GENETIC VARIATION IN WILD *ANOPHELES PHAROENSIS* THEOBALD (DIPTERA: CULICIDAE) OF MWEA IRRIGATION SCHEME.

BY

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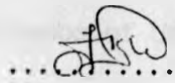
1989

DECLARATION

I hereby declare that the content of this Thesis is my original work and has not been presented in any other University.



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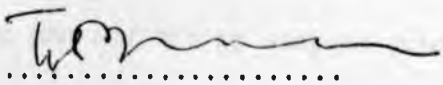

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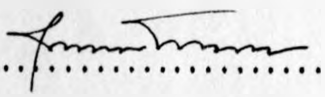

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DEDICATION

To my parents

Boaz and Grace Vulule

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ABSTRACT

Between October 1987 and June 1988, Anopheles pharoensis mosquitoes were sampled from four different villages in Mwea Irrigation Scheme, Kenya. Two major sampling methods were used, catching by miniature CDC light traps and pyrethrum spraying.

Laboratory colonisation was attempted with the mosquitoes collected from the wild. Female An. pharoensis showed reluctance to feed on a rabbit though they readily fed on human blood meal. Only females that were already gravid from the wild oviposited. Approximately 10% of the larvae matured into adults.

A notional estimate of polymorphism was established through an electrophoretic survey of four enzyme loci coding for β-Hydroxyacid dehydrogenase (β-HAD), α-Glycerophosphate dehydrogenase (α-GPDH), Aldehyde oxidase (AO) and Lactate dehydrogenase (LDH) from all the four villages. Variation was observed at all the loci. Mean heterozygosity ($\bar{H}$) for all the loci was 0.341 ±0.0653. The frequency of heterozygotes was much lower than expected at all the loci. From the results obtained, indications were that β-HAD had a dimeric enzyme structure since three band heterozygotes were observed. α-GPDH, AO and LDH molecules were monomeric in structure since the heterozygotes exhibited two band phenotypes.

Allele frequencies at all the loci for individual villages

were not in Hardy-Weinberg equilibrium. A possible explanation for this disequilibrium would be that each village does not constitute a distinct population of An. pharoensis. The possible reasons and implications of this observation are discussed.

Preliminary karyotype studies were also initiated by observation of ovarian polytene chromosomes from half-gravid females. The results did not establish a case for chromosomal polymorphism though more detailed studies remain neccesary.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Mosquitoes belong to the order Diptera. There are more than 3000 species of mosquitoes classified in 34 genera but less than 400 species in only 14 genera are regarded as pests or vectors of human diseases (White, 1982). Anopheles is one of the largest and best known genera. It has species of epidemiological significance in tropical and temperate parts of the world. Variation in geographic distribution according to season is a well established phenomenon in these mosquitoes.

Anopheles with few exceptions feed at night or in subdued light. Preference has been shown for warm blooded animals. Various species of Anopheles have been found to act as hosts for species of Dirofilaria and filarials especially Wuchereria bancrofti (Horsefall, 1955). Anopheles are also the main vectors of malaria.

Anopheles pharoensis Theobald belongs to the subgenus Cellia. It is common in nearly all unforested areas of Africa from the Nile delta to South Africa. It is also found in the Middle East. It is a medium sized cream coloured or greyish

anopheline with shaggy palps. It has tufts along each side of the abdomen. The palps have four main pale rings including one at the tip. It feeds on man inside and outside houses mainly at night and it rests outside amongst vegetation. It is an important local or occasional transmitter of malaria. It breeds mainly in swamps, ricefields and reservoirs (Gillet, 1971). In Kenya it is mainly found around the rice irrigation schemes (Mukiama and Mwangi, 1989a).

Malaria control measures in Africa have basically centred around spraying indoors with residual insecticides. This method has proved efficient in interrupting malaria transmission in regions where the vectors are endophillic. Transmission of malaria by exophillic mosquitoes cannot be effectively interrupted by this method since the vectors rest outdoors . Also, with vector populations becoming increasingly resistant to insecticides, more emphasis is being put on biological control. This in turn makes it neccesary for researchers to identify suitable markers in vector populations which can be used as parameters in studying the efficiency of various biological control measures. Genetic markers are thus becoming increasingly important in vector control. Enzyme mutants which can be detected using electrophoretic techniques can be effectively used as genetic markers in insect populations. This is feasible in An. pharoensis which is basically an exophillic mosquito and a suspected vector of malaria locally.

1.2 LITERATURE REVIEW

1.2.1 The ecology of An. pharoensis

An. pharoensis is endemic in Afrotropical regions (White, 1982). It is widely spread in Africa with its range extending from Egypt to South Africa and from Mauritania to Somalia (Gillies and de Meillon, 1968). An.pharoensis breeds in a variety of places, including rice fields (Khamala, 1971; Surtees, 1971); river fringes and swamps (Bevan, 1937; Lewis, 1948); lake shores and papyrus swamps (Bevan, 1937; Haddow, 1942; Garnham, 1945; Goma, 1960); inland swamps and reservoirs (Lewis, 1948, 1958; Goma, 1960); muddy puddles and open ponds (Khamala, 1971); and irrigated land and irrigation ditches (Lewis, 1948; El Safi and Haridi, 1986).

Mutero (1985), from his studies at Mwea, observed that out of 3447 mosquitoes sampled during the months of August - September 1983, An. gambiae s. l. formed 94.34%. Other anophelines, mainly An. funestus, An. rufipes and An. pharoensis comprised 1.65% of the catch. In the dry season of January 1984, no An. pharoensis were observed amongst the 334 mosquitoes sampled. He also did not observe any An. pharoensis in the samples caught from the Jimbo, Mishu and Jaribuni regions on the Kenyan coast.

An. pharoensis is strongly exophillic entering houses

mainly at night to feed (Haddow, 1942; Lewis, 1948; Smith, 1955; Krafsur, 1977; Ijumba, 1988; Mukiama and Mwangi, 1989a). This species nocturnal activity is a widely recognised phenomenon (Kerr, 1933; Haddow, 1942; Harney, 1960; Service, 1963; Haddow and Ssenkubuge, 1974). Daytime activity is normally restricted to resting outdoors amongst vegetation with only a small minority staying indoors (Gibbins, 1932; Lewis, 1948, 1958; Gillet, 1971).

Blood meal composition and biting behaviour in this species is well documented (Symes, 1931; Haddow, 1942; Lewis, 1948, 1958; Smith, 1955; Hammon 1963; Service, 1963; Garret-Jones, 1964; Garret-Jones et al., 1980; Chandler et al., 1975; Chandler et al., 1976; Krafsur, 1977; Boreham and Port, 1982; Ijumba, 1988; Mukiama and Mwangi, 1989a). Haddow (1942) observed that females entered houses mainly to feed and peak entry time was between 1200 and 0200 hours. On the contrary, Hamon (1963) caught 75% of all specimens in the first six hours of the night while Krafsur (1977) caught 72% of all specimens before midnight. Locally,in Mwea, Ijumba (1988) observed that peak biting activity occurred between 2200 and 2300 hours before the onset of short rains and between 1800-1900 hours during the short rain season. Two peak biting periods were observed during the long rainy season. Lewis (1958) noted that An. pharoensis was the principal man-biting anopheline outdoors in the Kosti area of Sudan in the evening hours but rarely bit indoors. Lewis (1948) also demonstrated its preference for man over calf. This is in contrast to Haddow (1942)

who demonstrated overwhelming preference for the calf. Garret-Jones et al. (1980) have reported an indoor sample of An. pharoensis from Ethiopia showing a man-fed index of 94%. Ijumba (1988), found that out of 26 blood meals tested in this species, 65.4% were positive for bovids, 11.54% were positive for humans and 3.85% were positive for dogs. This information indicates that An. pharoensis bites both man and bovids.

Females of An. pharoensis normally outlive the males because of a naturally longer lifespan and the ability to hibernate (Service, 1976).

1.2.2 Disease transmission potential of An. pharoensis

Vectorial capacity of An. pharoensis differs with different populations. Foote and Cook (1952) suggested that An. pharoensis could be an important vector of malaria in rice growing regions of Kenya based on its relative abundance. This species is a proven vector of malaria in Egypt and some West African countries (Gillies and de Meillon, 1968). It is treated as a secondary vector in many parts of Africa (Zahar, 1985a, 1985b), and is an important vector in Egypt (El Said et al., 1986).

