931-0535/52 Semi-Annual Progress Report

Year 2 July - Dec. 1972

PD-AAL-139

TO: Office of Science and Technology
U.S. Agency for International
Development--Washington, D.C. 20523

SUBJECT: Research Progress Report, Semi-annual

1. Project Title:

Ecological studies on Aedes aegypti in East Africa Preliminary to Genetic Control. Contract No. AID/csd 3159.

2. Principal Investigator and Contractor:

Professors G.B. Craig, Jr. and K.S. Rai
Department of Biology
University of Notre Dame
Notre Dame, Indiana 46556

3. Contract Period:

3 years, 1 July 1971 - 30 June 1974

4. Period Covered by Report:

2nd year, 1st half
1 July 1972 - 31 December 1972

5-8. Fiscal Data:

Not included in this report

9. Narrative Summary - Accomplishments:

In the first half of year 2 of this project, the staff has been completed and both laboratory and field site are well-established. Primary emphasis has been on monitoring specific village populations of A. aegypti to provide year-long baseline data on population dynamics. Life table data and patterns of movement and dispersal have been accumulated by capture-mark-release-recapture experiments. Earlier release experiments have been replicated in other villages. Experiments on the outdoor feral portion of the population have been conducted in outdoor cages and on canopy towers in a forest. It was shown that some gen exchange occurs between indoor and outdoor populations in the wet season but not in the dry season. In experiments to develop chromosome translocation stocks for genetic control,72 new translocations have been isolated in a Kenya background and two of these may now be available in homozygous condition. First attempts at mass production of mosquitoes for trial releases has been successful. We have accomplished colonization of four species of Aedes, all potential disease vectors, that have never been colonized in the laboratory before. Finally, an MBU consultant, Dr. W. Kilama of the Medical Faculty at Dar has shown that A. aegypti could be a significant vector of filariasis in East Africa.

Staff

By May 1972 the MBU staff was complete. No new people were appointed after that, although the numbers of casual helpers employed on a day to day basis for particular field experiments increased slightly towards the end of the year. The recruitment of local helpers was very valuable, especially when working in the villages and will continue in the future at an even slightly higher level.

By June most members of our ancillary staff had reached a satisfactory level of efficiency and competence. In the second half of this year we could therefore test them all individually at special tasks and entrust some of them with entire experiments or supervisory functions in field work. Based on their varying performance we reached the conclusion that we could reduce our staff by one laboratory and one field assistant without risk of losing efficiency. We expect rather that in future the remaining permanent staff will benefit through increased individual guidance. With upcoming changes due to the progress in some of our research projects our ancillary staff will be reduced by two people by the end of January 1973.

Laboratory facilities

In the past few months the MBU laboratory has experienced some significant changes:

1. The ICIPE governing board has decided that as of July 1, 1972, the laboratory of the Mosquito Biology Unit in Mombasa should be called ICIPE Coastal Research Station. This implies that one or two other ICIPE research projects will be accomodated on the same premises in the future.

2. The garage has been transformed into a mass production insectary.

3. A workshop for the construction of cages and laboratory furniture has been built in the garden surrounding the laboratory.

4. As agreed in the lease contract the rented building has been repainted inside and outside.

In order to accomodate a radiation source some additional minor changes will have to be carried out following specifications of the Kenya Radiation Protection Officer.

Equipment

1. MBU has finally obtained its third field vehicle, a Jeep Wagoneer. The vehicle is extremely comfortable on good roads and promises to be as tough and versatile in back country as the two Land Rovers. In view of its higher speed and greater comfort it will, however, mainly be used for long distance safaris and commuting between Nairobi and Mombasa.

2. A radiation source has been purchased and is now on the high seas. The arrival is expected for the beginning of February. The radiation physicist of the Kenyatta National Hospital will assist us with the clearing of the source through customs and with the dosimetry.

Relations

Visitors to MBU:

The flow of visitors experienced in the first half of 1972 continued at a steady pace also through the second half. A list of these visitors is attached. Especially mentioned should be the following:

July - August: Mr. S. K. Sempala, a medical entomologist of E.A. Virus Research Institute, Entebbe, stayed and worked with us for two weeks. During this period he got acquainted with our genetic marker stocks, our rearing techniques and our marking methods for mark-release-recapture experiments. His accounts of the bionomics of various *Stegomyia* in different parts of Uganda on the other hand provided much stimulation to us.

August: Ms. R. McDonald, a former laboratory technologist of Prof. Craig at Notre Dame and now with the Birkbeck College, University of London, spent her four weeks of leave with the McDonalds in Mombasa. She came to our laboratory almost daily and introduced our technicians to the acetone technique for preserving and mounting mosquitoes, and to other intricacies and finesses of mosquito work. We herewith wish to express our thanks to her for this to us most profitable visit.

August - September: Dr. Milan Trpis from the Vector Biology Laboratory of the University of Notre Dame was our only Project Consultant during the second half of 1972 (see annex 5). During his visit of 10 days we discussed several aspects of supportive research which is carried out at Notre Dame, especially with regard to host seeking behavior of the different *A. aegypti* populations found in the Rabai area. We also experimented with a simplified form of mosquito trap designed originally by Dr. Gouck from USDA, Gainsville Laboratories.

It ought to be mentioned especially that Dr. Trpis' visit to the E.A. Virus Research Institute in Entebbe, made during the same trip, was exceedingly well received. Both Dr. Mukwaya and Mr. Sempala have expressed their hope that future consultants to MBU would also pass by in their institute.

November: Mr. Philip Bebora, entomological field assistant of the Division of Vector Borne diseases, Ministry of Health, is working with us for a planned period of 6 months. During this time Mr. Bebora should learn the rearing of *Aedes aegypti* and other mosquitoes.

He should also become acquainted with various methods of catching mosquitoes in the field, with mark-release-recapture techniques, and with the recognition of mutations in A. aegypti. He should also learn to test mosquitoes for insecticide resistance. In addition he is expected to expand his basic knowledge in medical entomology.

So far Mr. Bebora has adjusted very well to our laboratory work. In the coming weeks he will now also become involved in field work.

Visits made from MBU:

Every month one or the other of the MBU scientists paid a visit to ICIPE headquarters in Nairobi. Since August these visits were coordinated with the monthly meeting of the ICIPE Science Council of which the MBU Project Leader or his representative is an ex officio member. These monthly visits serve not only to settle upcoming business matters but are also essential to maintain our close communication with the ICIPE scientists. The visits in Nairobi were usually combined with visits to the Division of Vector Borne diseases of the Kenya Ministry of Health and with contacts to teachers and students of the University of Nairobi.

In addition to these regular contacts Dr. D. Fanara paid a return visit to Mr. S. Magayuka in Amani and Mr. C. Garret-Jones in Tanga.

In November and December the project leader, Dr. W. Hausermann, having completed almost two years in Kenya, went on home leave for six weeks. 10 days of this period were spent at the Vector Biology Laboratory of Prof. Craig. Following this far too short stay at Notre Dame four days were spent attending the meeting of the Entomological Societies of America, Canada and Quebec in Montreal. Valuable new contacts were made during this time.

On the way back to Kenya a prolonged stopover in Switzerland permitted to visit the Swiss Federal Research Station for Plant Protection in Wadenswil. Here the sterile male technique had been successfully applied against the white grub cockchafer Melalontha vulgaris (Horber 1963) and plans are worked out to try the method against the cherry fruit fly Rhagoletis cerasi.

During the visits in USA and Switzerland two seminar talks were given, one in the Department of Biology at the University of Notre Dame and the other at the Swiss Tropical Institute in Basel. The first results of the research activities of MBU were the subject of the talks.

Research

The research work during the past six months has principally followed the project outlines presented in Annex I of the first Annual Research Report. The progress made in the various projects is discussed along these outlines.

Project 1: Routine monitoring of A. aegypti in Rabai location. Responsibility: W. Hausermann

For almost a year now, we have systematically monitored the fluctuations of the A. aegypti populations inside and outside houses. The following picture starts to form:

1. The indoor populations of A. aegypti are fluctuating widely from week to week or between villages in the same week. Seasonal trends, however, are almost absent, with perhaps one exception. Just after heavy rainfalls in May a series of peak landing-biting catches were made in June. These peaks coincided with a heavy build up of the outdoor population and are due to mosquitoes from outdoors entering houses (see also: Teesdale 1955; Van Someren et al. 1958; Fanara, pers. communication).

2. The outdoor populations of A. aegypti are naturally dependant on the rainfall and when being assessed in ovitrap collections, showed oviposition peaks about 2 weeks after rainfalls filled a large number of larval habitats. The highest oviposition peaks were observed in June after the heaviest rainfalls of the year in the second half of May.

It is notable also that during the observation period a shift in the species composition collected in ovitraps occurred. During the colder part of the year - May to August - A. metallicus was the second most common Stegomyis behing A. aegypti, while since November A. simpsoni has taken over this position and is even competing with A. aegypti for the number one spot.

It was decided to terminate the routine monitoring at the present scale by the end of January 1973, i.e. after 12 months of observations. However, in view of the rather common experience that every year is special, it is planned to combine an investigation into shifts in gene frequencies in indoor and outdoor populations with the collection of some census data from at least one village.

Project 2: Preparation of life tables for indoor and outdoor populations of A aegypti. Responsibility: W. Hausermann

The first mark-release-recapture experiment carried out in April and May 1972 is still under evaluation. The problem of dispersal and female insemination in the first few days after eclosion which was pointed out at that time was tackled in special experiments since.

In two release experiments, one with field produced A. aegypti and the other one with F_1 hybrids of two laboratory strains it was established that a) female insemination starts about 30 h after eclosion as in the laboratory and b) the percentage of inseminated females rises at a slower pace than in the laboratory. 72 hours after eclosion still less than 80% of the recaptured females were inseminated while in the laboratory cages close to 100% of the females were inseminated by this time (Gwadz 1968).

Dispersal from house to house also started about 30 hours after eclosion and within less than 96 hours after eclosion 32 and 36% of all mosquitoes recovered in the two experiments had dispersed to other houses. Trends for

different dispersal between sexes and between the native and the laboratory produced mosquitoes were noticed and will have to be studied in subsequent experiments.

