee and Hunt, 1985). The present observation of *An. quadriannulatus* resting indoors in houses solely occupied by humans corroborates previous reports by Sharp *et al* (1984) and Hunt and Mahon (1986).

The finding *An. merus* in this study is further documentation of the occurrence of the species this far inland. Mahon *et al* (1976) reported its occurrence in Zimbabwe at Hippo Valley, (21^0 7'S 31^0 38'E), and Coetzee *et al* (1993b) indicated its furthest inland distribution as Matetsi river mouth, (18^0 03'S $26^0$36'E). This is contrary to statements that describe *An. merus* as being confined to within 220 km inland from its preferred salty larval habitat on the (east) coast (Janssens and Wery, 1987). *An. merus* has been reported to be involved in malaria transmission in some localities (White, 1974b), but in this case the sample size would have been too small for establishing this aspect. The absence of *An. gambiae s.s.* in the two study sites is not surprising as it is often associated with humid areas like the equatorial rain forests (White, 1974b; Janssens and Wery, 1987). Even though this species has been recorded in Zimbabwe previously (Paterson *et al*, 1963; Paterson, 1964; Mahon *et al*, 1976; Taylor *et al*, 1986), the low densities recorded renders its possible role in malaria transmission of minor concern. It is possible that *An. gambiae s.s.*, like

An. funestus, was practically eradicated following the introduction of the house spraying programme. Mutero *et al* (1984) reported a high rate of human biting followed by outdoor resting by *An. merus* in Jimbo on the southern Kenyan coast.

Besides the *An. gambiae* complex, the other anopheline mosquitoes found during the study included the ubiquitous *An. pretoriensis* and *An. squamosus* and *An. funestus* type mosquitoes. *An. funestus*, *An. leesoni* and *An. rivulorum* have been documented as the most widely distributed members of the eight-member *funestus* group (Gillett, 1972). In Zimbabwe whereas Muihead-Thomson (1960) reported only two species, viz. *An. confusus* and *An. leesoni*, Gillett (1972) suggested *An. confusus* and *An. fuscivenosus* as the dominant members. *An. funestus* had been described as susceptible to the intradomiciliary spraying method of vector control. The possible vectorial, albeit secondary, role of *An. pretoriensis* has been mooted but its distinct preference for cattle hosts observed in this and previous study (Garrett-Jones, 1964) mitigates against this notion.

4.5.2 Temporal variation

The sampling peak of *An. arabiensis* occurred during the midst of the rainy season, whereas *An. quadriannulatus* appeared to peak soon after this period. This might indicate that *An. quadriannulatus* was better able to exploit the increasingly sparse water available after the cessation of the rains, and/or that the residual insecticide was having the desired impact on the endophagic *An. arabiensis* population available for sampling. *An. quadriannulatus* was found to breed in the greatest range of sites when compared to either *An. arabiensis* or *An. quadriannulatus* (Le Sueur and Sharp, 1988). Successive evaporation of stagnant water could lead to increased water salinity which would favour the breeding of the halophilic *An. merus* and the more tolerant *An. quadriannulatus* to the exclusion of the strictly fresh water breeding *An. arabiensis*.

4.5.3 Spatial variation

An. arabiensis was found more commonly in those houses situated close to semi-permanent water bodies. These were associated with the main rivers in both study areas, and swampy sites in Chireya. The comparatively high number of mosquitoes that was collected from house # 19 in Chireya was associated with the observed tendency for unfed '... mosquitoes to try to feed in the

first house which they encounter on leaving the breeding sites' (Smith *et al*, 1995). House # 19 was the closest to a breeding site in this part of the village. The houses adjacent to the irrigation fields in Siabuwa were also among the most productive for the area. Fortunately, the type of crops under irrigation do not favour flood irrigation unlike rice paddy fields which are notorious as breeding grounds for both malaria vector and nuisance mosquitoes (Surtees, 1970; Hunter *et al*, 1993). This phenomenon has given rise to the term "rice malaria" (Reuben, 1989; Service, 1989b).

4.6 CHROMOSOMAL INVERSION POLYMORPHISM

The observed floating arrangements in 2Rb and 3Ra have also been reported from Zimbabwe (Collins *et al*, 1988) and in other areas of continental Africa (Akoh *et al*, 1980; Petrarca *et al*, 1983, 1986, 1987; Coosemans *et al*, 1989; Mnzava and Di Deco, 1986; Sharp and Le Sueur, 1991). The frequency of the 2Rb arrangement (60%) is similar to the 57% recorded by Collins *et al* (1988) in third generation *An. arabiensis* from Chiredzi, Zimbabwe that was being colonized in the laboratory (Mpofu *et al*, 1993), 58% from Kisumu, Kenya (Petrarca and Beier, 1992) and 55% from several localities in Tanzania (Mnzava and Di Deco, 1986). This is further confirmation of the uniformity of *An. arabiensis* in eastern Africa (Collins *et al*, 1988), and even in western Africa for that matter considering the 2Rb frequencies averaging 50% recorded in Nigeria (Garki) by Coluzzi (1977). The 2Rb frequency was found to increase with altitude in the Awash Valley in Ethiopia (Mekuria *et al*, 1982). In Chireya inverted homozygotes constituted only 35% of the karyotypes contrary to the expected high frequencies (Ralisoa Randrianasolo and Coluzzi, 1987).

The 2Rb polymorphism could not be correlated with any behavioural tendency as previously noted with respect to feeding (Coluzzi *et al*, 1977; Petrarca *et al*, 1987; Ralisoa Randrianasolo and Coluzzi, 1987) and resting (Coluzzi *et al*, 1977, 1979). The absence of any scores for indoor resting samples precluded any comparative analysis between indoor- and outdoor-resting samples. The homogeneity between samples from exit trap and pit shelter collections is not unusual as house leaving mosquitoes eventually end up resting outdoors. Green (1982) considered as equivocal the relation between man biting and 2Rb polymorphism in *An. arabiensis* from Kanyemba, Zimbabwe. The same lack of a link between feeding and resting behaviour with polytene chromosome polymorphism was reported for *An. arabiensis* by Sharp and Le Sueur (1991) in KwaZulu\Natal,

South Africa and Mosha and Subra (1982) in Kisumu, Kenya. An indirect association may be inferred from the observed lack of animal-feds among the standard homozygote *An. arabiensis*. The human blood index of carriers of the standard 2Rb karyotype was 1.6 times higher than that of the inverted karyotype, with the heterokaryotype being intermediate among indoor resting *An. arabiensis* in Kisumu (Petrarca and Beier, 1992). Differences in frequencies of *Plasmodium* CS-protein indices between the karyotypes were not observed in Kisumu, however. In the work reported here the sample size was small, therefore, there is a need to conduct further larger scale investigations towards elucidating this issue. The *'f'* inversion on chromosome X observed in the local *An. quadriannulatus* confirms previous records by Coluzzi and Sabatini (1968), Green (1972) and Collins *et al* (1988).