Gibbins (1932) in dissections of 151 specimens of An. pharoensis caught around Jinja, Uganda, found monthly infection rates by Plasmodium to be between 2.7 - 8 %. Most of these

occurred in the stomach as oocysts. Krafsur (1977) on his work on An. pharoensis from Gambela region in Ethiopia, reported no infection rates in all the specimens dissected. Ijumba (1988) in his studies on the anopheline mosquitoes of Mwea irrigation scheme (Kenya), found none of the 85 specimens of An. pharoensis dissected during the short rains positive for sporozoites. All the 866 specimens tested by the ELISA method during this season were also negative. He also reports that the only two specimens dissected during the dry season were also negative. However, out of the 77 specimens tested using the ELISA in the long rain season, one was positive. He calculates the vectorial capacity of An. arabiensis in Mwea to be four times higher than that of An. pharoensis. From his estimations of vectorial capacity of An. pharoensis during the short rain season he states that a person was receiving at least one infective bite from this species every 33 days. Mukiama and Mwangi (1989a) on the other hand made an interesting observation in their studies of this species from the same region. Using the ELISA technique, they found that Plasmodium falciparum sporozoite rates for this species were 1.3%. These rates were found to be 0.68% by dissection. However for the ELISA technique these calculations were based on only 1 positive specimen out of a total of 77 specimens analysed while for the dissections this figure is based on only 1 positive specimen out of a total of 146 specimens dissected. These rates are however much lower than those observed in An. gambiae complex and An. funestus. Nevertheless, this would seemingly suggest the

possibility of An. pharoensis being a vector of malaria in Mwea especialy in view of the fact that it also feeds on man.

Garnham (1945) on observing variation in sporozoite infections in different populations of An. pharoensis suggested that varieties of the species with different vectorial capacities may exist. This is interesting especially in light of a recent publication by White (1982) in which it is observed that there are three species within the taxon An. pharoensis and only one of them is a vector of malaria.

1.2.3 Genetic variation in anopheline mosquitoes

Electrophoresis as a procedure for revealing genetic variation came into vogue in the late 1960s following the demonstration of its efficiency by Lewontin and Hubby (1966), Hubby and Lewontin, (1966) and Harris (1966, 1969).

Electrophoretic enzyme variation studies have been used for taxonomic purposes in several Diptera including Drosophila (Ayala and Powell, 1972) tephritid fruit flies (Berlocher, 1980, 1984) and black flies (May et al., 1977).

In Drosophila it has been demonstrated that Group 1 enzymes (those enzymes characterized by a singular physiological substrate which is usually generated and utilized intracellularly)

tend to be less variable than Group II enzymes, i.e. enzymes with multiple physiological substrates which reflect enviromental diversity (Gillespie and Kojima, 1968; Kojima et al., 1970).

In mosquitoes, enzyme variants have been considerably used for identification and seperation of various groups including the An. gambiae complex (Mahon et al. 1976; Miles, 1979), the An. maculipennis complex (Bullini and Coluzzi, 1978) and various Culex and Aedes species (Coluzzi and Bullini, 1971; Miles, 1976; Saul et al., 1977; Miles and Paterson, 1979; Munstermann, 1980). In Culex and Anopheles mosquitoes literature on enzyme variation and linkage relationships is summarised in the review by Narang and Seawright (1982).

Miles (1978), using electrophoresis worked on 22 enzyme systems in the An. gambiae . Six of the systems surveyed were found to exhibit inter- and / or intra-specific variation. Considerable information on enzyme characterisation in An. albimanus using electrophoretic studies is also available (Narang et al., 1981, 1983 and Narang and Seawright, 1983). Electrophoretic studies have also been carried out in anophelines and culicines to determine baseline esterase levels with respect to insecticide resistance (Hemingway and Georghiou, 1984).

The usefulness of electrophoretic studies of enzyme variation in mosquitoes has been discussed by various authors (Mahon

et al., 1976; Miles, 1978; Corsaro and Munstermann, 1984). Data derived from electrophoretic studies can be used in mapping of genes and elucidation of linkage groups. Enzyme variation can also be used in marking inversions and in localization of gene loci and chromosome breakpoints (Mukiama, 1987). This approach has been initiated in An. stephensi (Di Deco et al., 1978) and also has been extensively used in development of genetic sexing systems in An. albimanus for control purposes (Seawright et al., 1981). In biological insect control methods, isozymes could be used as genetic markers in sexing systems as follows: (1) as chromosome arm labels in special laboratory stocks, (2) for the identification of introduced or replacement strains, (3) for the measurement of introgression into target population after release and (4) for the assessment of migratory populations (Mukiama, 1983). With a genetic analysis follow-up of isozyme variants, all the above is feasible in An. pharoensis.

Locally, in Mwea irrigation scheme, genetic variation has been studied in An. arabiensis and some alleles found to be in Hardy-Weinberg equilibrium (Mukiama, 1987). No allozyme studies have been reported in An. pharoensis (White, 1982) and to date very few markers have been reported in this species.

Presently there are no reported studies on chromosomal polymorphism in An. pharoensis though this kind of polymorphism has been reported in a number of anopheline species notably the

An. gambiae complex (Coluzzi et al., 1979, 1985; Coluzzi, 1982, 1984; Mekuria et al., 1982; Mosha and Petrarca 1983; and Rishikesh et al., 1985).

1.2.4 The genetics of An. pharoensis

To date only five mutants have been reported in An. pharoensis (Kitzmiller, 1967, 1976). These are green larva (gl), white eye (w), non-stripe (st), DDT resistance gene (DDT) and dieldrin resistance gene (dl). Mason and Davidson (1966) have demonstrated both the green larva and white eye mutants of this species. Green larva is dependent on a single autosomal recessive gene linked with DDT and dieldrin resistance while white eye is sex linked and recessive. It is found in larvae, pupae and adults. Non-stripe is recessive, autosomal and is found in larvae and pupae. DDT resistance gene and dieldrin resistance gene are both autosomal. DDT resistance is suspected to be incompletely dominant, while dl is suspected to be recessive. The current knowledge of the genetics of An. pharoensis is summarised in the review by Narang and Seawright (1982).

There are three known species within the taxon An. pharoensis. Cross fertility is positive between some of the species and only two species are identifiable by cytotaxonomic methods using polytene chromosomes (White, 1982). Miles et al.

(1983), following cytogenetic observations on An. pharoensis, propose that there is a possibility of having two genetic species within this taxon.

1.3 The objectives of this study

1. To collect field samples of An. pharoensis and attempt laboratory colonisation.

2. To investigate the levels of genetic variation in wild samples of An. pharoensis by using electrophoretic techniques.

3. To carry out chromosomal observations on local members of the taxon An. pharoensis as a means to establish genetic composition of the species.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study area

Mwea Rice irrigation Scheme is situated 120km north-east of Nairobi. It is located at 0° 40° S, 37° 18° E near the foothills of Mount Kenya. The scheme covers approximatly 5,800 hectares of land and it sits astride one of the tributaries of river Tana called the Thiba river. The area is typically a riverine plain with an altitude of about 1500 metres above sea level. Annual precipitation averages 950mm and annual average temperature is 25°C. Maximum precipitation occurs in April/May and October/November. Humidity is between 50% and 80% with the higher levels occurring in the morning. Soils are of the black clay type. Other than cultivated vegetation, the area can be typically described as wooded grassland. Plate 1 shows the general study area.

3,235 families occupy the scheme (Ijumba, 1989) and their main occupation is rice growing. Other subsistence crops such as maize and beans are also cultivated. Horticultural crops are also grown in some areas.

Plate 1

Legend

A typical view of Mwea region with Mount Kenya in the background, a village and flooded paddies in the foreground.

Rice fields are flooded throughout the growing season from August to December. Water is drained off the paddies just before harvesting. Ploughing for the next season begins in March and this is done in phases. Flooding of paddies is also done in phases.

Mosquitoes were collected from three centrally located villages., Rurumi, Karima and Maendeleo; and another village Kimbimbi situated approximately 13 kilometres in a northerly direction from Maendeleo (Figure 1). The other three villages are situated 2-6 kilometres apart. Karima and Maendeleo are separated by rice fields and irrigation canals. In all the villages apart from Kimbimbi, rice fields are relatively close to human settlements. All the villages are densely populated with an average of seven persons per home. Houses are set 5-10 metres apart (Plate 2). In addition to human inhabitants, there are also bovids, donkeys, chickens and dogs. Collection of mosquitoes was done in two homes per village, the selection criteria being based on relative proximity to the rice fields.

2.2 Sampling techniques

Two major sampling methods were employed:

Light traps

Miniature CDC light traps (see plate 3) were placed in

Figure 1

A schematic representation of the Mwea Rice Irrigation Scheme showing the sampling sites.