Dr. McDonald has carried out an additional mark-release-recapture experiment in which he marked only newly emerged adults. The results of this experiment are presented in Annex 2. As the first mark-release-recapture experiment it indicated also an abnormal mortality in the first 24 hours after marking. After this first day the 24 hours survival rates corresponded closely to the results of the first experiment with about 0.89 for females and 0.62 for males.

Projects 3 and 4: Environmental definition of indoor and outdoor habitats and behavioral relationships between domiciliary and extra-domiciliary populations of *A. aegypti*. Responsibility: D.M. Fanara.

In the 1972 "Annual Research Report" this researcher reported on a pilot mark-release-recapture study. Since that experiment the three known strains of *Aedes aegypti* from the Changombe Location were colonized and a more extensive and intensive experiment was conducted. The more notable results are:

1. The marked, domiciliary, pale *A. aegypti* distributed itself throughout Mwamsabu Village after release. The numbers captured in any given house reflected more the attractiveness of the house to the mosquito than to the numbers released. The highest biting density of marked mosquitoes was recorded for a house in which no release was made, but which had a high density of *A. aegypti*. Figure 1 (Annex 3) lists the greatest distances into the forest travelled by this domiciliary strain. Both males and females were attracted to man in the forest. Figure 2 (Annex 3) depicts a simplified map of the location. The indoor landing-biting pattern (figure 3, Annex 3) of the unmarked domiciliary *A. aegypti* was sharply bimodal and contrasts sharply with the pattern of the marked strain. The distinctly contrasting landing-biting patterns may indicate that colonization and/or marking has influenced the behavior of *A. aegypti*.

2. An intermediate (in appearance) form of the extradomiciliary Bejumwa strain was co onized from larvae siphoned from a treehole during the wet season (Bejumwa Wet). Releases of this strain and the dark strain (Bejumwa Dry) were made at 50 meters intervals along the streambed (A through H, see figure 2 of Annex 3). The intermediate extradomiciliary strain ("marked" in figure 4, Annex 3) had a landing-biting pattern in the forest similar to that of the "unmarked" dark sylvan form. It was also taken indoors (33% for males and 38% for females). It appears that this strain may be the genetic link between the dark sylvan form and the pale domestic form of *A. aegypti* during the wet season.

3. The marked, colonized, dark, extradomiciliary Bejumwa Dry strain was recaptured in very low numbers (Figure 1, Annex 3). Eight males were captured in houses, but no females were taken indoors.

Current Projects:

1. A large, portable field cage (80 cm x 160 cm x 320 cm) has been erected in the Bejumwa Forest. It is being used for further studies into

the biting behavior of the dark extradomiciliary A. aegypti.

2. A 13 meter tower has been constructed into the canopy of the Bejumwa Forest. Marked males were captured at 7 meters altitude.

3. Three different genetic markers are being bred into the colonized strains of Changombe Location mosquitoes. An experiment will be conducted into the possibility of gene flow between strains.

4. An artificial mosquito resting site has been constructed in Bejumwa Forest.

5. An accurate detailed map of the Changombe Location, including Mwamsabu Village and Bejumwa Forest has been completed.

6. A series of experiments are being conducted in cooperation with Dr. W. Wood of the International Centre for Insect Physiology and Ecology in Nairobi. These experiments concern the attractiveness of water soluble extracts from the wood of treeholes utilized by A. aegypti.

Project 5: Genetic relation between indoor and outdoor population.
Responsibility: W. Hausermann

Between February and April 1972, when the hot dry season was at its peak, the A. aegypti collected with ovitraps placed outdoors, corresponded almost entirely to the color definition of ssp. formosus. The A. aegypti sampled from indoors represented the whole range from dark to pale A. aegypti type form. Although the mutants gold mesonotum and spot abdomen were encountered regularly in indoor samples, the mutants never occurred in the same individual as is the case in var. queenslandensis.

During the rains, which lasted intermittently from May to December this picture of two separate populations changed. A. aegypti type form mostly from the dark end of the range was quite commonly found in outdoor habitats and contributed about equally with ssp. formosus to the rise in outdoor oviposition registered in June.

Parallel to the increase of the outdoor population the indoor population registered a slight increase as well. Simultaneously dominant characters like black cuticle or black scales became more frequent in indoor samples and recessive characters like spot abdomen almost disappeared. It was therefore concluded that during the rains A. aegypti aegypti mated with ssp. formosus and exploited all available outdoor habitats at one end and mated and oviposited in indoor habitats on the other end, thus establishing a gene flow from ssp. formosus to indoor populations.

The infrequent presence of white scales on the first abdominal tergites in what was otherwise clearly ssp. formosus indicated at the same time a limited gene flow in the other direction. This gene flow was probably channeled mainly through the dark type form A. aegypti found in outdoor and indoor habitats. However, a single encounter of a ssp. formosus female displaying heterozygosity for the mutant gold mesonotum was very likely the result of a direct cross between a highly domestic A. aegypti and ssp. formosus.

According to Mattingly (1957) A. aegypti ssp. formosus varies from mainly domestic to fully wild and the type form from domestic to semi-wild behavior. Var. queenslandensis in turn is fully domestic. It seems that in the Rabai area each of the infraspecific categories cover the whole of its known adaptive range. This was demonstrated for instance in the host preference of laboratory strains established from field collected material. In both ssp. formosus and type form some Rabai strains showed a preference for man as host while others preferred birds or rodents over man (Trpis, personal communication).

In the past year sufficient evidence has been gathered to demonstrate a limited genetic exchange between wild A. aegypti ssp. formosus and domestic A. aegypti aegypti. However, attempts to estimate the rates of exchange in both directions have failed so far, partly because time was lost in search of known color mutants which could be used for such a study, but mainly because not enough time was available. With the planned reduction of routine monitoring program more time will be available for a study on gene frequencies and genetic exchange in domestic and wild A. aegypti.

Experiments to demonstrate the gene flow between indoor and outdoor populations through introduced genetic markers are planned by Dr. Fanara.

Projects 6 and 7: Production of translocation heterozygotes and selection of homozygotes and genetic engineering of release strains for translocations and sterile males. Responsibility: P.T. McDonald.

Work continues on the isolation of translocations, the maintenance of translocated stocks and the testing for homozygosis. To date the number of translocations recovered is 72, of which 28 are T(1:2), 14 are T(1:3), 26 are T(2:3) and 4 are multiple T(1:2:3). Of these 72 translocation stocks, 61 have begun the two-generation process of analysis for homozygosis at least once. Several have been tested for many generations. Results on 43 are in. Of these 43, one has given viable homozygotes but was lost subsequently. Two other, which have been analyzed just recently, appear to be giving homozygotes in preliminary testing.

The Africanization of marker strains has continued with three strains from indoors and three strains from outdoors. All are above 90% Africanized. Additional marker stocks have been added to this program.

Projects 8 and 9: Mass rearing for releases and release strategies. Responsibility: W. Hausermann

After the mass rearing room was established, the first attempts at mass rearing were started in September. So far three of our laboratory strains have been mass produced. Although in the mass production room - as in the insectaries - there is no temperature and humidity control, no problems for rearing and egg production have been encountered. In the best experiment we received about 60% pupation within 24 hours which is quite promising. Separation and sexing of pupae is not satisfactory yet. With the Gerberg pupae separator in use at present we suffer a substantial pupal mortality. It is planned to acquire a Sharma pupal separator shortly.

A first small scale release of fluorescent marked males and females, 2000 each, was made in five houses of a village comprising 32 inhabited houses. The mosquitoes released were F_1 hybrids of two "indoor" laboratory strains, one of which originated from the release village. Recovery of the marked mosquitoes was attempted with spray catches and was very modest with 11.9% for males and 8.2% for females. In the mark-release-recapture experiments with native mosquitoes recovery with the landing-biting catch was over time up to 25% for males and up to 50% for females. It was expected that with a spray catch similar recapture figures could be obtained at one go. Was the spray catch method at fault or the laboratory produced mosquitoes?? Further experiments should show. The only positive result of this "mass" release so far is the training of our field assistants in the spray catch technique.

Miscellaneous results

1. Colonization of other Stegomyia

Regular collection of eggs of other Aedes permitted almost weekly attempts to colonize species which rarely or never have been kept in laboratories before. One of the biggest obstacles to colonization is mating in laboratory cages, i.e. selection for stenogamy. Continuous attempts with new material seemed therefore more appropriate an approach to colonization than repeated forced mating.

A. (S.) vittatus was the first species to be colonized in our laboratory. The next two species were colonized almost simultaneously: A. (S.) metallicus and A. (S.) heischi. It is for the first time that these two species have been colonized.

The next species for which laboratory mating was obtained were A. (S.) simpsoni, A. (F.) fulgens and A. (D.) adersi. Unfortunately despite these initial successes no permanent colonies could be established yet. The experiments will continue.

2. Autogeny of A. (S.) vittatus

Initially some females of our A. (S.) vittatus colony exhibited facultative autogeny (Corbet 1967) and produced an average of 10.6 eggs. Attempts to establish an autogenous strain failed, however, and autogeny disappeared from the colony within 5 generations. The reason was that the offspring of autogenous females failed to reproduce autogenously and did not take a bloodmeal.

Autogeny was once also observed in A. aegypti. This, however, is not new. Trpis (personal communication) observed autogeny several times in newly colonized A. aegypti, but again was unable to select for an autogenous strain. It seems that in both species autogeny is lost very quickly under laboratory conditions.

3. Speed of development in different Stegomyia

From the regular ovitrap collections of the routine monitoring program we had the opportunity to rear almost all our coastal Stegomyia from the egg stage. A. aegypti ssp. formosus reached 90% eclosion in 6 to 7 days depending on the season. It was always about one day faster than A. aegypti type form and one to two days faster than A. simpsoni, A. metallicus, A. heischi, A. calceatus and A. soleatus. In A. vittatus the fastest individuals were as fast as ssp. formosus, however, eclosion of a rearing batch extended usually over three to four days while in ssp. formosus 90% eclosion was obtained within 24 hours.