4.7 FEEDING BEHAVIOUR

4.7.1 The precipitin tests

Blood meal analyses are fraught with problems during sampling of fed females, testing the smears and during the interpretation of results. Sampling bias arises from the fact that blood-fed mosquitoes tend to redistribute themselves in the interval between feeding and daybreak (Garrett-Jones, 1964). In addition a proportion of blood-fed females die in the sprayed huts, and hence are lost from the day-time resting sample (Garrett-Jones, 1964). This is particularly so when fast acting synthetic pyrethroids are used in indoor residual spraying. For instance, both blood-fed and unfed mosquitoes were found either dead or moribund in morning inspections of white calico sheets laid overnight in huts sprayed with lambda-cyhalothrin during the 1994-95 malaria season. While the three sources of blood-fed mosquitoes used here attempt to reduce the sampling bias, they are not exhaustive. The natural outdoor resting sites were not sampled as it was not convenient given the limited time. In addition the precipitin tests were not exhaustive because, to borrow from Boreham and Garrett-Jones (1973), a blood smear sample was 'discarded' once a positive reaction was obtained. Only the patent mixed blood for the limited antisera available could be discerned because the precipitin test is not suited to detect cryptic mixed meals.

The negative precipitin tests were most likely due to hosts for which no tests were made, or could have been due to various other factors. Service (1986) points out that while the precipitin

test has the distinct advantages that it is simple and inexpensive, it has limitations in that it lacks sensitivity and specificity. Well digested blood meals, the deterioration of the smears or manner in which the smears are prepared all have some effect on the precipitin test (Boreham, 1975; WHO, 1975). Eligh (1952) observed that precipitin tests of blood smears involving crushing the entire mosquito performed better than those in which the blood meals were isolated from the insect body and then smeared as was done in this study. Apparently the insect body debris reduced the adherence of blood to the filter paper and thus aided the extraction of blood at the time of testing. In *An. arabiensis* humans and bovids account for the larger number of feeds, other hosts are fairly incidental.

It has been observed that a mosquito species exhibiting a high attack rate results in lowered human blood indices as a result of increased avoidance by humans compared to other hosts (Burkot *et al*, 1989). This could result in mosquitoes having patent mixed blood meals as attempts to feed on humans are interrupted and are followed by feeding on animals. Boreham and Garrett-Jones (1973) forecast that interrupted feeding that results in cryptic multiple meals would effectively increase the vectorial capacity of the vector. Interestingly, Burkot *et al* (1988) suggest that interrupted feeding probably might not result in an increase but rather a reduction in transmission. They put forward the argument that partial blood meals may result in the gametocyte density failing to attain the minimum required for infectivity. Besides, interrupted feeds on humans sharing a house is not likely since the vector should exhibit the same degree of anthropophily towards all occupants. However, some studies have indicated that adults, mothers in particular, are more attractive than infants (Carnevale *et al*, 1978).

Cattle are preferred hosts in the absence of humans as was previously found in an entirely outdoor resting *An. arabiensis* population in Madagascar by Ralisoa Randrianasolo and Coluzzi (1987). Goats were found unattractive to what was most probably a mixture of *An. gambiae s.s.* and *An. arabiensis* in the Pare-Taveta area of Tanzania (Smith and Weitz, 1959). Green (1982) also reports on the lack of attractiveness of goats to anophelines. Even though goats and sheep are minor hosts, mosquitoes might have ended up feeding on them in the absence of suitable alternative hosts in some outdoor niches. Dogs, donkeys and pigs have also been found to be alternative hosts for malaria vector mosquitoes (Burkot *et al*, 1988).

Blood-meal tests on *An. pretoriensis* from one pit shelter in Chireya revealed that half the

sample (n = 8) had bovine blood, only one specimen had mixed bovine-human blood, while the remainder had blood from 'other' vertebrates which were most probably goats. Previously, Garrett-Jones (1964) reported on the absence of human blood meals in outdoor resting *An. pretoriensis* from Zimbabwe; 78% (n = 74) had fed on bovid blood. This species is unlikely to be involved in malaria transmission as it is not associated with humans in its feeding and resting behaviour. Gillies and De Meillon (1968) consider previously reported malaria infections in *An. pretoriensis* unlikely to have been of human origin.

4.7.2 Zooprophylaxis

Zooprophylaxis has been defined as involving *the use of wild or domestic animals, which are not the reservoir hosts of a given disease, to divert the blood-seeking mosquito vectors from the human hosts of that disease* (WHO, 1982). The role of animals in zooprophylaxis has been observed in agricultural development schemes where mechanization replaced draught animals to the point where the deficit in domestic animals resulted in originally zoophagic mosquitoes (for example, *An. aquasalis*) switching to feed on humans (Service, 1991). There is need to consider integration of livestock management in order to derive what Kay (1988) terms the 'free control option' from zooprophylaxis. Judicious mixing of selected animal types and locating them at suitable distances from homesteads are issues that have to be taken into consideration for zooprophylaxis to be effective. Studies on *An. aconitus* have shown that the distance between cattle enclosures and homesteads is much more important than the human:cattle ratio, (Kirnowordoyo, 1986, cited by Burkot *et al*, 1989). In Papua New Guinea pigs that spend the night under people's stilted houses diverted *An. farauti* from humans to the extent that a low HBI of only 9% was observed as compared with a HBI of 83% in villages where there were few pigs (Charlwood *et al*, 1985). It is estimated that a 60% change in mosquito feeding results in 84% reduction in vectorial capacity (Kay 1988). Not all animals are suitable for zooprophylaxis, however. For instance, while horses may provide protection against biting *An. bellator*, *An. darlingi*, and *An. pseudopunctipennis*, they may become infected with arboviruses that can also infect humans such as the equine encephalitis virus (Service, 1991). This might not be a problem in rural Zimbabwe where the horse is not ordinarily kept and the comparatively common donkey is refractory to the virus. Immunization of the animals would safeguard against such a zoonotic threat in case horses are kept.

The host choice of some mosquito species is apparently not affected by the presence of domestic animals. In Tennessee, in the United States of America, neither cattle nor horses could effectively divert from and provide protection to humans against *An. walkeri* (Bang and Simpson, 1942).

There is need for caution with zooprophylaxis, however, as mathematical models have shown that introduction of animals can lead to increased biting on humans and higher malaria transmission (Sota and Mogi, 1989 cited by Service, 1991). Such a situation would arise from increased fecundity of the vector mosquito following the provision of a readily available source of blood. Draper and Smith (1960) similarly observed that the frequent deviation of *An. gambiae* to cattle, in conjunction with resting in vegetation, would increase its numbers as well as assist its survival to an infective stage. Treatment of cattle with insecticide which has been reported to enhance the zooprophylactic role against *An. sinensis* (Self, 1987 cited by Service, 1991) might curtail such an unpleasant development. In this respect the present animal health legislation in Zimbabwe that requires regular cattle dipping to control tick infestation should inadvertently contribute towards the demise of the opportunistic feeding *An. arabiensis* as well.