Scale 1:88,000

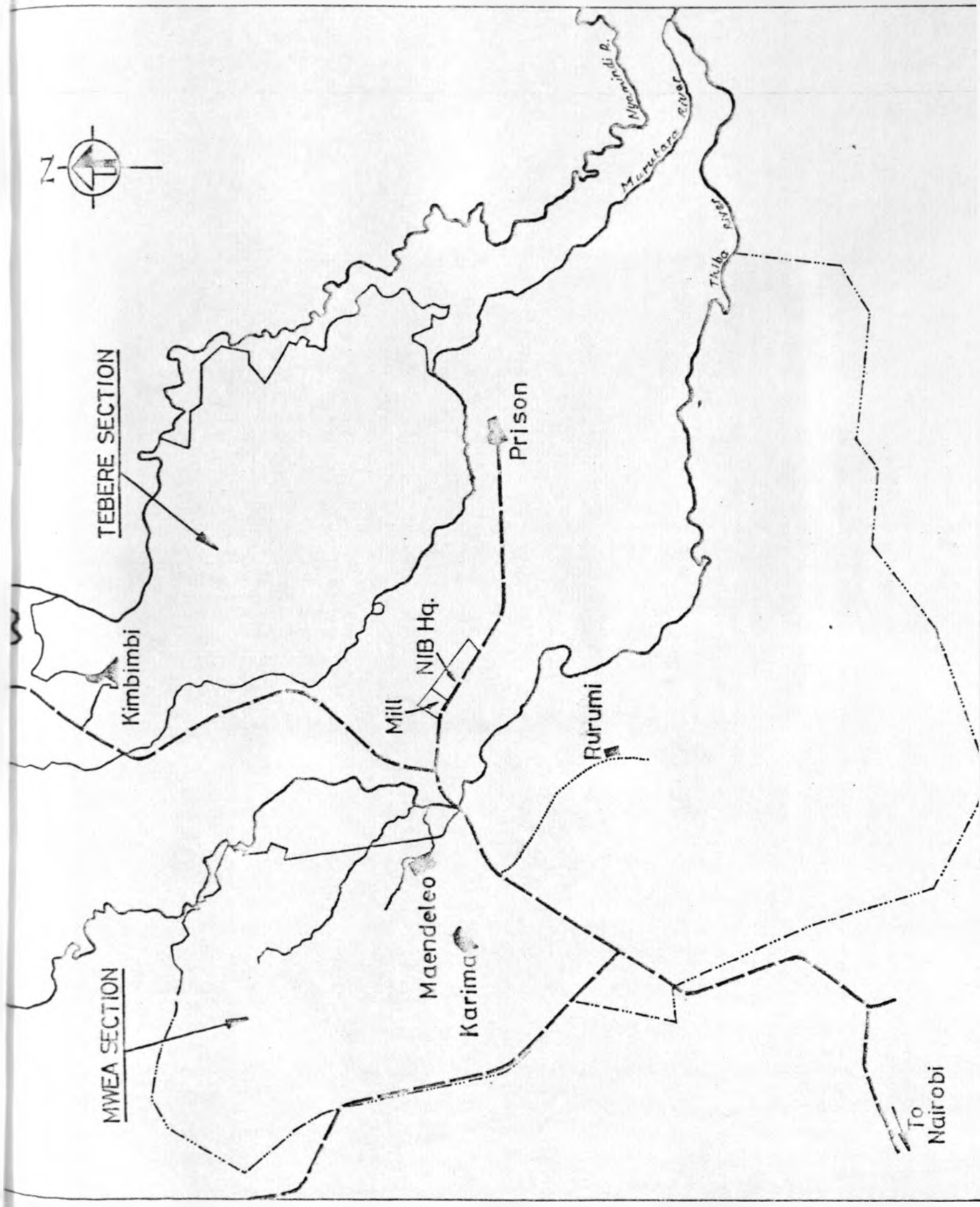


Plate 2

Legend

A typical homestead in Mwea Irrigation Scheme with a wooden house in the background (arrowed) where a light trap was placed overnight.

Plate 3

Legend

A miniature CDC light trap.

houses between 6.30p.m and 7.30a.m. Outdoor trapping was discouraged since light trap nets were normally damaged by moths. Trapping was also not done in kitchens and other smoky environs since mosquito numbers in such places were normally very low. Caught mosquitoes were aspirated into lantern chimmneys covered on either side with mosquito netting and transported to the laboratory.

Pyrethrum spraying

This was employed in houses where light traps had not been placed overnight. Spraying was done between 0730 and 0830 hours. Floors of houses to be sprayed were spread with white sheets. A local commercial preparation "Pyrethrol" was then sprayed and the doors and windows shut for 10 minutes. Knocked down mosquitoes were picked from the sheets and put into labelled McCartney bottles. These were placed in an ice box containing dry ice at approximately -20°C and transported to the laboratory.

2.3 Species identification

An. pharoensis was identified in the laboratory using ordinary taxonomic characters described by Gillies and de Meillon (1968).

2.4 Laboratory rearing

Mosquitoes were reared in cages 30 by 30 by 30cm in an insectary maintained at 28°C and 70% relative humidity.

Gravid females were placed in cages containing a petri dish filled with river water lined with 2 filter papers. Laid eggs were collected and put in rearing pans containing an inch depth of tap water. The trays were placed in an incubator set at 30°C until the eggs hatched. Larvae were transferred to new rearing pans containing the same depth of water and maintained at 30°C. This water was changed every three days. Rearing using distilled water and also river water was attempted but the two were found unsuitable. Larvae were fed on a commercial preparation "Tetramin" baby fish food and yeast in the ratio of 2:1, constituting a 2% suspension in water. Adults emerged in cages and were initially fed on a 10% glucose solution for three days. They were then bloodfed on a restrained white rabbit for 30 minutes once a day in the dark.

2.5 Electrophoretic procedures

Samples were run on a vertical electrophoretic system using a 7.5% resolving polyacrylamide gel and a stacking gel.

All gels and stock buffers were prepared according to the

following recipes outlined by Hames and Rickwood (1981) with slight modifications.

2.5.1 Gel buffer preparation

Stacking gel stock buffer.

6.0 grams of Trizma base was dissolved in 40 ml distilled water and the pH adjusted to 6.8 with 1M Hydrochloric acid (HCl). This solution was then made to 100 ml final volume with distilled water. The solution was then filtered thrpugh Whatman No. 1 filter paper and stored at 4°C. The final pH of the solution was 6.8.

Resolving gel stock buffer.

36.3 grams of Trizma base and 48.0 ml of 1M HCl were mixed and brought to 100 ml final volume with distilled water. The final pH of this solution was 8.8. Normally, this was adjusted using HCl to lower pH values corresponding with the optimum pH of the enzyme being assayed since the different enzyme systems resolved best at different pH values. This solution was filtered through Whatman No. 1 filter paper and stored at 4°C.

Reservoir stock buffer.

3.0 grams of Trizma base and 14.4 grams glycine were initially dissolved in 500 ml of distilled water and the final volume brought to 1000 ml using distilled water.

2.5.2 Electrophoresis

The apparatus used for the electrophoretic run was the Studier-type slab gel apparatus described by Hames and Rickwood (1981). This was locally made in the University Science workshop using Perspex while both the glass plates (notched and unnotched) were purchased from commercial dealers. All the Perspex spacer strips and comb were also made in the University science workshop. The spacer strips were 1.5mm thick while the comb had thirteen teeth.

An ISCO model 493 power pack was used for power supply throughout.

2.5.3 Preparation of slab gels.

Slab gels were prepared, with modifications according to techniques described by Hames and Rickwood (1981).

The Perspex spacer strips were slightly greased with "Vaseline" petroleum jelly and laid down on the sides and the bottom of the unnotched glass plate. The notched glass plate was carefully placed on top of this assembly taking care that the "Vaseline" only touches the plate edges. This plate assembly was then clamped together with strong metal clips positioned to press the sandwich over the spacer positions. The

whole assemblage was held vertically during the pouring of the gel which was done with the aid of a pipette.

The resolving gel mixture was prepared by adding the correct volumes of all the components as listed in Table 1 except TEMED which was added after degassing the solution for one minute. This solution was then poured into the space between the glass plates leaving a space of 3.5cm at the top for the stacking gel. After polymerization, which took place within 10-20 minutes, the stacking gel was prepared according to the protocol listed in Table 1. This was then added into the remaining space between the gel plates and the Perspex comb inserted immediately taking care not to trap air bubbles beneath it. This was left to polymerize after which the comb was removed and the wells rinsed with reservoir buffer. The gel was ready for use after removal of the bottom spacer strip.

2.5.4 Sample preparation for electrophoresis

The technique used here was basically that of Steiner and Joslyn (1979), with slight modifications. Whole mosquitoes were placed in a nylon block containing 8 rows and 8 columns of 1/4 inch holes and about 1/3 of an inch deep. This block had previously been kept in a fridge at 4°C overnight.. A nylon peg of 1/4 inch diameter was then inserted into the holes and used to homogenise the samples. 100 ul of 1:1 ratio glycerol to

Table 1

Preparation of stacking and resolving gels.