Joint research projects

Dr. Paul Rodriguez from the Vector Biology Laboratory in Notre Dame has recently tested 19 East African strains of A. aegypti for susceptibility to the filaria Brugia pahangi. 11 of these strains were susceptible while among 24 Asian, West African and American strains 22 were totally and two highly refractory. Could certain local populations of A. aegypti in East Africa play a significant role in the epidemiology of human Bancroftian filariasis? During his consultantship with MBU Dr. W. Kilama from the Faculty of Medicine in Dar es Salaam picked up this question. His report is included as Annex 4. He tested four of our laboratory strains and found two partially susceptible. Dr. Kilama has decided to continue this investigation in Dar es Salaam. Because of our interest in genetic differences between populations, MBU will support his project as far as our means permit.

Dr. Milan Trpis of the Vector Biology Laboratory, University of Notre Dame, made an extensive collecting trip in Kenya and Uganda to obtain new feral strains of Aedes aegypti. An account of his trip is included as Annex 5.

Mr. Leonard Munstermann of VBL, Notre Dame, spent the summer of 1972 at MBU, working on lizard malaria and its relation to disease transmission in Aedes aegypti. His report is included as Annex 6.

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Annex 1

List of visitors to MBU from July - December 1972

Visitor	Research Interest	Purpose Of Visit
Mr. M. Boppre + Ms. G. Kurz Max-Planck-Institut Seewiesen, W. Germany	Leidoptera Sensory physiology	Collecting
Mr. Ph. Bebora Division of Vector Borne Diseases, Ministry of Health Mombasa, Kenya	Medical entomology	Training
Prof. G.B. Craig, Jr. Vector Biology Laboratory University of Notre Dame Notre Dame, U.S.A.	Mosquito Genetics Population Biology Genetic Control	Principal Investigator
Dr. D. Elder ICIPE Research Scientist Nairobi, Kenya	Phytoecdysones	Collecting Plant material
Prof. T. A. Freyvogel Swiss Tropical Institute Basel, Switzerland	Mosquitoes Malaria transmission	Visit of Laboratory and field sites
Mr. C. Garret-Jones Welcome Trust Medical Research Council Tanga, Tanzania	Population structure of A. gambiae	Visit of Laboratory
Prof. W. Helle Lab. for applied Entomology University of Amsterdam Amsterdam, Holland	Cytogenetics Genetic control through translocations	Visit of Laboratory
Dr. W. Ma ICIPE Research Scientist Nairobi, Kenya	Sensory physiology	Visit of Laboratory
Mr. S. Magayuka E.A. Institute for Malaria and Vector Borne Diseases Amani, Tanzania	Mosquitoes Bionomics and control	Visit of Laboratory and field sites
Ms. R. McDonald Birkbeck College University of London London, England	Genetics	Visit of Laboratory Instruction of ancillary staff
Mr. L.F. Munstermann Vector Biology Laboratory University of Notre Dame Notre Dame, USA	Genetics of vectorial capacity	Reptile malaria Transmission by *A. aegypti*?

Visitor	Research Interest	Purpose of visit
Prof. J.W.S. Pringle ICIPE Deputy Director of Science Nairobi, Kenya	Electrophysiology	Inspection of Laboratory Facilities
Prof. K.S. Ra' Department of Biology University of Notre Dame Notre Dame, USA	Cytogenetics Genetic control	Principal Investigator
Mr. P. Scheltes ICIPE Research Scientist Nairobi, Kenya	Diapause in stemborer *Chilo zonellus*	Collecting, Visit of Laboratory
Mr. S. K. Sempala E.A. Virus Research Institute Entebbe, Uganda	Medical entomology Mosquito ecology	Training
Dr. J. Shepherd ICIPE Reserach Scientist Nairobi, Kenya	Sperm activation in Diptera and Lepidoptera	Visit of Laboratory
Dr. M. Trpis Vector Biology Laboratory University of Notre Dame Notre Dame, USA	Mosquito ecology and genetics	Consultant
Dr. S. K. Wangura Mr. P. K. Terer Mr. P. Kisia Kenyatta National Hospital Nairobi, Kenya	Radiation physics	Site inspection for installation of radiation source
Dr. W. Wood ICIPE Research Scientist Nairobi, Kenya	Biochemistry of Defense secretions	Collecting of millipedes

Report on a village mosquito population

By Paul McDonald

In an earlier attempt to determine the absolute population size of *Aedes aegypti* in an isolated village, a series of markings of landing-biting captures had been undertaken at Kwa Hamis Mtoro for several days. When the results of these mark-release-recaptures were analyzed it was found that the Lincoln Index estimates of population size were rather variable, ranging from 358 on one day to 1838 on the next day (table 1).

The Lincoln Index estimation of population size is dependent upon the recapture of marked mosquitoes. The recapture of mosquitoes in the population is dependent on the pattern of return of the mosquitoes, and this may be sex and age dependent. If a certain sex and age tends to return more often than another, then the population which has a higher percentage of that sex and age will appear to have a smaller population size.

The purpose of this second marking experiment was to mark all newly emerged mosquitoes in the village for several weeks until arriving at a point where almost 100 percent of the adult population was marked. At that time it would be possible, by remarking each individual upon recapture, to determine patterns of return with respect to sex and age. It would also be possible to see the age distribution of the village population over several weeks.

Another purpose of the experiment was the construction of adult survivorship curves for males and females. With such information it would be possible to determine the portion and life expectancy of fertile females that would be expected to be remaining in the population after releases of sterile males.

The relationship between newly emerged adults and landing-biting-captured adults over time could be estimated with the marking experiment on the basis of the village as a whole. The relationship between emergence and landing-biting-capture could be charted for each of the several houses as well.

Materials and Methods

The village of Mgandini, located in the western region of the MBU field study area, is an isolated village under coconut palms, set off from its nearest neighbors by approximately one half mile of grasses, maize and cassava in a very sparse cover of coconut palms. The village has a collection of 34 huts settled around cattle and goat pens. The initial survey of this village, made in July 1972, showed 50 water containers of which 46 had water and 32 had mosquitoes. Twenty houses were selected on the basis of productivity and location so that an even distribution of productive

houses in the village was obtained.

The experiment was begun on 12 October and terminated on 29 November. This was a period of intermittent light rainfall, moderate to high temperatures and good breezes. Pupae were picked on a daily basis from 12 October to 22 October and thereafter every other day. The pupae were taken from the twenty houses selected, and after 24 October from all the other houses in the village as well, although the other houses were relatively non-productive. Pupae from the 20 selected houses were kept in 20 cups, each marked with the house of origin and number of pupae collected.

Newly-emerged adult mosquitoes were counted and then painted with the date of painting. Painting was done on every other day with HUMBEROL spray paint applied in a small dot to the mesonotum. This technique has been described by Dr. Hausermann in the MBU Annual Research Report. All mosquitoes of one day's painting were given the same color on the same side of the mesonotum. The newly-emerged mosquitoes of two days later were painted with the same color but on the other side of the mesonotum. With twelve different colors mosquitoes were marked for 23 different days. After painting, mosquitoes were to be released in the same house from which they had been taken as pupae. The few mosquitoes taken as pupae from houses not selected for the experiment were killed.

Landing-biting (L-B) catches were organized so that two workers, each collecting 20 minutes in each of the 20 houses, could manage the collecting of mosquitoes as well as the releases at the end of the day. L-B catches were made every other day, on the same days that pupae were picked and newly-emerged adults marked. The mosquitoes from the L-B catches were examined for presence of marking; only those previously painted were painted again with the date of capture. All mosquitoes (1. unmarked from the L-B catch, 2. marked from the L-B catch and 3. newly-emerged marked) were released into the houses from which they came, and this was done only after all L-B catches of the day had been completed. Any mosquitoes dying during treatment were not counted as released for that day.

Results

Attempts to saturate village with marked mosquitoes

One major objective of this marking experiment was the saturation of the village population with marked mosquitoes, each marked with its date of emergence. Saturation did not occur. Table 2 shows a maximum of 36 percent marked in L-B catches at the termination of the experiment.

For the first few days, from 14 to 24 October, no collections were made in the houses not included in the 20 selected for surveillance. After that time pupae from these other houses were removed and killed. Even if 24 October is considered as the starting time for the saturation effect to begin, the saturation had not been achieved after 30 days time, which turned out to be the maximum lifetime for a mosquito in this study.

Soon after the experiment began daily collection of pupae had to be abandoned in favor of collection every other day. According to data collected in the first few days of the experiment less than 25 percent of pupae collected on one day will emerge on the next day. This could account for a greater than 25 percent unmarked L-B catch if these have greater survival than the marked.

Collection of pupae is dependent upon the efficiency of the field assistant, and this efficiency may be reduced when the water in the containers is murky.

It is possible that migration from either outdoor habitats near the village or from distant villages had occurred to some extent, thereby bringing into the L-B catches considerable numbers of unmarked adults.

Survivorship

Survivorship between release date and the next L-B catch two days later has been calculated for males released from 14 October to 19 November and females released between 14 October and 5 November. Accurate survival figures could be determined for only 19 groups of males and twelve groups of females, as will be shown in the next sections. Calculations of survival to two days after release are 139/676 or 21 percent for males and 119/456 or 25 percent for females. The possible explanations for this very low survival are 1) the marking treatment resulted in 67 percent mortality within two days or 2) early migration out of the study area occurred. The possibility of migration of newly emerged mosquitoes out of the study area is reinforced by the finding of a large discrepancy between total captures and marked captures in the L-B catches, suggesting that outsiders may be migrating in.

Survivorship curves for males have been made first with 15 groups of male releases, such that each group has the possibility of including all survivors up to the age of the oldest known male survivor. This curve is in figure 1-A with the survivorship on day two after release set to 100 percent. By assuming that the less-than-five percent survivors to the last four ages affect the curve only slightly, then 19 groups of male survivors can be graphed. This gives an essentially identical curve (figure 1-B) to that of 15 groups. Both curves match a survivorship of 60 percent per two-day interval over the time span covered.

Survivorship curves have been made for females, first with nine groups and then with twelve groups, assuming that the less-than-five percent survivors to the last three ages affect the curve only slightly (figure 2-A and 2-B). Both curves match a survivorship of 80 percent per two-day interval over the time span covered.

Survivorship and landing-biting catches

When the 15 groups of males were analyzed with regard to known survivors of an age compared with number of those known survivors caught in a L-B catch at that age, the graph of figure 3 resulted. This indicates that approximately 50 (47 on the average) percent of known survivors of each age were captured in L-B catches under the conditions of this experiment.