4.8 RESTING BEHAVIOUR

Partial exophily occurs as demonstrated by the preponderance of human-fed females in exit trap catches and in outdoor resting sites. The exodus of blood-fed mosquitoes even from unsprayed houses strongly suggests that this is a natural behavioural pattern in *An. arabiensis*. Similar observations were made in KwaZulu\Natal by Sharp and le Sueur (1991) and Sharp *et al* (1993). The high HBI among indoor resting mosquitoes suggests that human-vector contact occurs indoors, and also that deltamethrin does not appear to irritate mosquitoes into exiting before feeding (Dartigues, 1987). The low vapour pressure of deltamethrin would explain its lack of a fumigant action. However, Lindsay *et al* (1991) found evidence that vapour from the solvent in the formulation contributed to the deterrency/repellency properties of synthetic pyrethroids in freshly treated bednets lasting a few months. On the hand deterrency of several months was contributed by airborne effects in pyrethroids as documented by Somboon, (1993). Coosemans and Sales (1977), cited by Dartigues (1987), made the following observations about deltamethrin: it decreased the intra-dwelling anopheles density; both *An. arabiensis* and *An. funestus* which entered treated

dwellings exit rapidly, and *An. arabiensis* and *An. funestus* did not remain in sufficient contact with the product to be killed after 24 hours. The observations made in the present study appear to support the last point mentioned above. It has been hypothesized that in situations where insecticide spraying discourages feeding (eg., Smith and Chabeda, 1969), extra-domiciliary malaria transmission would ensue (Mosha *et al*, 1992). This would be facilitated by human behaviour (Gillett, 1985); a lot of outdoor activity was evident after dusk in the two study areas. However, sleeping outside as a habit is not prevalent among the people of Chireya and Siabuwa. Regardless of exophily, indoor spraying with synthetic pyrethroids has an adverse effect on the not so domestic *An. arabiensis* as illustrated by the mortalities in collections of unfed mosquitoes that were kept for a 24-hr holding period. Those that survived the holding period probably had not absorbed sufficient insecticide despite coming into contact with treated walls. Mosquitoes may have avoided the insecticide altogether by resting on untreated surfaces that abound in the average home (see section 4.12). Alternative resting surfaces exist on beds, wardrobes, cupboards, storage boxes, lines of hanging clothes, carrier bags, goat skin mats and picture frames. Some of these are among the items that are (needlessly) moved out of the houses to allow the thorough spraying of walls and thatch and then returned. This is most prevalent when insecticides which leave unsightly deposits are used; the wettable powder of DDT is well known for this. This highlights the need to use an insecticide (such as a synthetic pyrethroid) which is not only effective but is also acceptable for spraying on furnishings to increase the available insecticide area.

Re-plastering was not a serious problem in the two study areas as evidenced by the low recorded - only 5% in Siabuwa and none in Chireya. The KAP study revealed that the spray teams effectively explained to the community the need to avoid (or at least delay until after six months) re-plastering houses. Re-plastering has been attributed to sprayed insecticide accentuating the bed bug nuisance problem indoors as was found in KwaZulu\Natal where DDT is used for residual spraying (Sharp *et al*, 1988, 1990). Another common reason for re-plastering was also the unacceptable insecticide deposits for which DDT, HCH and malathion are examples (Reuben, 1989). However, re-plastering of walls might not have such a negative impact if the majority of the vector mosquitoes prefer resting on thatch rather than on walls as was found in experimental huts in Umbugwe, Tanzania where 80% *An. gambiae* were found on the roof (Smith *et al*, 1966). In contrast the finding of indoor resting collections in re-plastered DDT sprayed huts indicated that

An. arabiensis was resting on the walls and not on the sprayed roof (Sharp *et al*, 1990). This resting behaviour can only be gauged by meticulous use of the hand catch method for sampling indoor resting mosquitoes in dwellings.

Exit window traps on houses with either asbestos or corrugated iron sheet roofing were more productive than their thatched counterparts and the exodus of mosquitoes from the former is presumably triggered by the comparatively abrupt changes in indoor temperature during the course of day in the former house type. Smith *et al* (1966) observed similar reduced numbers of *An. gambiae* resting on corrugated iron roofs as a result of higher temperatures during the day. A greater saturation deficit was observed for the greater part of the day in a house with a corrugated iron roof than in a thatched one in Chireya; from 04:00 to 19:00 hours, and from 21:00 to 01:00 hours.

A seasonal perspective of the abdominal conditions among daytime indoor resting females could not be obtained as the present studies were conducted during the wet season only. The proportion of fed:gravid has been found to vary with season (Coosemans *et al*, 1989). Gonotrophic dissociation was inferred from the increased proportion of gravid female resting indoors observed during the dry season. Could this indicate the period during which the vector is most vulnerable to residual insecticide in such instances?

Several workers have reported the collection of outdoor resting mosquitoes from cattle enclosures (eg., Ralisoa Randrianasolo and Coluzzi, 1987; Service, 1976). The cowsheds in Madagascar had roofs, unlike the kraals found throughout in rural Zimbabwe (Appendix 4, Plate II). A resting mosquito would derive little, if any protection from either sun or wind in the animal enclosures found in this study area. It is therefore not surprising that no mosquitoes were found in these animal shelters despite repeated searches. The abundant vegetation would provide a more suitable micro environment.

4.9 SURVIVAL OF EXITING FEMALE MOSQUITOES

The fate of mosquitoes that leave houses is illustrated by observations on survival rates of exit trap mosquitoes kept over a 24-hours holding period. Unfed female mosquitoes which were found in exit traps died irrespective of the spray status of the house from which they were obtained.

These unfed females were probably irritated by insecticide or were discouraged from feeding indoors by host avoidance behaviour. Outdoors these would have sought an alternative meal (from either plant or mammal) or would have perished from starvation. A higher proportion of unfeds in exit window trap collections in sprayed houses would reinforce the idea that *An. gambiae s.l.* fled treated dwellings before feeding (Dartigues, 1987). Smith (1964) proposed a repellency index (RI) based on the relative numbers of unfed mosquitoes leaving sprayed and unsprayed houses. A high feeder-survivor index, FSI, (Hudson and Esozid, 1971) was estimated from the blood-fed females that survived the 24-hr holding period. This implies that those human-fed vector mosquitoes which exit following short resting durations indoors escape the adverse effect of the residual insecticide. It has been observed that in experimental huts window traps delay mosquitoes leaving houses. This results in their increased exposure to insecticide as their normal escape route is blocked by the traps (Coz, 1971, cited by Service, 1976). The presence of both door and eave gaps would offer abundant alternative exit points unless the exiting mosquitoes have a peculiar preference for window gaps. The low numbers of dead mosquitoes found in exit traps could be due to the fact that they were removed by scavenging ants before the traps were emptied.