The various ingredients for both the stacking and resolving gels were mixed in the following proportions as indicated:

	Gels	
Ingredients	Stacking	Resolving
Acrylamide-bisacrylamide (30:0.8 i.e 30 grams of acrylamide and 0.8 grams of bisacrylamide in 100ml water)	2.5 ml	7.5 ml
Resolving gel buffer	-	3.75 ml
Stacking gel buffer	5.0 ml	-
1.5% ammonium persulphate	1.0 ml	1.5 ml
Water (distilled)	10.0 ml	17.25 ml
NNN'N-Tetramethyl ethyleneadiamine (TEMED)	0.015 ml	0.015 ml

water at 4°C was used in homogenising the samples.

50 ul of sample homogenate was loaded into individual wells using a microlitre syringe. A marker dye (Bromophenol blue) was then introduced into one of the wells.

2.5.5 The electrophoretic run.

The whole glass plate assemblage was then attached to the electrophoretic apparatus using "Vaseline" and strong metal clips. Reservoir buffer was then poured into both the lower and upper tanks of the apparatus until both the lower and upper ends of the gel were in contact with the buffer. The whole system was then connected to a power supply and put in a refrigerator at 4°C. A current of 20mA was then applied for 30 minutes. This enabled the samples to stack at the point of contact between the stacking and resolving gels. A constant current of 40 mA was then applied. Distance migrated by the samples was estimated from the distance migrated by the marker dye. When the marker dye had migrated 10cm from its point of origin, the power supply was disconnected and the gel recovered for staining.

2.6 Stain buffer preparation and enzyme staining.

Buffer stock preparation and actual staining were according to the following techniques described by Steiner and Joslyn (1979) with slight modifications. Lactate dehydrogenase staining was adapted from Othmar (1972) with modifications.

2.6.1 Stain buffer preparation

Trizma buffer pH 8

24.2 grams of Trizma base and 11.8 ml concentrated HCl were mixed with 1000 ml of distilled water.

Trizma buffer pH 7.5

12.1 grams of Trizma base and 7.5 ml concentrated HCl were mixed with 1000 ml distilled water and the pH adjusted to 7.5.

Wash solution

10% glycerol was mixed with 8% glacial acetic acid to the required volume.

2.6.2 Enzyme staining

(i) β-Hydroxyacid dehydrogenase (β-HAD) E.C. 1.1.99.6

To 35 ml of Trizma-HCl buffer pH 8 the following were

added;

(a) 100mg of D-Gluconic acid sodium salt.

(b) 10 ml sodium chloride (10gr NaCl/100 ml distilled water).

(c) 0.5 ml of magnesium chloride (2.03gr MgCl/100 ml distilled water).

(d) 2 ml Nicotinamide adenine dinucleotide (NAD) 1gr NAD/100 ml distilled water.

(e) 1 ml 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) 1gr MTT/100 ml distilled water.

The solution was poured over the gel and the gel incubated for 1 hour at 37°C. 0.5 ml Phenazine methosulfate (PMS) 1gr PMS/100 ml distilled water was then added. Incubation was carried out for another 30 minutes and the gel was then immersed in the wash solution for 30 minutes and observed.

(ii) α-Glycerophosphate dehydrogenase (α-GPDH) E.C. 1.1.1.8

To 35 ml of Trizma-HCl buffer pH 7.5, the following were added;

(a) 50mg DL-α-glycerophosphate Disodium salt

(b) 1 ml MTT (1gr MTT/100 ml distilled water)

(c) 2 ml NAD (1gr NAD/100 ml distilled water)

The solution was poured over the gel and incubated at

37°C for 30 minutes. 0.5 ml PMS (1gr PMS/100 ml distilled water) was then added and the gel incubated further for a period of 1 hour. The gel was then put in a wash solution for 1 hour.

(iii) Aldehyde oxidase (AO) E.C. 1.2.3.1

To 35 ml of Trizma-HCl buffer pH8, the following were added;

(a) 0.5 ml benzaldehyde

(b) 2 ml MTT (1gr MTT/100 ml distilled water)

This was poured over the gel and incubated for 30 minutes at 37°C. 0.5 ml PMS (1gr PMS/100 ml distilled water) was then added followed by a further incubation period of 15 minutes. The gel was then put in a wash solution for 1 hour and observed.

(iv) Lactate dehydrogenase (LDH) E.C. 1.1.1.28

To 35 ml of Trizma-HCL buffer pH 8, the following were added;

(a) 300mg DL-lactic acid Lithium salt

(b) 2.0 ml NAD (1gr NAD/100 ml distilled water)

(c) 1 ml MTT (1gr MTT/100 ml distilled water)

The solution was poured over the gel which was then incubated for 1 hour at 37°C. 0.5 ml PMS (1gr PMS/100 ml distilled water was added followed by a further incubation period of 30 minutes. The gel was removed and put in a wash solution for 1 hour and observed.

2.7 Enzyme nomenclature.

The genetic nomenclature used for the identification of allozymes (hence alleles) was that of Ayala et al. (1972). In this method the commonest allozyme from a region is arbitrarily designated 100. Other allozymes are accordingly designated 101, 102, 103, etc. if their mobility was +1mm, +2mm, +3mm etc. faster than the commonest allele i.e. 100. Similarly, other alleles are designated 99, 98, 97 e.t.c. if their mobility was -1mm, -2mm, -3mm etc. slower than allele 100.

2.8 Data analysis

2.8.1 Calculation of allele frequencies (after Lewontin and Hubby, 1966).

The proportion of all alleles at the locus of interest gives a notion of the genetic variation. The frequency of any prescribed allele in a sample is equal to twice the number of homozygotes for that allele plus the number of heterozygotes for that allele divided by the sample size. Consequently, allelic frequencies were calculated as follows: If in a population sample of 20 individuals, 10 were homozygous for the allele β-Had^{100} (i.e genotype 100/100) and the other 10 heterozygous for the allele β-Had^{90} and β-Had^{100}

(i.e genotype 100/90), then since every allele is represented twice in a diploid individual, a total of 40 alleles were sampled.

The frequency of $\underline{\beta\text{-Had}}^{100}$ is;

$(10 \times 2) + (10 \times 1)/40 = 0.75$

while that of allele $\underline{\beta\text{-Had}}^{90}$ is;

$(10 \times 1)/40 = 0.25$.

The standard error for this estimates was obtained by the established formula; S.E. $= \sqrt{r(1-r)}/n$ where r is the estimate and n the sample size.

2.8.2 Calculation of heterozygosity values.

Heterozygosity values for the various enzymes from different villages were calculated after Nei and Roychoudhury (1973) using the formulae;

$H = hi/r$

where $hi = 1 - \sum x^2 i$

and r = no. of loci examined, and

x = frequency of the i^{th} allele.

2.8.3 Calculation of goodness of fit (after Bailey, 1981)

To determine whether different enzyme loci were in Hardy-Weinberg equilibrium for the different villages, the following methods were used:-

(a) Calculation of expected number of individuals for every allele of a given enzyme from earlier calculated gene frequencies:

If the frequency of allele $\underline{\beta\text{-Had}}^{100}$ was 0.75 in a population of 30 individuals, then the expected number of individuals to be homozygous $\underline{\beta\text{-Had}}^{100}$ was calculated as follows:

$(0.75)^2 \times 30 = 16.875$ individuals. Similarly, expected number of individuals homozygous for the allele $\underline{\beta\text{-Had}}^{90}$ if its frequency was 0.25 would be:-

$(0.25)^2 \times 30 = 1.875$ individuals while the expected number of heterozygotes would be:-

$2(0.25 \times 0.75) \times 30 = 11.25$ individuals.

(b) A Chi-Square analysis was then used to calculate the goodness of fit using the formulae:

$$\chi^2 = \Sigma(O-E)^2/E$$

whereby O = observed number of individuals

E = expected number of individuals.

The answer would then be tested using χ^2 tables at 5% significance level and 1 degree of freedom to determine if the deviation was significant.

2.9 Chromosomal preparations

2.9.1 Stain preparation

5 grams orcein was dissolved in 125ml of acetic acid. This procedure took 2-3 hours after which 125ml lactic acid was added and allowed to dissolve for 20 minutes. The stain was then filtered and ready for use.

2.9.2 Carnoys fixative preparation

Mosquitoes for chromosomal observations were preserved in Carnoys fixative at 4°C. The fixative was made as follows: Ethanol and acetic acid were mixed in the ratios of 3 ethanol to 1 acetic acid.