When nine groups of females were analyzed with regard to known survivors of an age compared with number of those known survivors caught in a L-B catch at that age, the graph of figure 4 resulted. This indicates that approximately 35 (34 on the average) percent of known survivors of each age were captured in L-B catches under the conditions of the experiment. There does appear to be a higher percent captured on day two followed by lower percentages on days four and six, suggestive of the effect of taking a blood meal on day two after release.

The close correspondence of survivorship curves, which require individual marking - at present practiced only with painting - and L-B catch curves which require group marking only - practical with dusts - suggests that in the future survivorship curves can be approximated, especially for genetic release males, without resort to more laborious marking methods.

Pattern of return in landing-biting catches

Groups of released males were analyzed with regard to their age at time of L-B catch and their probability of return in the next L-B catch. This information is given in table 3. Since the probability of survival for males is the same regardless of age, the figures represent behavioral characteristics. The overall return is nine percent (based on 15 groups); that for young males two and four days after release is ten percent (based on 20 groups). It appears that return of males is independent of age. The estimation of probability of return is based on 14 returnees.

Groups of females were analyzed with regard to their age at time of the L-B catch and their probability of return at the next L-B catch. The information is given in table 4. With a constant survival factor for females, the figures represent behavioral characteristics. The overall return is eight percent (based on nine groups) while females two days after release show a 19 percent return (based on 19 groups); four to ten days after release, ten percent return (based on 19 groups). Very young females have a markedly increased probability of return compared with older females.

It is interesting to note that even when the number of female returnees is eleven (nine groups) the ratio between percent young females returned to percent older females returned is 2 to 1 which approximates the ratio of 1.9 to 1 based on 27 returnees (19 groups).

The probability of return on the average is only slightly different for males (nine percent) and females (eight percent). Male probability of

return is independent of age. However, young females have a probability of return more than twice as great as that of females in general.

Age distribution

The average of the age distribution of known survivors over several days would be expected to reveal a curve of age distribution which reflected the survivorship curve for each sex. Such age distributions have been calculated as far as possible. Averages of three successive catches, four successive catches and five successive catches for males as well as for females are given in tables 5 and 6. These tables clearly show the variability of average age distributions. The variability is due to the particular patterns of emergence of adults into the population. With more catches included in the average the female age distribution averages come more quickly into order than those for males, reflecting 1) the greater longevity of the females compared with the males and 2) a different pattern of emergences. With nine or ten catches averaged the age distribution curves come to closely resemble the survivorship curves (figure 5 and 6).

In general it seems that more stable age distribution averages are obtained if the first day considered is one period (two days) after the emergence of many mosquitoes preceded by a few periods of low adult production and followed by two low production periods. Such a day was 3 November and the period November 3 though November 7 including three catches gave age distributions of both males and females that were reflective of stable age distribution.

The Lincoln Index could best be done with three markings and four catches. Marking is to be done on the catch following a peak of emergence and for two subsequent catches. Recaptures should be made two days after each marking, all during a lull of adult emergence. This would insure that the female age distribution was normal and generally 36 percent of those females would have a high probability of return while 64 would have a lower probability of return.

Population dynamics

The fluctuations in total L-B catch and marked L-B catch, after 24 October (when the collection of all pupae in the village was begun on a regular basis) have been determined and are displayed in figure 7. These fluctuations are generally but not precisely responsive to fluctuations in the numbers of pupae. The fact that the L-B catch is rather constant may be a reflection of a relatively stable population size brought on by the selection out of most pupae and their subsequent dehabilitation as newly-emerged adults by the marking treatment, possibly resulting in the loss of up to 67 percent of the males and females within two days. Those pupae emerging from the water containers in the houses, together with immigrants would then furnish the 64 percent or more of the population which would give shape to the L-B catch fluctuation.

The fluctuations for both male and female numbers of released and captured mosquitoes can be calculated for the marked subpopulation of Mgandini alone. Figure 8 compares 1) the number of those released on a date that are captured at some time later, with 2) the number of known survivors on a date, and 3) the number of marked in the L-B catch. For males it is apparent that after 18 October the number of known survivors reflected the previous released by an interval of a two-day period. Furthermore the L-B catches of the males reflected quite closely the known survivors. For females from 24 October the number of known survivors reflects the releases by an interval of two two-day periods. However, the female L-B catch does not reflect the known survivors closely.

The total capture to survivor figure from 16 October to 9 November is 241 to 657 or 37 percent. The male catches average 52 percent of male survivors while the female catches average 30 percent of female survivors. These figures are comparable for values obtained with several release groups analyzed over their lifetimes, giving 47 percent for males and 34 percent for females (figures 3 and 4).

The attempt to estimate population size by using two methods is illustrated in figure 9. By the first method the number of males in the L-B catch is multiplied by two and the number of females is multiplied by 3. In the second method the number of males plus females is multiplied by 2.7 (100 percent/37 percent). Both estimates are compared with the known survivors curve for both sexes together. The two methods are very little different in results. An accurate catch by catch estimation cannot be made in either case.

The number of newly-emerged adults marked and the number of marked adults taken in L-B catches has been determined from data of the 20 houses used in this study. The relationship has been plotted in figure 10. Generally the adults are distributed in the same way as the pupae; however, there is the possibility that a house with high pupal production and adult emergence may give few adults in the L-B catch (No. 2) and a house with low breeding will give many adults in the L-B catch (No. 6). Overall, 25 percent of the L-B catch were marked, but this was not true for each house. Some houses gave considerably lower percentages of marked mosquitoes, suggesting that these houses may have been difficult for pupae picking or especially attractive to immigrants.

Conclusions

1. At most 36 percent of captured mosquitoes in L-B catches were marked, after the marking of all newly emerged adults collected as pupae in the village of Mgandini for six weeks.

2. The survivorship of males is 60 percent for a two day interval from two days after release. Survivorship for females is 80 percent for a two day interval from two days after release. If natural survivorship for the two days after release (emergence) is also 60 percent for males and 80 percent for females, then 67 percent mortality was caused to both sexes by the marking treatment.

3. Landing-biting catches of marked mosquitoes closely resemble survivorship curves, especially for males. This suggests that group marking may be successful in determining survivorship curves.

4. The probability of return in a subsequent L-B catch was nine percent for males and eight percent for females. Return probability is independent of age for males but young females have greater than twice the probability of return compared with older females.

5. Stable age distribution occurs over three consecutive catches if the catching begins two days after a peak emergence preceded by a lull and followed by a lull. This would be the best time to do a Lincoln Index.

6. An estimation of the number of marked survivors of the age of two days after release (emergence) and older can be determined by multiplying the number of marked captures by 2.7 under the sampling regime of this experiment.

7. The distribution of marked adults caught in L-B catches generally corresponds to the distribution of newly-emerged adults released in this village.

Table 1

Lincoln Index values for Kwa Hamis Mtoro

Date	Interval between catches	
	one day	two days
19 July	605	---
20	590	679
21	430	1008
22	358	629
23	1838	1126
24	---	1047

Table 2

Date	Percent L-B catch marked
14 October	0
16	9
18	22
20	20
22	31
24	17
26	30
28	20
30	25
1 November	29
3	29
5	34
7	18
9	19
11	35
13	25
15	29
17	21
19	30
21	18
23	35
25	29
27	27
29	36

Table 3

Age of male (days after first released)	Probability of return in next L-B catch: 15 groups	Probability of return in next L-B catch: 20 groups
2	5/38 (9%)	7/74 (9%)
4	5/37 (14%)	5/44 (11%)
6	1/17 (6%)	1/23 (4%)
8	1/9 (10%)	1/9 (10%)
10	0/7 (0%)	
12	0/1 (0%)	
14	0/1 (0%)	
16	0/3 (0%)	
18	0/1 (0%)	

Table 4

Age of female (days after first released)	Probability of return in next L-B catch: 9 groups	Probability of return in next L-B catch: 19 groups
2	5/37 (14%)	15/78 (19%)
4	0/17 (0%)	3/42 (7%)
6	1/15 (7%)	2/33 (6%)
8	2/15 (13%)	4/22 (18%)
10	1/12 (8%)	3/28 (11%)
12	0/7 (0%)	
14	0/8 (0%)	
16	1/6 (16%)	
18	1/5 (20%)	
20	0/2 (0%)	
22	0/1 (0%)	
24	0/2 (0%)	
26	0/2 (0%)	
28	0/0 (0%)	
30	0/1 (0%)	

Table 5: Average age distribution of males

Dates	Percentage at ages							
	2	4	6	8	10	12	14	16
Three catches:								
1, 3, 5 Nov.	51	29	11	0	2	2	2	2
3, 5, 7 Nov.	49	26	16	5	0	0	2	2
3, 7, 9 Nov.	34	31	19	9	3	0	0	3
7, 9, 11 Nov.	65	19	7	7	2	0	0	0
9, 11, 13 Nov.	55	33	8	2	2	0	0	0
11, 13, 15 Nov.	58	25	14	3	0	0	0	0
13, 15, 17 Nov.	36	33	16	11	4	0	0	0
15, 17, 19 Nov.	36	24	19	12	8	2	0	0
Four catches:								
1, 3, 5, 7 Nov.	47	28	13	4	2	2	2	2
3, 5, 7, 9 Nov.	48	25	15	6	2	0	2	2
5, 7, 9, 11 Nov.	57	24	10	5	2	0	0	2
7, 9, 11, 13 Nov.	51	32	11	5	2	0	0	0
9, 11, 13, 15 Nov.	56	25	13	4	1	0	0	0
11, 13, 15, 17 Nov.	52	27	11	7	2	0	0	0
13, 15, 17, 19 Nov.	30	33	19	10	7	1	0	0
Five catches:								
1, 3, 5, 7, 9 Nov.	54	24	11	4	3	1	1	1
3, 5, 7, 9, 11 Nov.	60	22	10	4	1	0	1	1
5, 7, 9, 11, 13 Nov.	47	33	13	4	1	0	0	1
7, 9, 11, 13,15 Nov.	53	25	14	7	1	0	0	0
9,11, 13, 15,17 Nov.	51	27	11	8	3	0	0	0
11,13, 15, 17,19 Nov.	44	28	14	7	5	1	0	0