4.10 VECTOR SUSCEPTIBILITY TO INSECTICIDE

Presently, any apparent inadequacies in malaria vector control in Zimbabwe cannot be attributed to vector resistance to insecticides. Deltamethrin, the insecticide used in house spraying was acceptable to the community as it does not leave unsightly deposits on treated surfaces unlike DDT. The absence of resistance to deltamethrin in this study is not surprising as malaria vector mosquitoes have not been reported to be resistant to any of the pyrethroids (WHO, 1992; Roush, 1993). However, evidence of pyrethroid resistance has been found in *An. gambiae* from Nigeria, *An. arabiensis* from the Sudan and *An. stephensi* from Pakistan through selection in the laboratory WHO (1980). Recent biochemical tests have further confirmed the susceptibility of *An. arabiensis* collected from Kamhororo area of Gokwe North (F. C. Murahwa, pers. comm*.). The shortcomings associated with using wild caught specimens in bioassays also apply to insecticide susceptibility tests (Green, 1981; Hunt and Mahon, 1986). The use of progeny from individually identified *An. arabiensis* for the susceptibility tests as first recommended by WHO (1975) and

adopted by Sharp *et al* (1990) is the best approach. This approach could however not be adopted in this study because the author's identification skills were inadequate at the time field exercises started. Roush and Miller (1986) provide a critical analysis of the LD_{50} measure and the sample size in using these standard tests for resistance management. They point that huge sample sizes (approx. 100's) are necessary to detect resistance at the nominal low (phenotypic) frequency of 1%. After resistance frequencies reach this level, control can theoretically be lost in as little as 1 to 6 generations, depending on the circumstances (Georghiou and Taylor, 1977).

The mechanisms involved in DDT resistance do not, apparently, confer cross resistance to synthetic pyrethroids in *Anopheles* (WHO, 1987; Malcom, 1988). This is so despite the similarity in mode of action of the pyrethroids and DDT which both involve action on the sodium channels of the nerve axon (Roush, 1993).

The contribution of insecticides used in agriculture in selecting for resistance in mosquitoes has been documented (Roush, 1993; WHO,1987). Insecticides used in cotton-growing areas in Central America and the Sudan, have been found to be a factor in the selection for resistance in malaria vectors (Reuben, 1989). The following insecticides are used for cotton pest control in the Chireya: dimethoate (for aphids), carbaryl (for leaf eaters) and fenvalerate (a broad-spectrum contact synthetic pyrethroid insecticide). Given the outdoor resting behaviour of *An. arabiensis*, these pesticides should adversely impact on the insecticide resistance status of the vector mosquito in cotton growing areas. Roush (1993) suggests that for those species that have shown a propensity for resistance, the solution lies in assuming that resistance will occur and adopting a preventive strategy. Organophosphate insecticides are theoretically considered as suitable substitutes for pyrethroids in the event of resistance developing to the latter.

Another potential threat as far as the development of resistance concerns the uncontrolled use of larvicides of the same or similar class of insecticide as the adulticides (Fontaine, 1983, p. 78). The risk of resistance development is low in area where control is limited to the adult stage. It is preferable to use larvicides that do not eventually compromise the adult vector control method.

4.11 INSECTICIDE RESIDUAL EFFICACY

The results of bioassay tests provide further evidence that deltamethrin deposits on both thatch and mud walls were still active two months after spraying. The vapour pressure of deltamethrin (1.5×10^{-8} mm Hg at 25^0C) is lower than that of DDT (1.5×10^{-7} mm Hg at 20^0C). On this basis alone deltamethrin should have a residual action comparable to that of DDT. However, rate of erosion for deltamethrin on mud and thatch surface in hot weather is presently unknown. In this time-dependent procedure, it was assumed that the amount of insecticide on sprayed surfaces did not change over the two days of conducting the tests. The use of wild caught mosquitoes for the bioassay is, however, associated with several drawbacks: firstly, the mosquitoes are of an unknown age, secondly, they could have absorbed lethal doses of insecticide from treated houses prior to capture, and thirdly, pertinent in this instance, the sibling species constituting the *An. gambiae* complex cannot be known until after specimens are identified in the laboratory. But the low mortalities that were recorded with specimens randomly taken from the same source in the control unsprayed houses is a clear indication of the presence and effectiveness of insecticide deposits in the sprayed houses. Ideally bioassay tests require laboratory-reared mosquitoes of known susceptibility, but favourable results from insecticide susceptibility tests lend confidence to the use of the wild caught mosquitoes. Standard WHO (1975) insecticide susceptibility tests conducted on site in Chireya indicated that the wild caught *An. gambiae* complex mosquitoes from an exit window were susceptible to deltamethrin. In retrospect, it is noted that the majority ($>80\%$) of the exit trap mosquitoes collected from hut 19 were *An. arabiensis*.

4.12 MOSQUITO ENTRY/EXIT AND HUT PROFILES

There have been several studies on the subject of the role played by human dwellings in facilitating vector-borne disease transmission in general (eg., Kroeger, 1980; Schofield and White, 1984; Garcia-Zapata *et al*, 1992) and malaria transmission in particular (eg., Gillies, 1988; Ault, 1989). In this study the proximity of breeding sites to homesteads coupled with the availability of numerous entry ports on houses evidently contributed towards the presence of mosquitoes in the latter. The absence of any semblance of house-proofing is attributed to lack of awareness, poverty within and neglect of the rural community by the appropriate authorities. The size of the houses

and the material used in their construction did not appear to affect the mosquito yield in daytime resting catches although it is strongly felt that the effect of corrugated iron roof on temperature in house # 19 in Chireya probably contributed towards a mass exodus of freshly fed mosquitoes collected from the window trap. Grass thatched roofs, in contrast, have been observed to provide a comparatively much more stable thermal environment (Kroeger, 1980). However, Gillies (1988) observes that the house material is a primary determinant of indoor resting rather than micro-environmental factors. Rough plastered walls are preferred to their smooth rendered counterparts as resting sites (Gillies and Coetzee, 1987). In addition the type of house material influences on the effectiveness of residual insecticides (eg., Ault, 1989). Sorptive soils on walls tend to reduce the longevity of insecticides whereas there is considerable persistence of toxicity of many formulations on grass thatch (Smith *et al*, 1966).

Increasing wall height has been reported to effectively deny entry to some mosquito species (Snow, 1987). The role played by uncut grass or bush around the home appears to have been emphasized in old literature on tropical hygiene and sanitation (eg., Jordan, 1950) but this is hardly mentioned in recent malaria guides (eg., Gear *et al*, 1988). Grass certainly plays a role as an outdoor resting site, at least for certain species (Gillies, 1988).

The tendency for mosquitoes to be associated with exit traps on the western side of houses observed in the present study contrasts with previous observations on experimental huts by Taylor *et al* (1981). *An. arabiensis* preferred exit traps on the upwind side from both DDT and decamethrin sprayed experimental huts. In the present study the preferred western side of the houses was generally towards the leeward side. The prevailing wind in the study areas was notably north-east.