2.9.3 Mosquito dissection and chromosome staining

This procedure was modified from Coluzzi and Sabatini (1967). Half gravid females were transferred from the fixative into a solution of distilled water in a petri dish, then to a microscope slide. The ovaries were dissected out using pins and a dissecting microscope. A drop of 50% propionic acid was put onto the ovaries and left for 2 minutes. A drop of stain was then added and left for 5 minutes. The stain was washed off using 50% propionic acid and excess stain wiped off. A cover

slip was placed on the slide and tapped about 30-40 times with the blunt end of a pencil, then squashed under a blotting paper using the palm and excess stain wiped off. Small quantities of lactic acid were added along the edge of the coverslip to prevent evaporation and the slide examined.

2.10 Gel and chromosome photography

Gels were photographed using an "Ilford Pan F" film of speed 50 while chromosome photography was done using an "Ilford Fp4" film.

CHAPTER 3

RESULTS

3.1 Laboratory Rearing

Wild collected females of An. pharoensis showed marked reluctance to feed on a restrained rabbit in the dark though they readily bit on a human forearm placed on top of the cage even in light conditions. Gravid wild caught females oviposited after an average of 2-3 days while females bloodfed in the laboratory oviposited after an average of 6 days. Larvae hatched 2-3 days after oviposition but in some cases, this took up to 4 days. Instar stages took an average of 2 days each and pupation occurred between the 10th and 12th day after egg hatch. Pupal duration averaged 3 days after which adults emerged.

In the first rearing trial, out of the second batch of eggs, only five pupae survived to give rise to adults. This represented approximately 10% of the initial number of 1st instar larvae.

In the second rearing trial, out of an original 97 larvae, only 10 adults were realised. This represented approximately 10% of the original number of 1st instar larvae.

In both cases, the laboratory emerged mosquitoes failed to bloodfeed both on a restrained rabbit and on a human forearm. They died after an average of 10 days in the insectary.

One green larva mutant was observed during the rearing experiments, but died at the fourth instar stage. Larval mortality was highest between the second and third instar stages, with generally very few deaths occurring during the first instar stage.

3.2 Enzyme electrophoresis

3.2.1 β-Hydroxyacid dehydrogenase (β-HAD)

Two β-Hydroxyacid allozymes were scored. All the samples assayed from the four different locations in Mwea exhibited single band homozygous slow (S) or fast (F) phenotypes, or three band heterozygotes (SF). The fast allele was the most common and thus was designated 100. The slow allele was designated 90. Table 2 shows the frequency of the two different alleles from the four different locations in Mwea.

In all the cases observed, the homozygous β-Had^{100} and β-Had^{90} exihibited a dark single conspicuous band while the heterozygous β-Had$^{100/90}$ had three equally stained lighter bands. After the electrophoretic run, allele β-Had^{100} was detected 4.5cm from the point of sample origin while allele β-Had^{90} was detected 3.5cm from the point of its sample origin. In the case of the heterozygotes i.e β-Had$^{100/95}$ the central band was observed 4.0cm from the point of sample origin. The band phenotypes are shown in Plate 4.

Table 4 shows the number of individuals observed as being SS (allele 90/90), FF (allele 100/100) and SF (alleles 100/90) for the individual locations and the number of individuals expected to

Plate 4

Legend

Band phenotypes at the β-Had locus

Key:

1,3,4 and 9 are FF (i.e β-Had$^{100/100}$)

2,6 and 7 are SF (i.e β-Had$^{100/90}$)

5,8 and 10 are SS (i.e β-Had$^{90/90}$)

K = Karima

Ki = Kimbimbi

M = Maendeleo

R = Rurumi

1	2	3	4	5	6	7	8	9	10
K	K	K	Ki	Ki	Ki	R	R	M	M

Samples

be of genotypes SS, FF, and SF as calculated from the allele frequencies.

Heterozygosity values for the individual villages are indicated in Table 6. Allele frequencies in individual villages were found to be in Hardy-Weinberg disequilibrium with data showing significant deviations as indicated on Table 7.

3.2.2 α-Glycerophosphate dehydrogenase (α-GPDH)

Only two allozymes, slow (S) and fast (F) were observed from the four different locations. The phenotypes were either single band homozygotes or two band heterozygotes (SF). Allele F was designated 100 while the other allele was designated 95. Table 2 shows the frequency of the two alleles from different locations in Mwea.

In the homozygous condition, alleles, α-Gpdh^{100} and α-Gpdh^{95} exihibited darkly stained bands, while the heterozygous α-Gpdh$^{100/95}$ bands were of a lighter intensity. In some cases these two bands were not easily discernible and had to be interpreted using a luminous gel reader. Allele α-Gpdh^{100} was normally detected 3.6cm from the point of sample origin while allele α-Gpdh^{95} was detected 3.1cm from the point of sample origin. The band phenotypes are shown on Plate 5.

Plate 5

Legend

Band phenotypes at the α-Gpdh locus

Key:

1,2,3,4,5,6,7,9 and 10 are FF (i.e α-Gpdh$^{100/100}$)

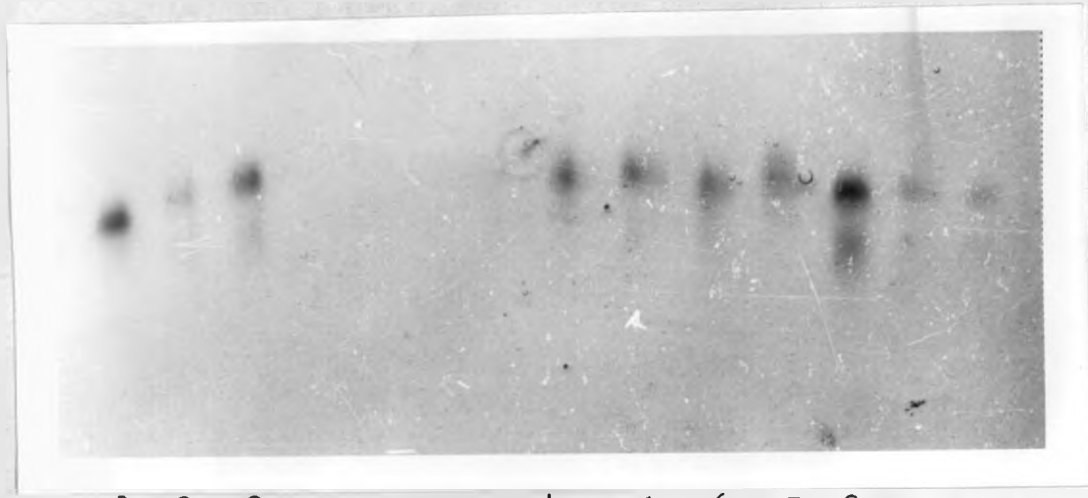
8 is SF (i.e α-Gpdh$^{100/95}$)

K = Karima

Ki = Kimbimbi

M = Maendeleo

R = Rurumi



1	2	3	4	5	6	7	8	9	10
K	K	K	Ki	Ki	Ki	R	R	M	M

Samples

Table 4 gives both the number of individuals observed and expected to be SS, FF, and SF. The expected number of individuals is calculated from the allele frequencies.

Heterozygosity values for the various locations was as indicated on Table 6. Allele frequencies from all the locations were in Hardy-Weinberg disequilibrium (Table 7).

3.2.3 Aldehyde oxidase (AO)

Two allozyme variants of this enzyme were detected. The mosquito specimens typically resulted in two band heterozygous (SF) phenotypes or single band homozygous SS or FF phenotypes. Allele $\underline{Ao}^{100}$ was the fast type. The slow allele was designated $\underline{Ao}^{95}$. The frequencies of the two alleles from different locations in Mwea are given in Table 3.

The fast band was detected 4.3cm from the point of sample origin while the slow band was normally detected 3.8cm from the point of its sample origin. Plate 6 shows the band phenotypes.

The number of sample specimens observed as being SS (allele 95/95), FF (allele 100/100) and SF (alleles 100/95) are given on Table 5. The same Table gives the number of individuals expected to be SS, FF, and SF.