Table 6: Average age distributions of females

Dates	Percentage at ages			
	2	4	6	8
Three catches:				
22, 24, 26 Oct.	36	28	18	18
24, 26, 28 Oct.	32	30	23	15
26, 28, 30 Oct.	20	30	29	21
28, 30, 1 Oct.-Nov.	23	20	29	28
30, 1, 3 Oct.-Nov.	37	20	17	26
1, 3, 5 Nov.	46	29	14	12
3, 5, 7 Nov.	36	31	22	11
Four catches:				
22, 24, 26, 28 Oct.	33	27	22	18
24, 26, 28, 30 Oct.	27	28	25	21
26, 28, 30, 1 Oct.-Nov.	25	28	26	22
28, 30, 1, 3 Oct.-Nov.	32	21	23	23
30, 1, 3, 5 Oct.-Nov.	35	26	19	20
1, 3, 5, 7 Nov.	39	28	20	13
Five catches:				
22, 24, 26, 28, 30 Oct.	29	26	24	22
24, 26, 28, 30, 1 Oct.-Nov.	29	26	23	21
26, 28, 30, 1, 3 Oct.-Nov.	31	27	23	20
28, 30, 1, 3, 5 Oct.-Nov.	31	26	23	19
30, 1, 3, 5, 7 Oct.-Nov.	32	26	22	20

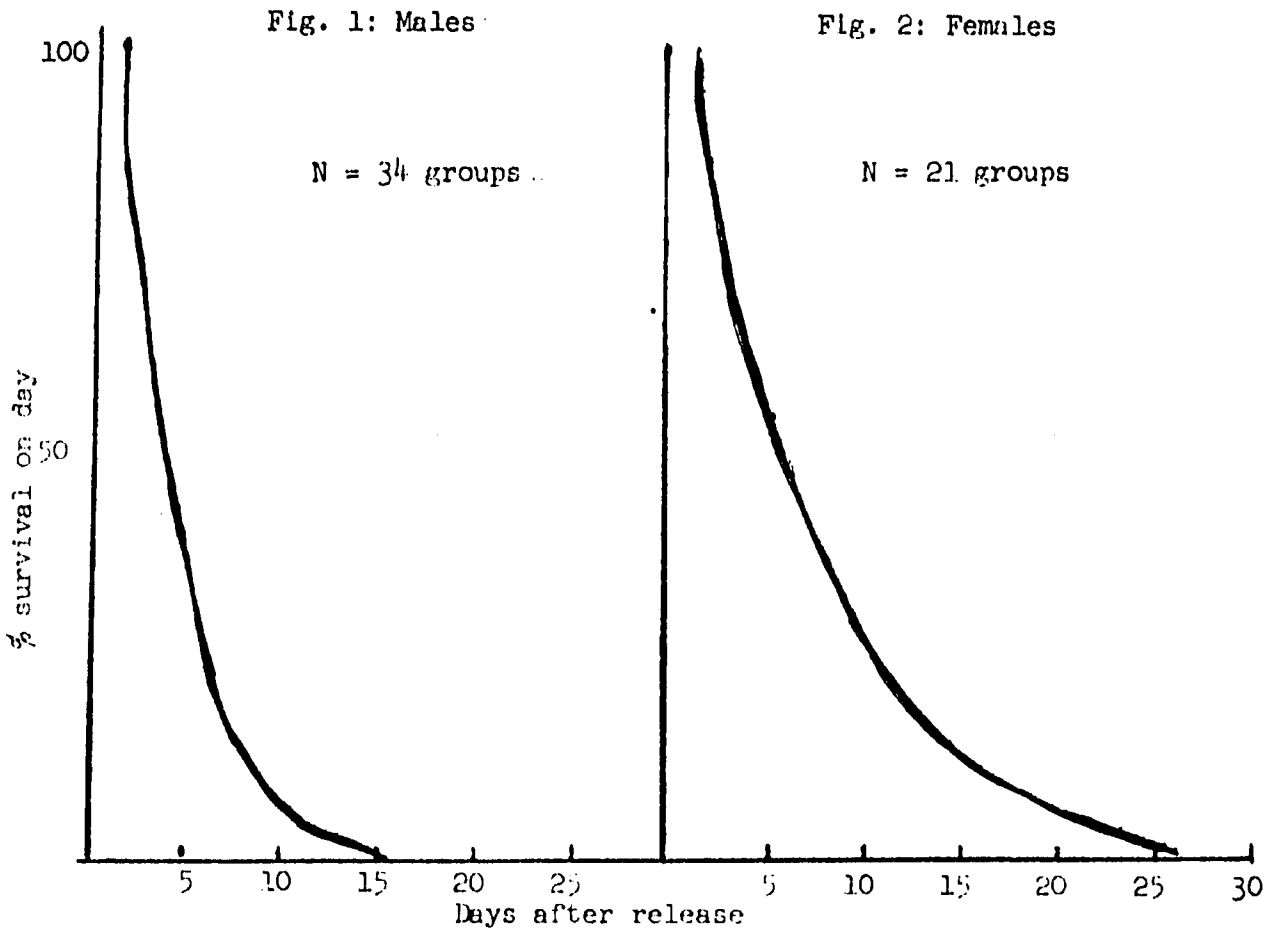


Fig. 1: Males
Fig. 2: Females
N = 34 groups
N = 21 groups
100
50
% survival on day
5
10
15
20
25
30
Days after release

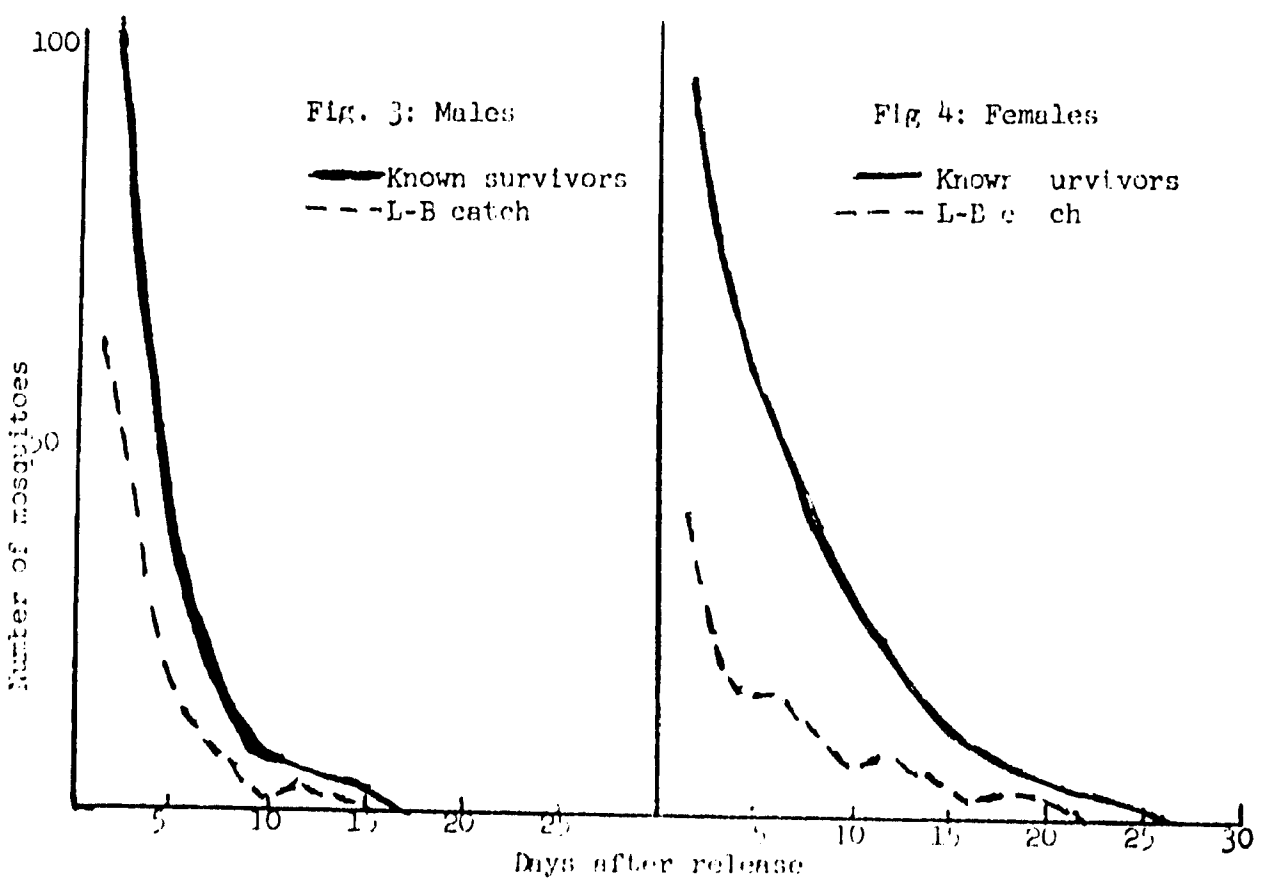


Fig. 3: Males
Fig 4: Females
Known survivors
L-B catch
100
50
Number of mosquitoes
5
10
15
20
25
30
Days after release