There were insufficient data to assess the extent to which the number and age of occupants of houses affected the number of mosquitoes collected either resting indoors or from exit window traps. These factors are associated with inter-house variation in mosquito densities (Smith *et al*, 1995).

4.13 PEOPLES' PERCEPTION AND BEHAVIOUR IN RELATION TO MALARIA TRANSMISSION AND CONTROL

The aim of this study was to ascertain the peoples' perceptions of disease transmission, the house spraying exercise and practices in relation to their behaviour within the scope of their socio-economic environment. The people in both localities were arguably aware of the basics of malaria transmission, treatment and control efforts. The night time activities that could aid their becoming infected include the fetching of water (mostly womenfolk), bathing and occasional social gatherings. While there is hardly any sleeping outdoors in Chireya, the same cannot be said about Siabuwa. In Chireya the practice of preparing the evening meal outdoors is common, thus allowing at least 3 hours of outdoor biting time for both vector and nuisance mosquitoes.

The indifferent attitude towards house spraying probably reflects the people's endurance of the seemingly unending programme. It has been noted that non-compliance with house spraying sets in once people do not recognize the need nor see any tangible benefits of spraying that has been in practice for a long time (Reuben, 1989). Moreover, the spraying coincides with the period when people are busy preparing for the festive season, or are in the fields. Spraying also necessitates the moving of furniture and other possessions and the provision of generally scarce water. This can lead to apathy, such as was noticed in the very early days of spraying in Zimbabwe (Alves and Blair, 1953). Spraying is thus an inconvenience to the householder, and therefore appropriate health education is a requisite on the part of the spraymen. The value of insecticide-treated bednets (Bradley *et al*, 1986; WHO, 1987; Self, 1987; Snow *et al*, 1988; Rozendaal, 1989; Sexton *et al*, 1990; Curtis, 1992; Service, 1992), coils (Charlwood and Jolley, 1984; WHO, 1984; Sloof, 1987;), and repellents (Yap, 1986; Frances, 1987; Sloof, 1987; Sholdt *et al*, 1988; Harbach *et al*, 1990; Mani *et al*, 1991) as protection against mosquitoes is well documented. These different personal protective measures are considered low priority in the present study despite the apparent awareness of the role played by mosquitoes both in malaria transmission and as a nuisance. In West Africa low use of bednets was compensated for by the easily accessible mosquito repellents (Aikins *et al*, 1994). In Zimbabwe, the use of bednets is not associated with any socio-cultural value, unlike what was found in The Gambia (Aikins *et al*, 1994). Bednets have been found useful for malaria control in those communities where the nets are accepted items of living (Davidson, 1989). In The Gambia and Guinea Bissau bednets were also associated with additional benefits like privacy, warmth and

protection from falling roof debris. The last aspect is one which also featured in the proposed ceiling net (Masendu, in prep.). The ceiling net is designed to block and divert mosquitoes attempting to enter houses through the eaves into a gap left between the thatch and the ceiling net where they will be exposed to the treated ceiling. The efficacy of repellent soap might be improved by the provision of adequate supplies of the soap and, most importantly, strict compliance with the timing of its application to take into account the peak biting times for both nuisance and malaria vector mosquitoes at each locality. This should reflect the average six hours' protection time (Mani *et al*, 1991) and also recognition of a possible shift in the biting cycle as a result of insecticide use (WHO, 1987). It is presently not known whether inhaled smoke emanating from coils presents any health hazard. Irritation of the eyes and nose similar to hay fever has been reported by people sleeping in a room where coils containing pyrethrins were burned. The constituents of the smoke other than the insecticides should not be assumed harmless, since Schoental and Hibbard (1967) after consideration of the high incidence of nasopharyngeal cancer amongst the Chinese, found carcinogens in the smoke from incense sticks (Hudson and Esozid, 1971).

Other aspects of this study attempted to ascertain the willingness of the people to participate in community-based control measures; source reduction (with its rather limited applicability) and introduction of an element of self-reliance. The introduction and development of the primary health care concept is designed to end costly vertical control programmes (Gratz, 1985; Gish, 1992). Further, according to the Global Malaria Control Strategy, the fight against malaria will be considered within the context of other health problems, rather than in isolation as has been the practice in the past (WHO, 1993). The *health for all by the year 2000* concept envisages a greater role in the participation of individuals and families in disease prevention (Gratz, 1985; WHO, 1993). Gish (1992) discusses arguments proffered for and against both the verticalist and integrationist approach to disease control. A multi-pronged approach is required for future sustainable malaria control; and this encompasses health education, environmental management, use of bednets, therapy and vector control by chemical and biological agents (Davidson, 1982; Sloof, 1987; Service, 1992).

The SPf66 vaccine which is still under evaluation brings hope alongside the prospects of genetically engineered mosquitoes that are refractory to malaria infection (Service, 1992, Alonso *et al*, 1994). There is little, if any participation for affected communities as far as these highly

technological solutions are concerned. The use of larvivorous fish as a biological control measure (Davidson, 1982) is not suitable in the majority of mosquito breeding sites, as these are so shallow (Plate VIII) and ephemeral in nature that introducing *Gambusia affinis* would be inappropriate. Opportunities for larviciding are optimal during the dry season when residual breeding sites are more defined and limited in their extent. There are prospects for incorporating Geographic Information Systems (GIS) in mapping these dry season water bodies for concerted larviciding.

4.14 HOUSEHOLD MALARIA PREVALENCE

The high malaria prevalence observed in Siabuwa is surprising considering the comparatively low vector population that was sampled in the area. The few mosquitoes available must exhibit a high vectorial efficiency which is, perhaps, aided by the also very high gametocyte rate (30.9%). Another contributing factor that might help explain differences in malaria prevalence between the two areas could be the lower spray coverage of only 53.3% in Siabuwa as compared to 78.9% in Chireya. The small proportion of slide positive men in Chireya probably reflects a higher level of acquired immunity for this age and sex group. This would probably arise from this age group being more exposed to malaria parasites as hypothesized by Molineaux and Gramiccia (1980). This is certainly possible considering that the rural population is generally not on prophylaxis which has been associated with lowered immunity (Greenwood, 1984; Gardiner *et al*, 1984). In meso- and holoendemic situations adults would be expected to have a higher immunity by virtue of their age (Sharp *et al*, 1988) and therefore longer exposure to infection. Individual differences in getting bitten by mosquitoes is probably related more to the variation in individual irritability and defensive behaviour than to height, weight or blood group (Wood *et al*, 1972; Burkot *et al*, 1988).