Plate 6

Legend

Band phenotypes at the Ao locus

Key:

2,3,5,6,7,8,9,10,11 and 12 are FF (i.e $Ao^{100/100}$)

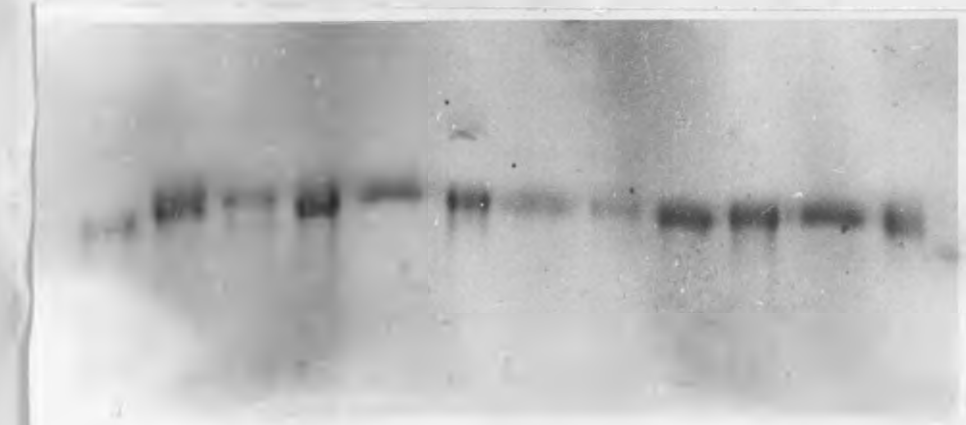
1 and 4 are SS (i.e $Ao^{95/95}$)

K = Karima

Ki= Kimbimbi

M = Maendeleo

R = Rurumi



1	2	3	4	5	6	7	8	9	10	11	12
K	K	K	Ki	Ki	Ki	R	R	R	M	M	M

Samples

Table 6 shows the heterozygosity values of this enzyme from the individual locations in Mwea. Allele frequencies for this enzyme from individual locations in Mwea were not in Hardy-Weinberg equilibrium. This is indicated in Table 8.

3.2.4 Lactate dehydrogenase (LDH)

There were two lactate dehydrogenase allozymes present. Band phenotypes were either single band SS or FF homozygotes or two band heterozygotes (SF). The fast allele ($\underline{Ldh}^{100}$) was the most common while the slower allele was designated $\underline{Ldh}^{96}$. The frequencies of the two alleles from the four different locations in Mwea are given on Table 3.

Homozygotes exihibited darkly stained band phenotypes while heterozygote bands were lighter in colour. The fast band was usually detected 3.2cm from the point of sample origin while the slow band was detected 2.8cm from the point of its sample origin. Plate 7 shows the band phenotypes.

Table 5 gives the number of individuals observed and the number expected as SS (allele 96/96), FF (allele 100/100) and SF (alleles 100/96).

Heterozygosity values for individual locations were as

Plate 7

Legend

Band phenotypes at the Ldh locus

Key:

1,2,3,4,5,6,7,9,11 and 12 are FF (i.e $Ldh^{100/100}$)

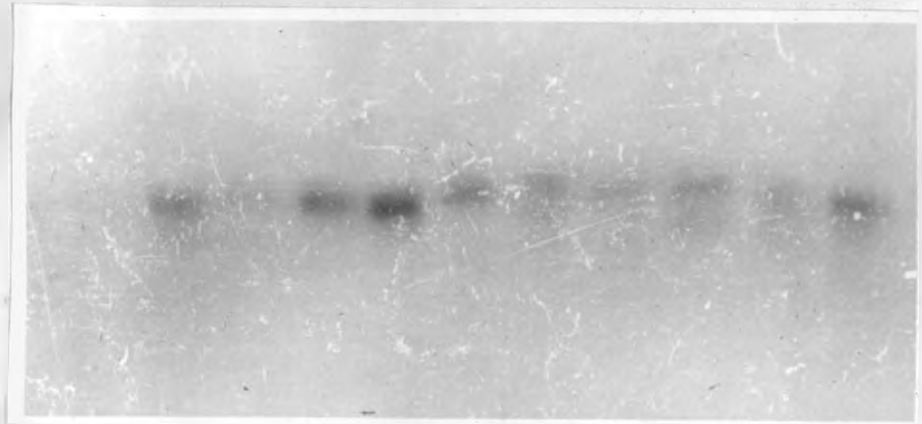
8 and 10 are SF (i.e $Ldh^{100/96}$)

K = Karima

Ki = Kimbimbi

M = Maendeleo

R = Rurumi



1	2	3	4	5	6	7	8	9	10	11	12
K	K	K	Ki	Ki	Ki	R	R	R	M	M	M

Samples

Plate 8

Legend

Ovarian polytene chromosomes of An. pharoensis. The X-chromosome is arrowed.

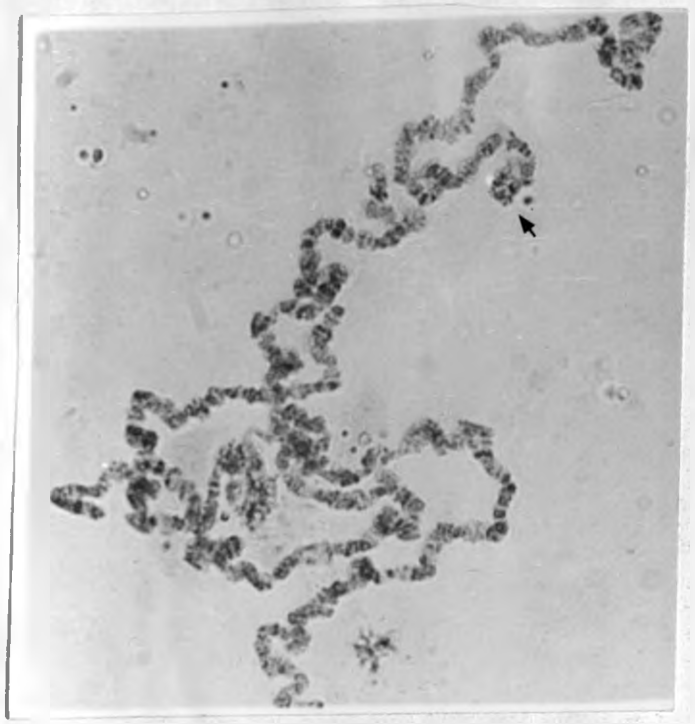


Plate 9

Legend

Ovarian polytene chromosomes of An. pharoensis showing the banding patterns.

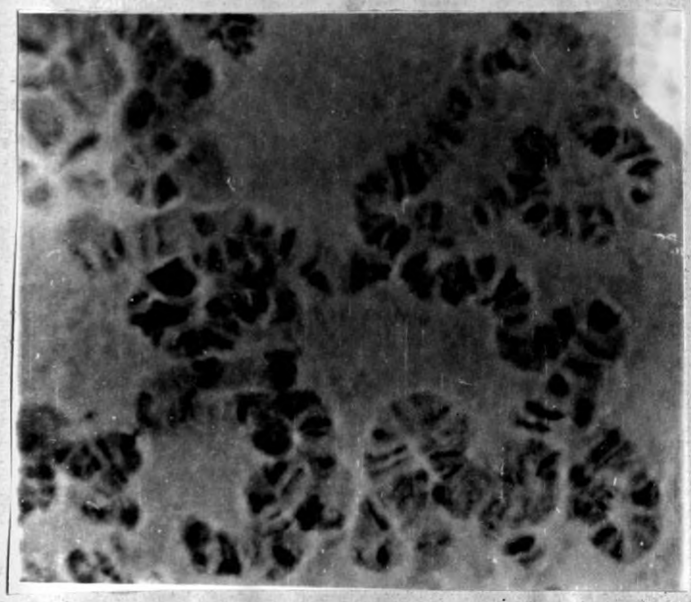


Table 2

Allele frequencies at the β-Had and α-Gpdh, loci in An. pharoensis

Locus β-Had

		Alleles		S.E.
Village	n	100	90	
Karima	51	0.549	0.451	0.0697
Kimbimbi	51	0.461	0.539	0.0698
Rurumi	55	0.545	0.455	0.0671
Maendeleo	51	0.520	0.480	0.0700

Locus α-Gpdh

		Alleles		S.E.
Village	n	100	90	
Karima	64	0.852	0.148	0.0444
Kimbimbi	64	0.789	0.211	0.0510
Rurimi	64	0.840	0.160	0.0458
Maendeleo	59	0.670	0.330	0.0612

Table 3

Allele frequencies at the Ao and Ldh loci in An. pharoensis

Locus Ao

		Alleles		S.E.
Village	n	100	95	
Karima	54	0.770	0.230	0.0573
Kimbimbi	54	0.926	0.074	0.0356
Rurumi	54	0.852	0.148	0.0483
Maendeleo	42	0.810	0.190	0.0605

Locus Ldh

		Alleles		S.E.
Village	n	100	96	
Karima	44	0.807	0.193	0.0595
Kimbimbi	44	0.886	0.114	0.0479
Rurumi	44	0.852	0.148	0.0535
Maendeleo	48	0.771	0.229	0.0606

Table 4

Observed and expected* numbers of individuals at the β-Had and α-Gpdh enzyme loci for An. pharoensis