Fig 5: Males

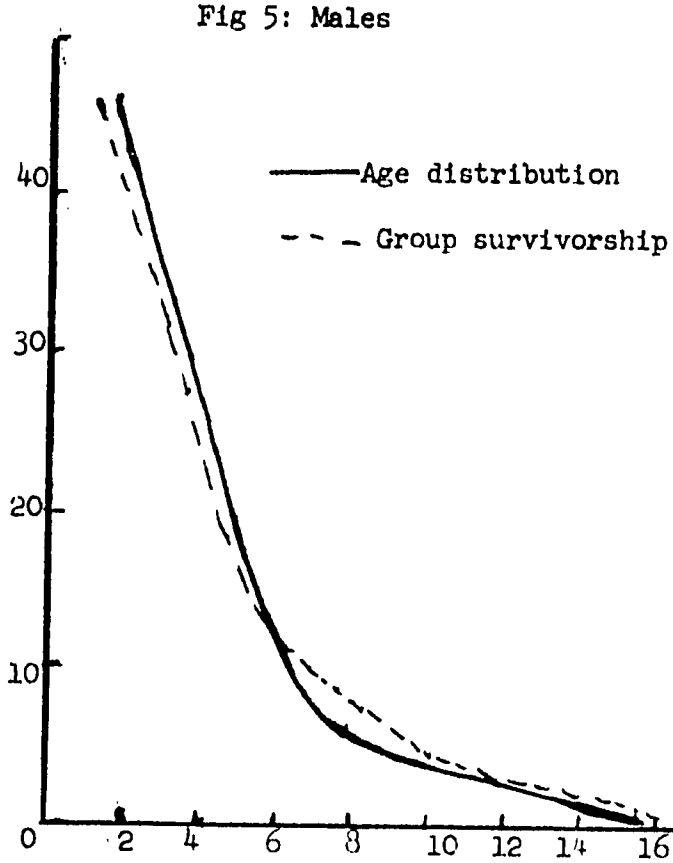


Fig.6: Females

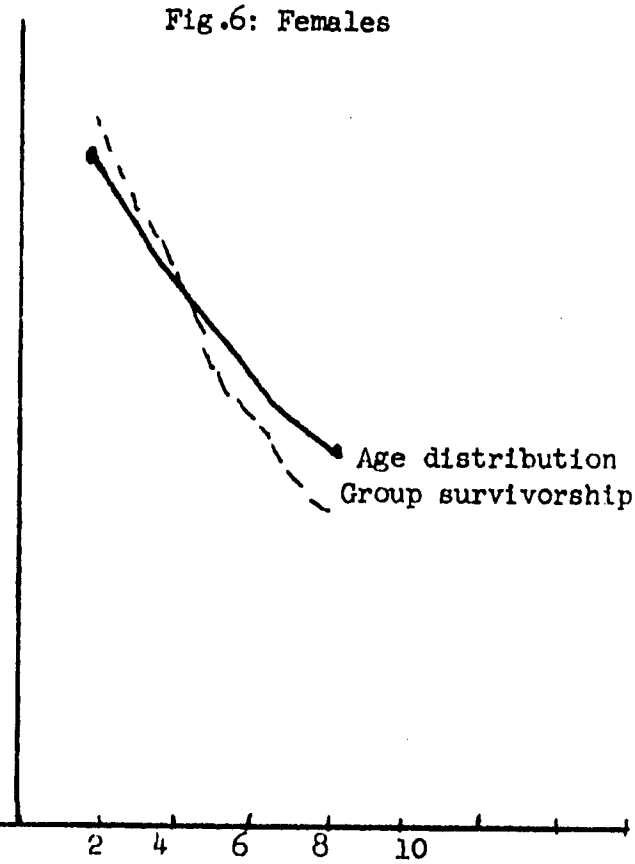


Fig. 7

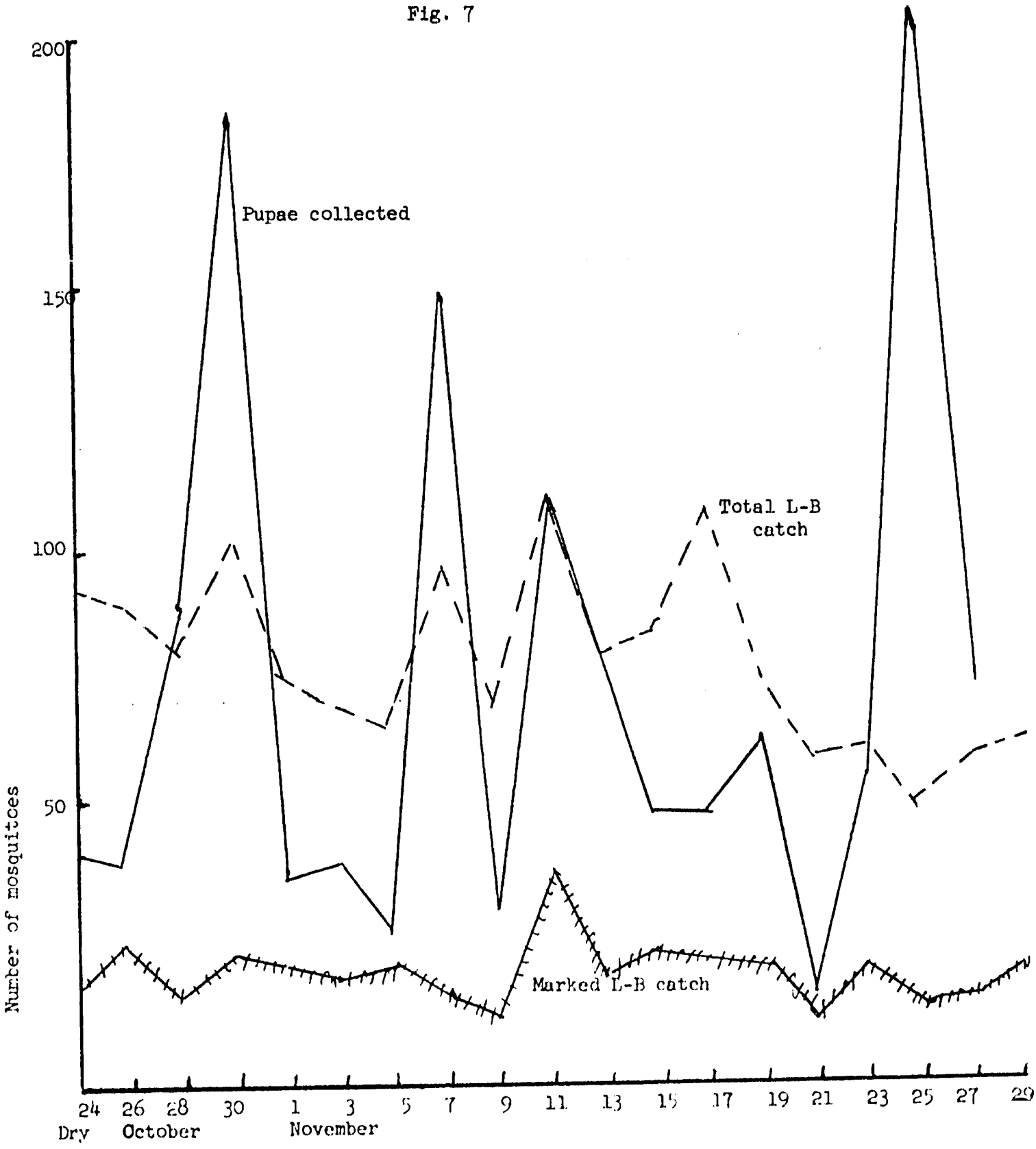

200
150
100
50
Number of mosquitoes
Pupae collected
Total L-B catch
Marked L-B catch
24
26
28
30
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
Dry
October
November

Fig. 3

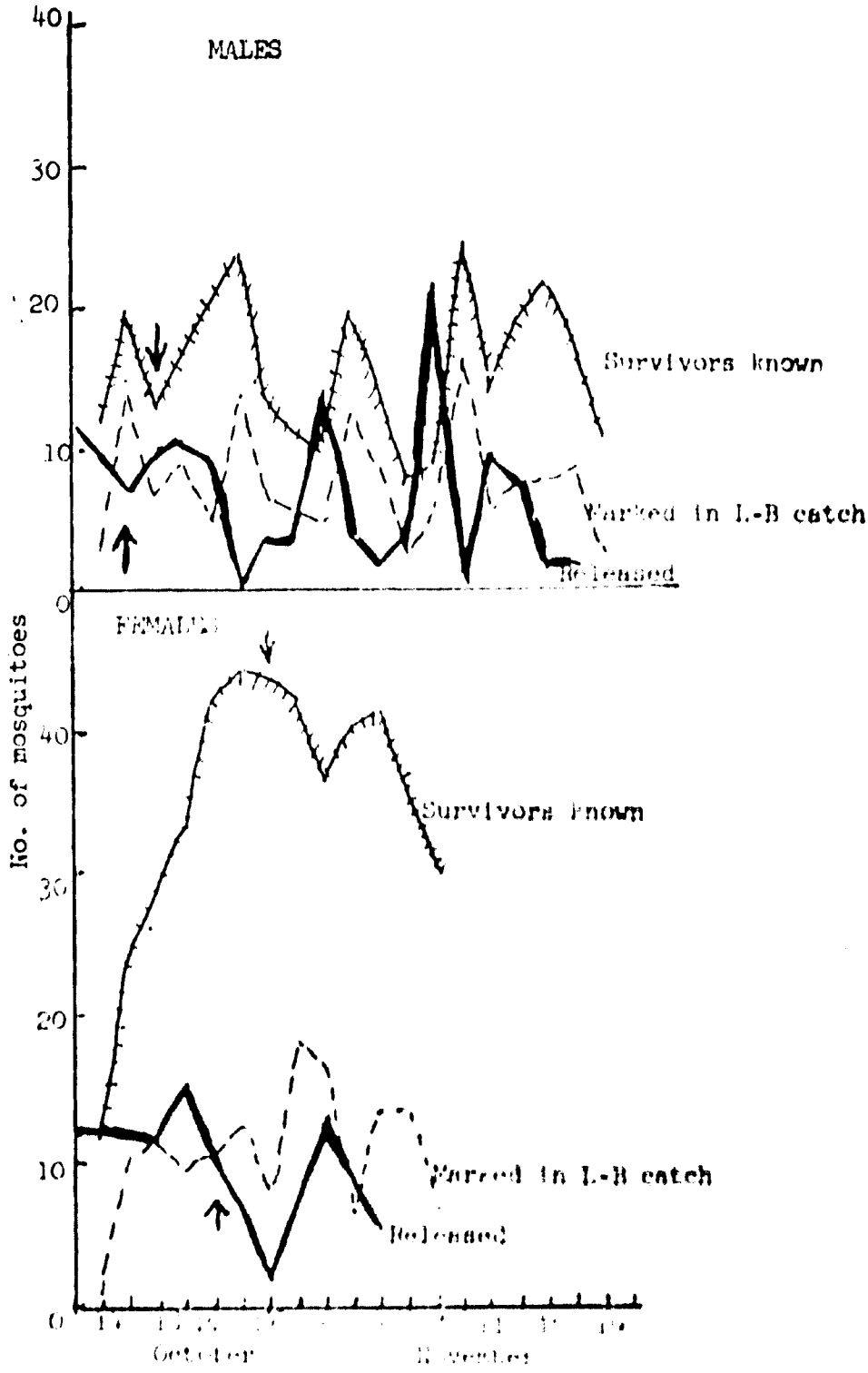

MALES
40
30
20
10
0
Survivors known
Marked in L-B catch
Released
FEMALES
No. of mosquitoes
40
30
20
10
0
Survivors known
Marked in L-B catch
Released
October