Chapter 5
CONCLUSION

The following strengths and weaknesses emerged from this study of malaria transmission and vector control in Chireya and Siabuwa. The population is not protected against mosquitoes both indoors or outdoors. Virtually all human-vector contact takes place indoors, by virtue of the absence of outdoor sleeping habits, and the peak biting time(s) of the vector. The following conclusions emerged in favour of the vector control intervention effort:

i) *An. arabiensis* is susceptible to both the synthetic pyrethroid deltamethrin and DDT;

ii) while the formulation and the dosage of deltamethrin appear to be suitable for indoor residual spraying, there is little evidence on thoroughness during its application;

iii) community compliance during the spraying exercise varies from place to place, but is generally high. This is complemented by their attitude towards spraying and a high awareness among the population of aspects such as the role of mosquitoes in transmission and disease etiology.

Two major threats to vector control and malaria transmission stem from the resting behaviour of the vector mosquito and the unimpeded opportunity for human-vector contact indoors. The principal vector *An. arabiensis* is partially exophilic, consequently it is not fully amenable to control by indoor application of residual insecticides. This is evidenced by the fact that those freshly fed mosquitoes which leave houses seem to have a high probability of survival. The absence of mosquito-proofing on rural homes is further aggravated by the total absence of any form of personal protection. The absence of personal protective measures is attributed to a multitude of factors: poverty, a lack of motivation and the low priority that is given to disease prevention practices.

It is strongly felt that the future of control lies in concerted health education, improved housing and the involvement of the community in disease control measures. Mosquitoes invariably breed away from peoples' homes and yet they are expected and permitted to come indoors by the proponents of indoor residual spraying. Intradomiciliary spraying has undoubtedly made an impact on malaria transmission, but with the problem of exophily it is worthwhile shifting emphasis towards measures designed to curtail critical human-vector contact within an integrated malaria control programme. One of the weaknesses which militates against the adoption of house-proofing

and use of personal protective measures is the stark poverty of the rural population. Beach *et al* (1993) estimate that it costs 30% less to curtain an average house than to provide bed nets to all occupants of the same house. The material requirement for ceiling nets would exceed that for curtains, but it anticipated that the ceiling net would last longer and retain insecticide better than curtains. Few of the breeding places found in the average rural environment lent themselves to pond-filling or the introduction of larvivorous-fish type of source reduction control approaches.

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APPENDICES

Appendix 1. Published and unpublished work on Zimbabwean vectors; an (chronological) annotated bibliography

Leeson (1931). Leeson conducted the first extensive vector investigations following an outbreak of blackwater fever in Zimbabwe and implicated *Anopheles gambiae* and *Anopheles funestus* as vectors. He reported the annual wet weather invasion through river valleys by *An. gambiae* from the lower altitudes to the north and south of the central plateau, towards the central highveld, an observation which was to form the basis of barrier spraying in the 1950s.

Alves and Blair (1953). Described the 1945 "Pilot-plant" experimentation on the use of DDT for residual spraying. The success of the 1949 Mazoe Valley Project persuaded government to set up an organization within the Health Department to undertake the control of malaria in rural areas. DDT was dropped in favour of BHC, and the small number of indoor resting *An. gambiae* observed was interpreted as an indication of a change in habit to zoophily. The country was epidemiologically zoned by altitude into the malaria-free high plateau at 1 200 - 1 500 metres above sea level, and the malaria prone lowveld.

Alves and Blair (1955). Documented the expansion of malaria control from the Mazoe Valley to the south east, north east and eastern border areas of the country. They further described the 'peripheral barrier scheme' of malaria control whose rationale was the supposed vector migration between the two altitude zones described by Leeson (1931).

Paterson *et al* (1963). Presented evidence for the existence of a new member of the *An. gambiae* complex, form C, and further pointed out its significance in relation to the so-called behaviour changes in '*An. gambiae*' following anti-malarial spraying operations. They also noted form C's breeding, host preferences, exophily and sympatry with other members of the complex. Their

observations were based on studies in Chirundu, Chipinda Pools, Mazoe Valley, Uzumba and Save/Runde river confluence.

Ramsdale (1965), cited by Zahar (1985), highlighted the paucity of indoor resting females in Uzumba; and in the south east lowveld observed a low human blood index among specimens found resting in experimental huts.

Green (1970). Reported the presence of cytogenetically identified species B (*An. arabiensis*) in Gutsa Irrigation Scheme (16^0 20' S 30^0 57' E), 1000 ft (= 305 m) above sea level. These observations supported the view that the species was largely exophilic and catholic in its host choice. Green expressed surprise at the apparent absence of species A (*An. gambiae s.s.*) in an area not subjected to residual insecticide spraying. The sampling techniques used included: indoor catches, pit shelters, human, and animal baited nets indoors and outdoors.

Green (1972). He presented an X chromosome map for species C (*An. quadriannulatus*) from ovarian nurse cells.

Harwin and Goldsmid (1972). Described the occurrence of imported malaria on the Mafungabusi and Charama plateaux (1000 m above sea level) in Gokwe. Epidemiologically malaria is not expected in such high altitude areas.

Hunt (1973). Described a modified cytological technique for the study of members of the *An. gambiae* complex.

Mahon *et al* (1976). Developed an electrophoretic technique for routine identification of members of the *An. gambiae* complex, and indicated its application in insecticide resistance studies of vector mosquitoes.

Green (1981). Illustrated the need for sound genetic species identification in making

practical operational decisions: the identification of *An. arabiensis* among survivors from a dieldrin susceptibility test provided the impetus to abandon this insecticide in favour of DDT.

Crees and Mhlanga (1985). Reporting on the 1983 malaria prevalence survey, they described *An. arabiensis*, as controllable but refractory to eradication by domiciliary insecticidal spraying. The inference was conjectural as no entomological investigation accompanied the survey.

Mpofu (1985). Described seasonal variation in vector population densities in relation to disease incidence patterns in Uzumba. The sympatry of the three fresh water breeders of the *An. gambiae* complex was noted. The sampling techniques used were pit shelters, human and animal baited net catches indoors and outdoors.

Mpofu and Masendu (1986). Described a human- and animal- baited trap for the sampling of members of the *An. gambiae* complex.

Mpofu *et al* (1993). Attempted the laboratory colonization of *An. quadriannulatus* from wild caught *An. gambiae* complex females.

Publications pertaining to insecticide efficacy evaluations:

Taylor *et al* (1981). Reported on the behaviour of laboratory reared *An. arabiensis* in DDT and decamethrin treated experimental huts.

Taylor *et al* (1986). Reported on the efficacy of encapsulated deltamethrin on *An. arabiensis* and *An. gambiae* and the suppression of disease prevalence.

Mpofu and Gomo (1990). Reported on the efficacy of lambda-cyhalothrin (Icon^{R}) in vector control.

Mpofu *et al* (1991). Reported on the efficacy of bendiocarb (Ficam VC^{R}) for vector control.

Appendix 2. Questionnaire: Human behaviour, knowledge, attitudes and practices on malaria transmission and control.