Village	Karima	Kimbimbi	Rurumi	Maendeleo
Locus β-Had				
n	51	51	55	51
genotypes				
100/100	21 (15)	16 (11)	22 (16)	19 (14)
90/90	16 (11)	20 (15)	17 (12)	21 (12)
100/90	14 (25)	15 (25)	16 (27)	11 (25)
Locus α-Gpdh				
n	64	64	64	59
genotypes				
100/100	52 (47)	49 (40)	50 (45)	38 (27)
95/95	7 (1)	12 (3)	7 (2)	18 (6)
100/95	5 (16)	3 (21)	7 (17)	3 (26)

*Expected values are in parenthesis

Table 5

Observed and expected* number of individuals at the Ao and Ldh enzyme loci for An. pharoensis

Village	Karima	Kimbimbi	Rurumi	Maendeleo
Locus Ao				
n	54	54	54	42
genotypes				
100/100	36 (32)	48 (46)	38 (39)	31 (27)
95/95	7 (3)	2 (0)	5 (1)	5 (2)
100/95	11 (19)	4 (8)	11 (14)	6 (13)
Locus Ldh				
n	44	44	44	48
genotypes				
100/100	35 (29)	39 (34)	37 (32)	37 (29)
96/96	8 (1)	5 (1)	6 (1)	11 (2)
100/96	1 (14)	0 (9)	1 (11)	0 (17)

* Expected values are in parenthesis.

Table 6

Heterozygosity values at the β-Had, α-Gpdh, Ao and Ldh loci in An. pharoensis from four different villages in Mwea Rice Irrigation Scheme.

Location/Locus	β-Had	α-Gpdh	Ao	Ldh	Mean H	S.E.
n	208	251	204	180		
Karima	0.495	0.252	0.354	0.310	0.353	±0.0655
Kimbimbi	0.497	0.333	0.137	0.202	0.292	±0.0623
Rurumi	0.496	0.269	0.252	0.252	0.317	±0.0632
Maendeleo	0.499	0.442	0.308	0.353	0.401	±0.0693
			overall	$\bar{H}$	0.341	±0.0653

Table 7

values with 1 d.f. at the .05 significance level for β-Had, α-Gpdh, for An. pharoensis from four villages of Mwea Rice Irrigation Scheme.

Locus β-Had

Location	n		P	D.F
Karima	51	9.512	*	1
Kimbimbi	51	7.939	*	1
Rurumi	55	8.815	*	1
Maendeleo	51	16.376	*	1

Locus α-Gpdh

Location	n		P	D.F
Karima	64	44.094	*	1
Kimbimbi	64	44.453	*	1
Rurumi	64	18.937	*	1
Maendeleo	59	48.828	*	1

* Significant deviation

Table 8

values with 1d.f. at the .05 significance level for Ao and Ldh in An. pharoensis from four villages of Mwea Rice Irrigation Scheme.

Locus Ao

Location	n		P	D.F
Karima	54	9.202	*	1
Kimbimbi	54	12.489	*	1
Rurumi	54	16.668	*	1
Maendeleo	42	8.862	*	1

Locus Ldh

Location	n		P	D.F
Karima	44	62.313	*	1
Kimbimbi	44	25.735	*	1
Rurumi	44	34.872	*	1
Maendeleo	48	59.707	*	1

* Significant deviation.

CHAPTER 4

DISCUSSION

An. pharoensis was a difficult specimen to rear in the laboratory, with the adult females showing marked reluctance to feed on a restrained rabbit. This was also noted by Theordor and Parsons (1945). However, the mosquito readily fed on human blood when the forearm was placed in the cage. This occurred readily at any time of the day even under light conditions. Similar observations were recorded by Theodor and Parsons (1945). Klein et al., (1982) on colonizing strains of An. nivipes and An. phillippinensis from Thailand found that An. nivipes showed reluctance to feed on a hamster but avidly fed on a human blood meal. The same authors report that in the initial colony, less than 10% of both strains fed on hamsters.

Gravid wild An. pharoensis females laid eggs after an average of 2-3 days. This is in close agreement with the findings of Ijumba (1988) on this species, who observed that eggs were oviposited after an average of 2.4 days. Klein et al. (1982) working on strains from Thailand found that 98% (241 specimens) of An. phillippinensis oviposited within 1-6 days while 2% (5 specimens) oviposited within 7-10 days. It was observed that females bloodfed in the laboratory oviposited after an average of 6 days but this could take up to 8 days. In this study, egg hatch

CHAPTER 4

D I S C U S S I O N

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took place within 2-3 days after oviposition, larval instar stages took an average of 2 days each and pupation took place between the 10th and 12th days. Mukiama (1987) observed that emergence in An. arabiensis occurred after the 9th day. Adult mosquitoes hatched in the laboratory, survived an average of 10 days in an insectary maintained at 28°C and 70% relative humidity.

The high larval mortality recorded in this study could be attributed to either lack of optimum conditions for this species in the laboratory especially temperature and relative humidity, or the diet. A third possibility could be the tap water which was used in rearing the larvae. Due to the fact that tap water is chlorinated, larvae might not have found it a suitable medium. All the larvae died when rearing was attempted using river water and also distilled water. In the former, this could possibly have been as a result of micro-organisms rapidly colonizing the river water. The rapid proliferation of the micro-organisms might have lead to depletion of oxygen in the water therefore making it an unsuitable medium for larvae. In the case of distilled water, larval death may have been due to lack of optimum conditions in the laboratory.

In this study, approximately 10% of the 1st instar larvae matured into adults. Mukiama and Mwangi (1989b), on their field studies of larval An. arabiensis Patton of Mwea Irrigation Scheme, estimated pupal productivity for the Mwea rice fields to be 1% of the total immature population.

One green larva mutant was observed during the rearing. This mutant has also been demonstrated by Mason and Davidson (1966) and its mode of inheritance described in the review by Narang and Seawright (1982).

Theodor and Parsons (1945) attributed An. pharoensis initial reluctance to feed to low populations which did not facilitate swarming since initially, few specimens were put in the cages for rearing. In this study, it would have been desirable that greater population numbers be introduced into the rearing cages to see if this would have an effect on the feeding patterns.

Electrophoretic enzyme variation was detected at all the four enzyme loci assayed from the four different villages in Mwea. All the four loci were dimorphic.

β-Hydroxyacid dehydrogenase which mediates the oxidation of β-hydroxyacids (Narang and Seawright, 1983) was observed to have either single band homozygotes (β-Had$^{100/100}$, β-Had$^{90/90}$) or three band heterozygotes (β-Had$^{100/90}$). A three band heterozygous phenotype indicates a dimeric enzyme structure. This has been suggested for a variety of enzymes in mosquitoes notably Alcohol dehydrogenase (ADH) in An. stephensi (Iqbal et al., 1973); Aldehyde oxidase (AO) in An. albimanus (Narang and Seawright, 1982); β-HAD in the same

(Narang and Seawright, 1983); Octanol dehydrogenase (ODH) in An. gambiae and An. arabiensis (Miles, 1978); 6-Phosphogluconate dehydrogenase (6-PGD) in An. albimanus (Narang et al., 1983) and ADH, AO, β-HAD, Isocitrate dehydrogenase (IDH), ODH and 6-PGD in An. arabiensis Patton (Mukiama, 1987). The same has also been suggested for ODH, α-Glycerophosphate dehydrogenase (α-GPDH) and Malate dehydrogenase (MDH) in three species of tsetse flies (Gooding, 1981).

No nulls were observed for β-HAD. Null variants have been observed in field populations of other Anopheles species thus indirectly suggesting that β-HAD is dispensable in anophelines (Narang and Seawright, 1983).

α-Glycerophosphate dehydrogenase, Aldehyde oxidase and Lactate dehydrogenase exihibited single band homozygotes or two band heterozygotes. This indicates that their molecules are monomeric in structure. The same has also been suggested for esterases in An. albimanus (Narang and Seawright, 1982), Phosphoglucomutase (PGM) in the An. gambiae complex (Miles, 1978; Bullini and Coluzzi, 1978), esterases in An. arabiensis (Mukiama, 1987) and PGM in the same (Mukiama, 1987). Gooding (1981) also suggests this structure for Alkaline phosphatase (ALKPH) in three species of tsetse flies.

Data for molecular structure of enzyme systems for various

Anopheles and Culex mosquitoes is summarised in the review by Narang and Seawright (1982).