Fig. 9

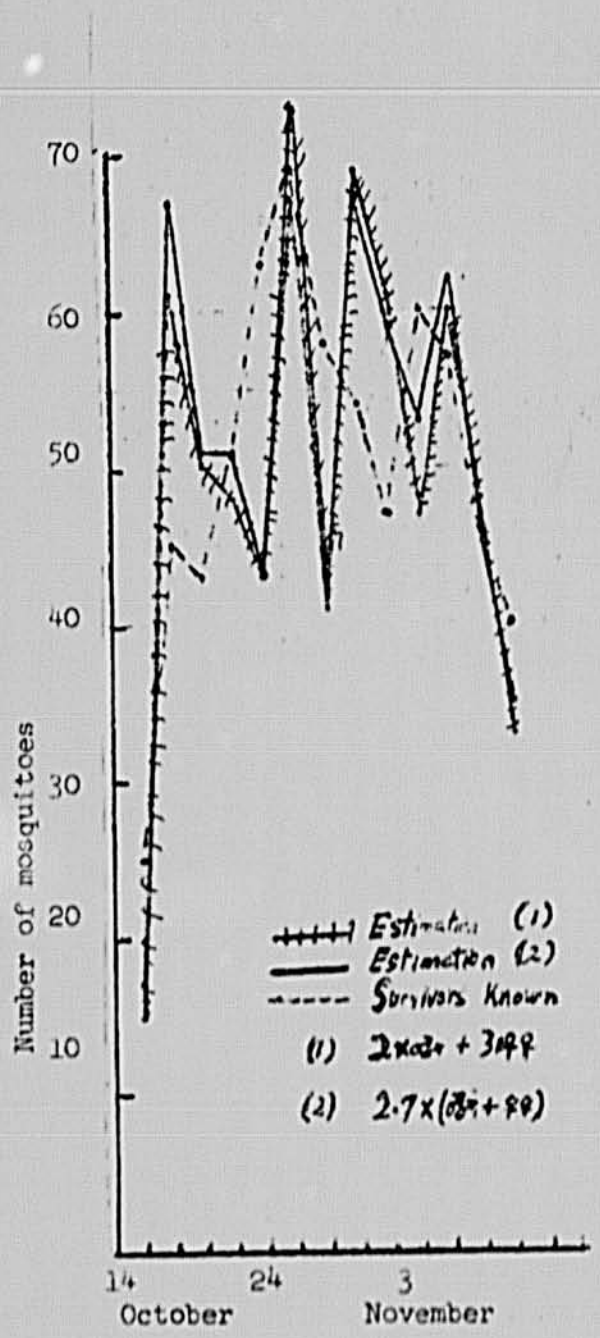
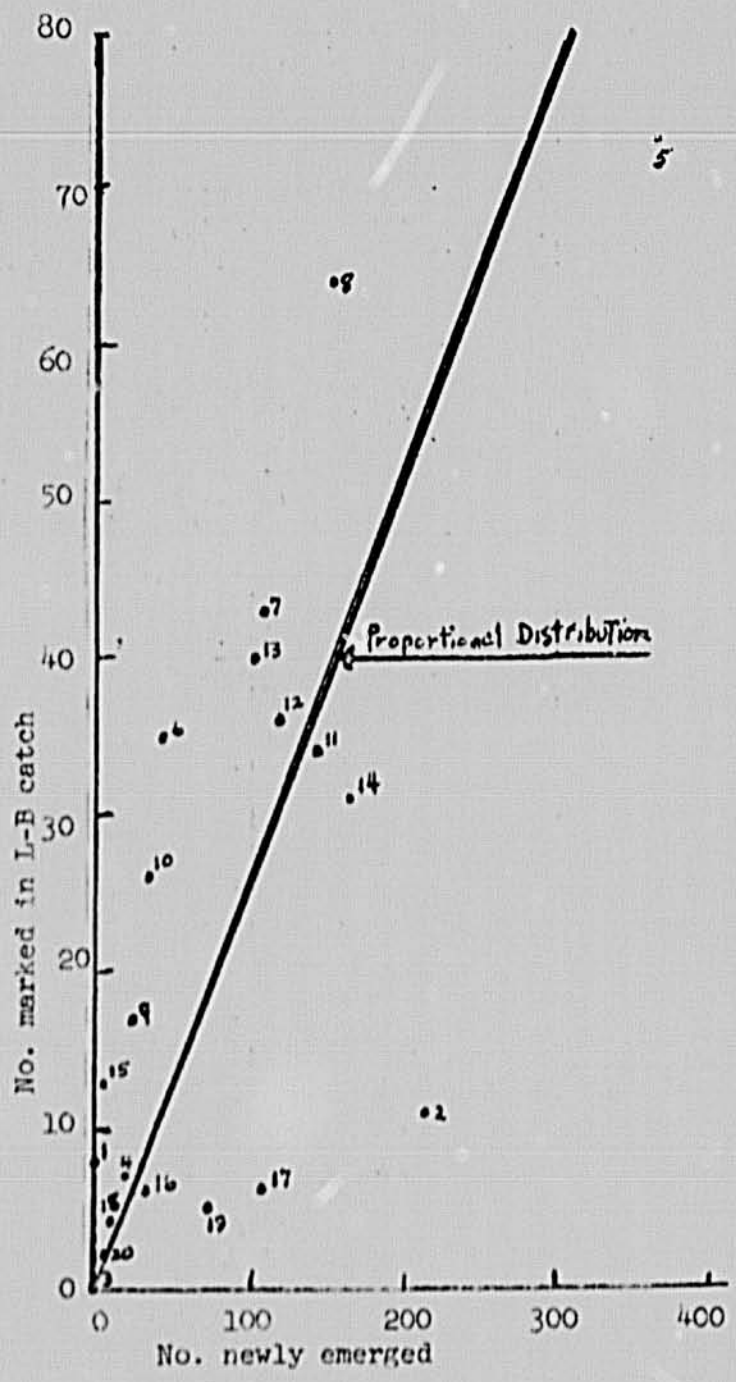

Number of mosquitoes
70
60
50
40
30
20
10
14
24
3
October
November
Estimation (1)
Estimation (2)
Survivors Known
(1) 2×[illegible] + 3[illegible]
(2) 2.7×([illegible]+[illegible])

Fig. 10


No. marked in L-B catch
80
70
60
50
40
30
20
10
0
100
200
300
400
No. newly emerged
Proportional Distribution

ANNEX 3: Studies in the behavior of domicilliary and extradomicilliary strains of *Aedes aegypti* in the Changombe Location.
Responsibility: D.M.Fanara

FIGURE I DATA ON CAPTURE OF MARKED *AEDES AEGYPTI*, CHANG'OMBE LOCATION.

AEGYPTI STRAIN	SEX	RELEASE SITE	RELEASE TOTAL	CAPTURE TOTAL	CAPTURE PERCENT	GREATEST DISTANCE	SITE	ELAPSED DAYS
Pale-	M	House	999	255	25.5	96 m	Forest	2
Domicil.	F	House	965	209	21.7	176 m	Forest	1
Inter.-	M	Forest	457	72	15.8	196 m	House	8
Extradom.	F	Forest	545	61	11.2	196 m	House	5
Dark-	M	Forest	1,349	36	2.7	97 m	House	4
Extradom.	F	Forest	503	5	0.99	None taken in houses		

FIGURE 2 SKETCH OF MAP OF CHANG'OMBE LOCATION.