Date:

Area:

Interviewer:

Part I. Background Information

1. Sex: female - 0; male - 1.
2. Age: ..... years
3. Education: none - 0; primary - 1; secondary - 2; tertiary - 3.
4. Occupation: self employed - 0; public - 1; other - 2.
5. Size of household: Children < 5 ; Girls ; Boys ; Men ; Women .
6. Religion: none - 0; Christian - 1; Pentecostal - 2; Traditional - 3.
7. Duration of stay in area: years.
8. Crop yields obtained last season (Bales/ bags): Cotton ; maize .
9. Crop yields expected this season (Bales/ bags): Cotton ; maize .
10. Number of cattle ; kraal distance from home .
11. Number of goats ; kraal distance from home .
12. Number of donkeys ; kraal distance from home .
13. Number of pigs ; kraal distance from home .
14. Number of pole and dagga and thatch houses: .
15. Number of mould and thatch houses: .
16. Number of brick and thatch houses: .
17. Number of brick and asbestos houses: .

Part II. Knowledge, Attitudes and Practices

Malaria

18. How do you tell someone has malaria?
    Vomiting - 0; Headache - 1; Fever - 2; Weak joints - 3.
19. Number of febrile episodes in the past 2 months ;
20. Drugs taken to treat said febrile illness:
    Chloroquine - 0; Other antimalarial - 1; Analgesic/antipyretic - 2.
    Herbal drugs - 3; None - 4; Unknown - 5.
21. Use of prophylactics: no - 0; yes - 1.
22. Frequency of prophylaxis use: irregular - 0; regular - 1.
23. What causes malaria? Wrong answer - 0; correct answer - 1.
24. Distance from health center: Far - 0; average - 1; near - 2.

Night time behaviour

25. Did you sleep indoors last night? no - 0; yes - 1.
26. Do you always sleep indoors at night? no - 0; yes - 1.
27. Why sleep outdoors? cooler - 0; bedbugs - 1; space problems - 2; other - 3.
28. Other reasons 'forcing' you to sleep/venture outdoors at night;
    Field - 0; church - 1; rituals - 2; other - 3.
29. Frequency of fetching water between dusk and dawn:
    Always - 0; occasionally - 1; seldom - 2; never - 3.
30. Frequency of bathing between dusk and dawn:
    Always - 0; occasionally - 1; seldom - 2; never - 3.
31. Approximate time of getting indoors last night .
32. Approximate time of sleeping indoors last night .
33. Approximate time of getting up today .....
34. Nocturnal distribution of under fives from dusk to 9pm last night:
    Indoors awake - 0; indoors asleep - 1; outdoors awake - 2;
    outdoors asleep - 3; away from home - 4.
35. Nocturnal distribution of boys from dusk to 9pm last night:

Indoors awake - 0; indoors asleep - 1; outdoors awake - 2;
outdoors asleep - 3; away from home - 4.

36. Nocturnal distribution of girls from dusk to 9pm last night:
Indoors awake - 0; indoors asleep - 1; outdoors awake - 2;
outdoors asleep - 3; away from home - 4.

37. Nocturnal distribution of men from dusk to 9pm last night:
Indoors awake - 0; indoors asleep - 1; outdoors awake - 2;
outdoors asleep - 3; away from home - 4.

38. Nocturnal distribution of women from dusk to 9pm last night:
Indoors awake - 0; indoors asleep - 1; outdoors awake - 2;
outdoors asleep - 3; away from home - 4.

Mosquito problem and control

39. Is your house sprayed? No - 0; yes - 1.

40. Why is your house sprayed?
Correct reason - 0; wrong reason - 1; don't know - 2.

41. Does it help to have your house sprayed? No - 0; yes - 1.

42. Rating of mosquito problem:
mild - 0; moderate - 1; severe - 2.

43. The most affected members of the family:
children - 0; adults - 1; all - 2.

44. Most problematic time of day for mosquitoes:
Day - 0; night - 1; day and night - 2.

45. Perceived number of bites per night:
Low - 0; moderate - 1; high - 2; don't know - 3.

46. Attitude towards residual spraying:
Negative - 0; moderate - 1; positive - 2.

47. Willingness to pay for residual spraying: No - 0; yes - 1.

48. Willingness to participate in community source-reduction control measures:
No - 0; yes - 1.

49. Use of repellent soap:

    Never - 0; occasionally - 1; often - 2; daily - 3.

50. Use of lotions:

    Never - 0; occasionally - 1; often - 2; daily - 3.

51. Use of nets/screens:

    Never - 0; occasionally - 1; often - 2; daily - 3.

52. Use of coils:

    Never - 0; occasionally - 1; often - 2; daily - 3.

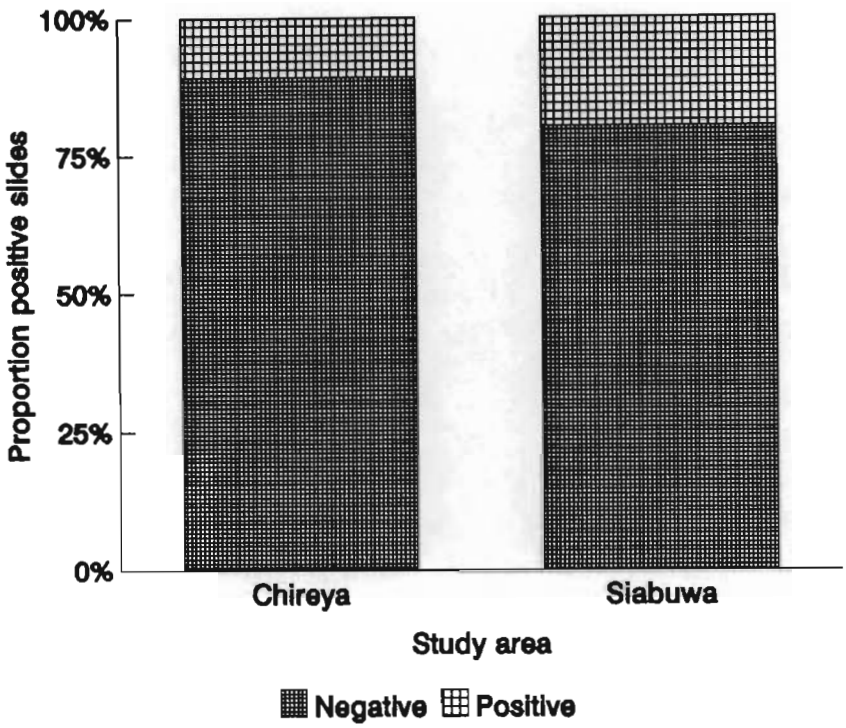
53. Comments from respondent:

    Suggestions - 0; questions - 1; criticism - 2.