Heterozygosity values for the different enzymes varied from village to village (see Table 6). Overall mean heterozygosity was 0.341 ± 0.0653. β-HAD was the most variable enzyme while AO was the least variable. The mean heterozygosity observed in this study was much higher than that found by Dobzhansky et al. (1977) which was 0.134 for 51 species of invertebrates. Miles and Paterson (1979) in their studies on the Culex pipiens group of species found the mean heterozygosity to be 0.296 ± 0.036. The greatest heterozygosity occurred in Cx. pallens Coq with a value of 0.349. Tabachnick et al. (1979) found the mean expected heterozygosity from six populations of East African Aedes aegypti at three seperate gene loci for hexokinase-2, hexokinase-3 and hexokinase-4 to be 0.348 while the mean expected heterozygosity at the Mdh loci was 0.452. The mean heterozygosity over the four enzyme loci in this study compares favourably.

The high heterozygosity values in this species from Mwea could be attributed to a high degree of genetic diversity, possibly due to the fact that An. pharoensis is endemic in the Afro-Tropical regions, and thus well established locally. Introduced populations generally exihibit low genetic diversity as observed by Tabachnick and Powell (1979) who found lack of genetic

diversity in Asian populations of Ae. aegypti because of its recent introduction from East Africa. The four enzyme loci examined in this study were chosen non-randomly and it is therefore misleading to extrapolate from the average heterozygosity of allozymic variation observed here to a total genomic heterozygosity of An. pharoensis from Mwea Irrigation Scheme. A study over a larger number of enzyme loci remains desirable to see if this would differ with the mean heterozygosity observed in this study.

An. pharoensis is known to make mass migrations over long distances. Such tendencies would favour genetic substructuring of its population, thus introducing greater degrees of heterozygosity. Harrington et al. (1984) observed that the genetic homogeneity in Houston populations of Ae. aegypti could be partly attributed to the short flight range (2km) of this species, a factor which would reinforce any tendency to become genetically substuctured.

All individual village populations were found to be in Hardy-Weinberg disequilibrium for the different enzyme systems sampled (Tables 7 and 8). At every locus there was a deficiency in the number of heterozygotes (Tables 4 and 5). This peculiar observation can be attributed to a number of factors since was hypothesized that individual villages would be in Hardy-Weinberg equilibrium for the various enzyme systems. For a population to

meet Hardy-Weinberg expectations, assumptions are that mating has to be random, there should be no selection, no random genetic drift, no mutations and no differential migrations.

The genus An. pharoensis consists of 3 subspecies of which two can be distinguished by cytotaxonomic methods using polytene chromosomes (White, 1982). Miles et al. (1983) on their studies of ovarian polytene chromosomes in this species from 8 different regions in Africa, observed two different X-chromosome arrangements. Notable, was the lack of heterozygotes for these arrangments. Crosses between the two produced sterile F1 males suggesting that the two X-chromosome arrangments mark two species within this taxon. Should a possibility of having more than one subspecies of this mosquito in Mwea exist, then two or more reproductively isolated subspecies might have been misclassified as belonging to one population. This would interfere with the goodness of fit in Hardy-Weinberg equilibrium since in such a population, mating would not be random, but rather there would be reproductive isolation between the different subspecies. Callard et al. (1986) made similar observations on two cryptic species of Psychodophygus. Using cellulose acetate electrophoresis, they compared eleven enzyme systems of the two species. Two enzyme systems were completely diagnostic. Three other loci gave evidence of reproductive isolation. At the Phosphoglucomutase locus, P. carrerai departs from Hardy-Weinberg equilibrium. It was proposed that this might be due to natural selection or

lack of random mating which could be a result of recent gene flow or two or more reproductively isolated populations within this genus.

Deviations from the Hardy-Weinberg equilibrium can also be expected if the four different locations from which the specimens were collected constitute one freely intermixing population other than four different stable panmictic populations as earlier expected. Garret-Jones (1950) observed that An. pharoensis is capable of making mass migrations using the wind. He sampled An. pharoensis 18-28 miles from their nearest known breeding ground. Ijumba (1988) in his studies on anophelines from Mwea using mark-release-recapture method, caught a single female bloodfed An. arabiensis 2km upwind from its point of release. No detailed studies of this nature are reported for An. pharoensis. It is possible that not only An. arabiensis migrated, but also An. pharoensis especially considering the fact that this species is a strong flier. In view of the fact that some of the villages sampled were only 3km apart, this strongly increases such a possibility. The four different villages would then constitute one population and an attempt to subdivide it into four distinct populations would possibly result in Hardy-Weinberg disequilibrium. However, it was observed that pooled data still showed significant deviations from the Hardy-Weinberg equilibrium thus suggesting that other factors may be responsible for the Hardy-Weinberg disequilibrium observed.

Detailed studies involving a larger number of specimens remains necessary.

An. pharoensis has been found breeding for up to nine months a year in Mwea rice irrigation scheme (Mukiama and Mwangi, 1989a). During the course of this study, samples of An. pharoensis were caught over three seasons : the short rainy season, the long rainy season and the dry season in between the two. Most of the specimens were caught during the short rainy season while only two specimens were caught in the months of January and February. Mutero, (1985) caught no samples of An. pharoensis in Mwea during the month of January 1984. The long rainy season did not yield high numbers of An. pharoensis. The scarcity of this species in the months of January-March was due firstly to the lack of rains, and secondly to the fact that the rice fields were not flooded thus ensuring that there were no larval breeding grounds. Mukiama and Mwangi (1989a) found that the flooding phase in August maintains the link between the long rainy season and the short rainy season providing a continuous breeding ground for the mosquitoes. It would have been desirable to catch a larger number of An. pharoensis during the long rainy season. A comparison between the samples caught during the long rains and the short rains could then be made to find out whether the variation observed was seasonal. Dobzhansky and Ayala (1973) reported seasonal variation at the Phosphoglucomutase-1 and Malic-2 enzyme loci in D. pseudoobscura and D. persimilis.

Only two sampling techniques were employed in catching mosquitoes from all the four different villages. Using the spray catch method, it was observed that smoky houses yielded very few numbers of mosquitoes. Ijumba (1988) also observed the same. During the short rains, An. pharoensis constituted approximately 10% of light trap catches. Studies on An. arabiensis of Mwea by Ijumba (1988) revealed that there were two populations of this species during the long rainy season. One was endophillic and the other exophillic. However, these two populations were indistinct during the short rainy season. No similar observations were made on An. pharoensis. It would be desirable to see if the same phenomenon does exist in An. pharoensis since this might affect variation.

Seasonal studies on karyotype distribution and their frequencies and also karyotype studies involving indoor and outdoor resting specimens of An. pharoensis remain desirable. Coluzzi et al. (1979) observed in the An. gambiae complex that certain inversion karyotypes did not distribute at random in relation to human environment but rather were specific being significantly more frequent in indoor than outdoor samples, or vice versa. Seasonal karyotype variation has also been observed in indoor resting An. gambiae in Nigeria (Rishikesh et al., 1985).

The fact that the number of heterozygotes observed at all loci was less than expected might seem to signify that mating in this species was not random. Either there was reproductive isolation or selection against heterozygotes existed. Miles et al. (1983) observed that there is a possibility of having two genetic species within the taxon An. pharoensis. White, (1982) observed that three species are known within this taxon and cross fertility is positive between some of them. There still remains a possibility that more than one species of this taxon does exist locally and that these species might be reproductively isolated.

The few slides prepared for chromosomal observations were not satisfactory. Bands were clearly discernable and could be used in mapping of the chromosomes. The X-chromosome was also observed in some slides. Nevertheless, it was not possible to see inversions or separate chromosomes into their distinct arms since the squash preperations were not well spread out. Hamed et al. (1973b) have shown the salivary gland chromosome complement of An. pharoensis larvae. Miles et al.(1983) from their genetic observations on this taxon observe that there exists a possibility of having two species within the taxon. The slides prepared during the course of this study did not facilitate similar observations and thus did not rule out the possibility of subspecies existing within this genus locally. Should that be the case, the other factors discussed in this study would only be

secondary in contributing to the Hardy-Weinberg disequilibrium. However, studies on a larger sample size remain neccesary.

CONCLUSIONS

Rearing of An. pharoensis in the laboratory proved to be difficult thus curtailing more effective studies on this species. Laboratory colonisation therefore remains a priority in order to carry out more effective genetic observations.

All the four enzymes studied from four different locations in Mwea were found to be variable. The different village populations were found to be in Hardy-Weinberg disequilibrium for the different enzyme systems tested and there was always a deficiency in the number of heterozygotes observed at all the loci.

Chromosomal studies were not conclusive and it remains neccesary that more extensive karyotype studies be carried out to see whether more than one subspecies of An. pharoensis does exist locally.

The observations of this study suggest that An. pharoensis from Mwea does not form different stable panmictic populations in the various villages studied. More information on the ecology of this species in Mwea still remains desirable especially data on its flight range.

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