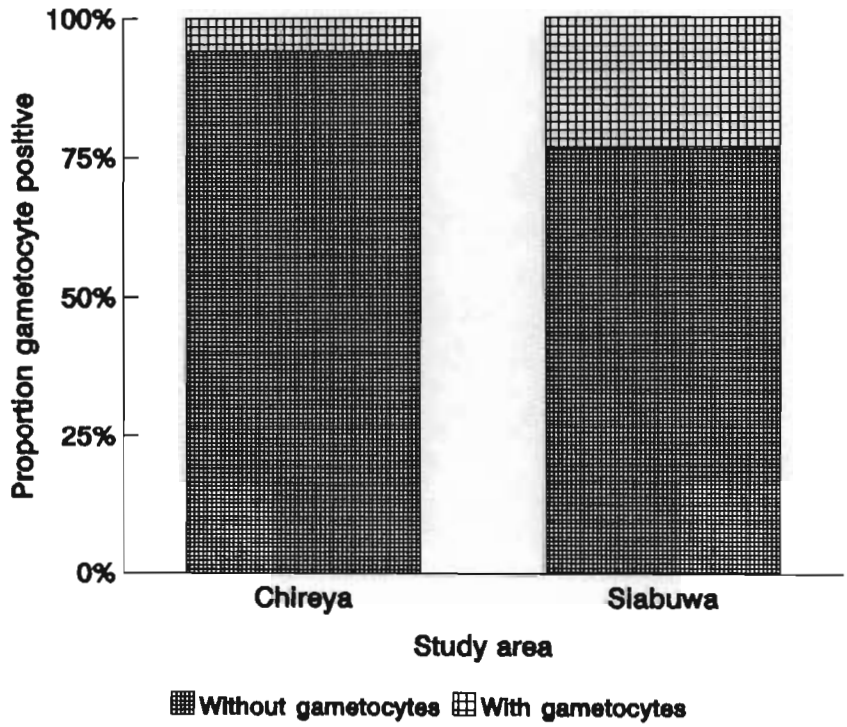
Thank you for your cooperation.

Appendix 3a.
Sketch map of the study area in Chireya showing the mosquito sampling stations

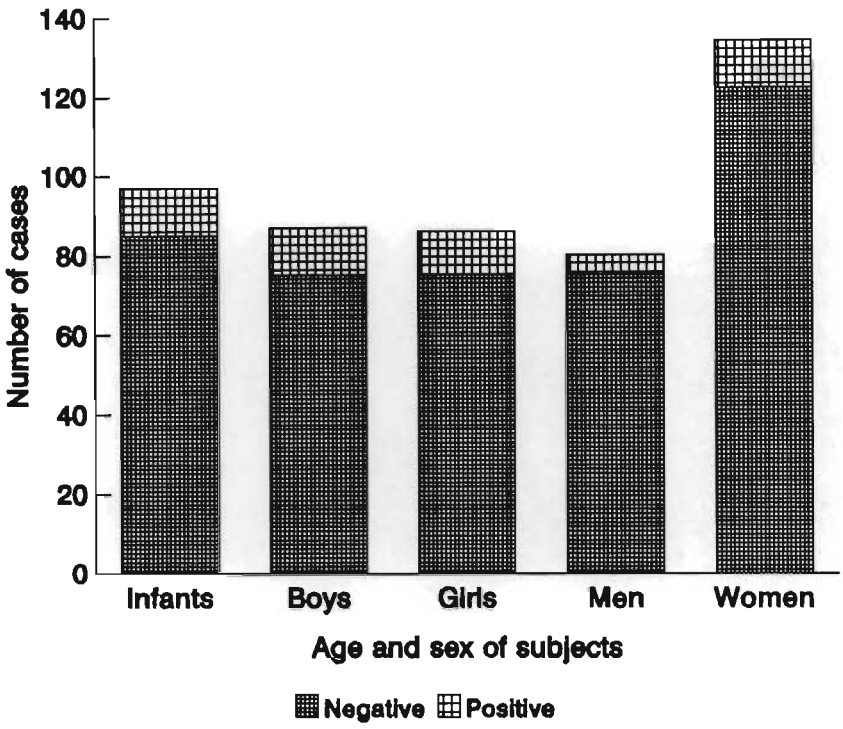
Appendix 3b.
Sketch map of the study area in Siabuwa showing the mosquito sampling stations



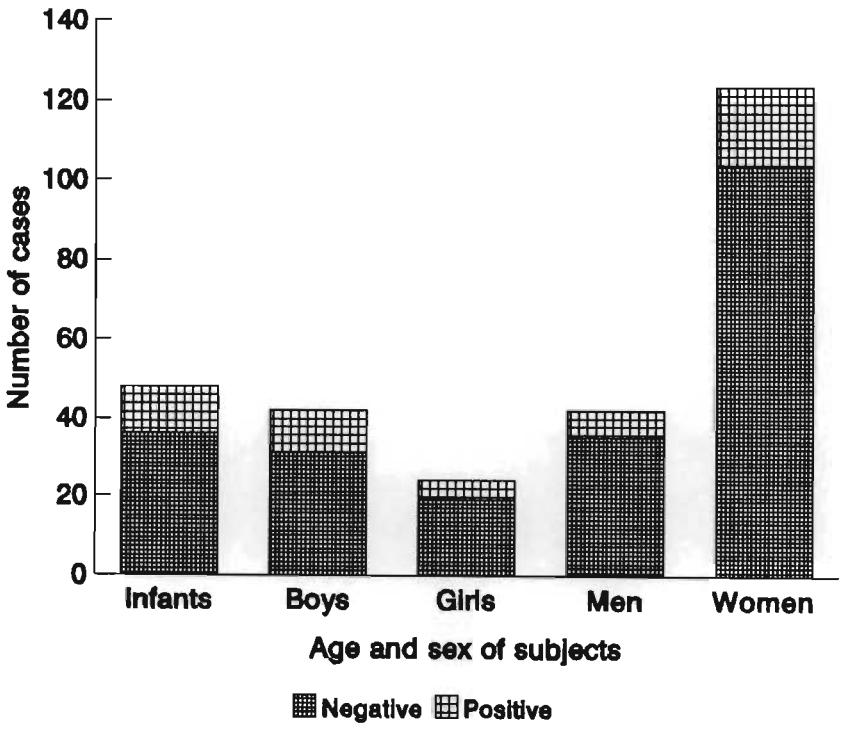
Appendix 4a. Malaria slide positivity in Chireya and Siabuwa; showing greater prevalence in Siabuwa



Appendix 4b. Gametocyte rates in Chireya and Siabuwa; showing greater gametocyte rate in Siabuwa



Appendix 4c. Malaria prevalence in Chireya; showing the similar infection rates in all age and sex groups except for comparatively lower rates in men



Appendix 4d. Malaria prevalence in Siabuwa; showing the similar infection rates in all age and sex groups

Appendix 5. Plates showing:

Plate I. Typical rural house in the study areas

Plate II. Typical cattle kraal,

Plate III. River bed excavation (*mufuku)*; ideal for mosquito breeding

Plate IV. Muirhead-Thomson pit shelter

Plate V. CDC light trap used

Plate VI. Muirhead-Thomson exit window trap

Plate VII. Mosquito bait-net described by Mpofu and Masendu (1986).

Plate VIII. Temporary breeding site derived from a hoof-print