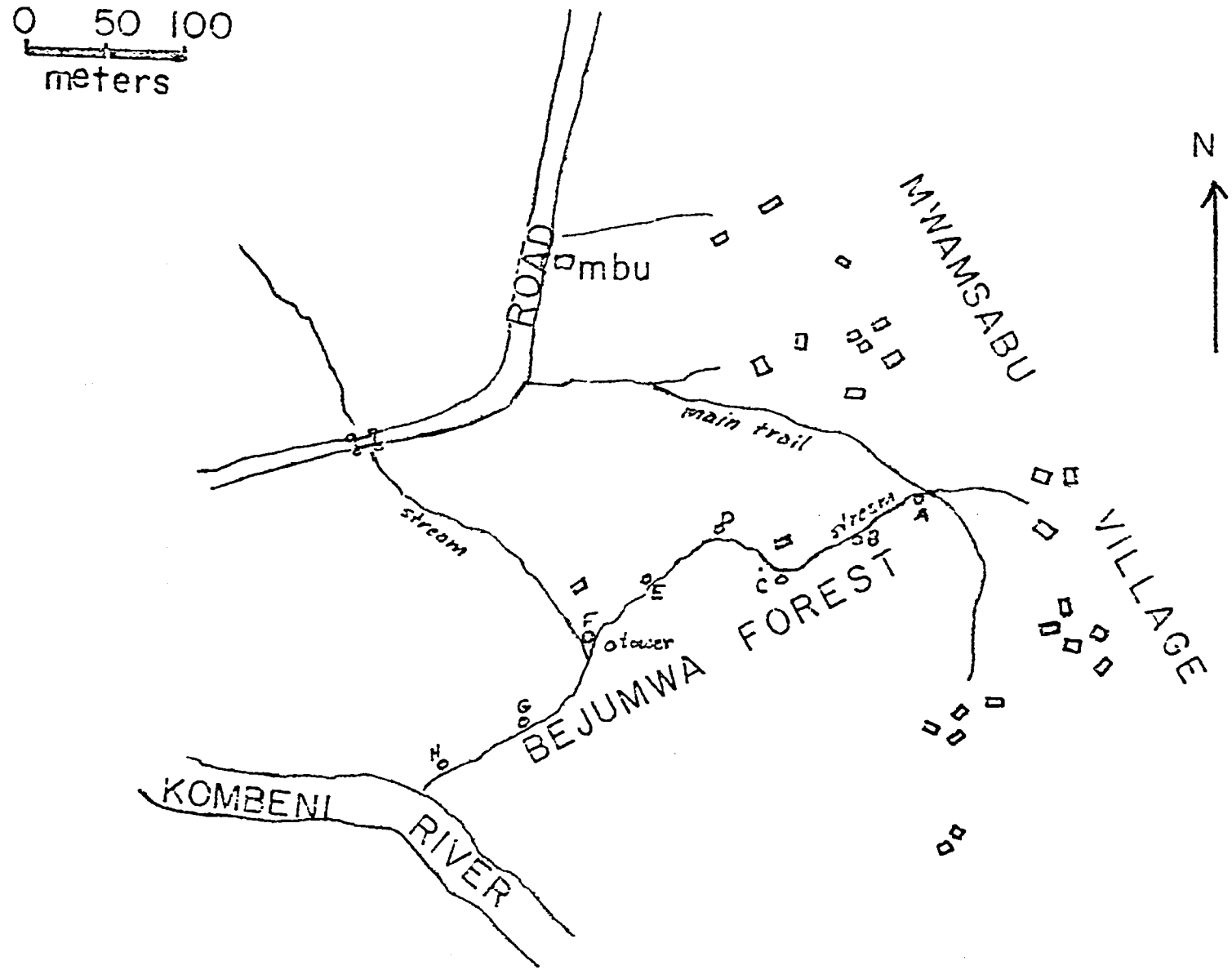


Fig. 3. INDOOR LANDING-BITING PATTERN, CHANGOMBE

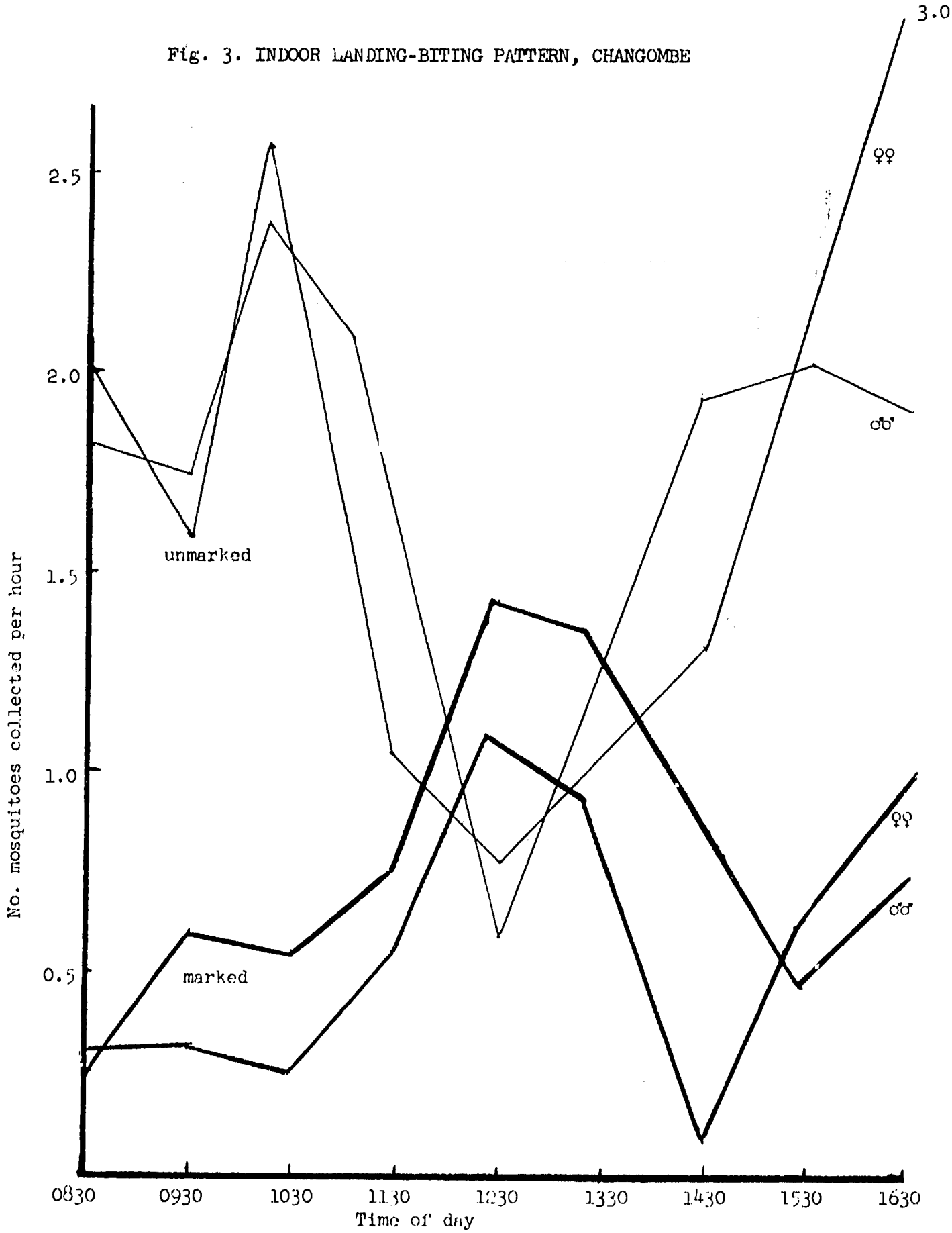


FIGURE 4 FOREST LANDING-BITING PATTERN

ANNEX 4

VARIATION IN SUSCEPTIBILITY OF *AEDES AEGYPTI* (L.) STRAINS TO *WUCHERERIA BANCROFTI* (COBBOLD)

by

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Consultant's Report to MBU/ICIPE,

Summer, 1972

INTRODUCTION

From a conversation with Professor Craig, I learned that Dr. P. Rodriguez of the Vector Biology Laboratory, University of Notre Dame, has recently demonstrated that Aedes aegypti formosus strains are more susceptible to infection with Brugia pahangi than the type form Aedes aegypti aegypti.

Thinking that the same phenomenon might hold for W. bancrofti, this short project was therefore undertaken to study the susceptibility of Aedes aegypti to W. bancrofit, emphasis being placed on comparisons of the type form strains with the feral strains.

MATERIALS AND METHODS

The strains of Aedes aegypti mosquitoes used are shown in Table I. They were all obtained from colonies maintained by the Mosquito Biology Unit of the International Centre of Insect Physiology and Ecology, at Mombasa, Kenya. The mosquitoes were reared in an insectary maintained at a tropical ambient temperature of about 25°C and about 70% relative humidity.

When approximately five days old, the adult female mosquitoes were deprived of sugar and water for a period of about 14 hours, then they were fed between 21 and 23 hours (local time) on the arm of a man naturally infected with periodic W. bancrofti. The enclosed photograph shows Mr. Jefa Wawara of Garashi Location, Malindi District who kindly supplied the microfilariae. Mr. Waware did not have a previously recorded history of treatment for filariasis. All mosquito feeding proceeded inside a laboratory room, just adjacent to the insectary.

Before the mosquito feeding began, a temporary wet blood film was made by pricking the tip of a finger and examining the blood for microfilariae. At the same time, thick blood films were made for future references. These thick films were later stained with Weigert's Homatoxylin in the Department of Parasitology and Medical Entomology Laboratory, Faculty of Medicine, University of Dar-es-Salaam. (It was not necessary to stain the thick smears at Mombasa since in this part of the world live W. bancrofti microfilariae are easily recognized on site). No attempt was made at determining the microfilarial densities, this can be estimated from the accompanying stained blood films.

The mosquitoes were allowed to engorge on the donor's arm, which was introduced through the sleeve of a 'gallon' cage. In all the three feedings, the two strains being compared were engorged within thirty minutes, (two attempts at feeding Culex pipiens fatigans which we previously collected as immatures from tyres with residual rain water, completely failed after exposing an arm for at least 30 minutes on each occasion).

Because of unforeseen timing difficulties the infected mosquitoes were held on sugar and water for 18 to 22 days (instead of 9 to 11 days) inside the insectary, after which the surviving ones were dissected, the abdomens examined immediately and the thoraxes and heads fixed in 85% alcohol, then shipped to Dar-es-Salaam. In the Dar-es-Salaam laboratory, the fixed mosquitoes were brought down to water, then stained for 24 hours in Mater's Acid Hemalum, after which they were washed in tapewater to "blue" the stain, and then stored in glycerol for subsequent dissections (Shute and Maryon, 1966).

RESULTS

Three of the enclosed microscopic slides show W. bancrofti microfilariae on the three days of mosquito feeding. On these occasions, an average thick drop would contain more than 50 microfilariae.

Two of the enclosed microscopic slides show infective larvae of W. bancrofti.

Table II shows the susceptibility of four strains of Aedes aegypti to W. bancrofti. Two of the strains (Ganga and Tree-Hole) did not show any larvae. The Ganga strain did not show any larvae in two separate infection attempts. However, two other strains (Mkwaja and Mnazi) developed infective larvae. These infective larvae were found mainly in the head, although a few were still in the thoraxes. From abdomen examinations of all these mosquitoes, Dr. Hausermann recorded two infective larvae from the haemocoel of one Mnazi female.

Each infected mosquito had one to four infective larvae, the majority having two.

Neither first nor second - stage larvae were observed in any of the mosquitoes.

DISCUSSION

Two of the strains showed no filariae at all. One of these strains (Genga) was Aedes aegypti var. queenslandensis, the other (Tree-Hole) was Aedes aegypti formosus. Therefore susceptibility like refractoriness is not restricted to one subspecies.

Nelson et al. (1962) site workers in West Africa, the Congo and in the U.S.A. who succeeded in infecting Aedes aegypti with periodic W. bancrofti. However, Nelson found no natural infections of W. bancrofti in Aedes aegypti in Kenya; he also failed to infect Aedes aegypti with W. bancrofti in the laboratory. Those apparently contradictory results are not surprising since different mosquito populations differ in their vectorial capacity (Ramachandran et al. 1960; Kilama, in press). The dichotemous results presented in this report therefore confirm the view that different mosquito populations differ in their vectorial capabilities. As Kilama and Craig (1969) said, " the occurrence of simple monofactorial

factors controlling vectorial capacity should give pause to those medical entomologists who, on the basis of a few transmission experiments with insects from a single laboratory colony, quickly decide that a given species is or is not a potential vector of a given pathogen." It is very likely that the observed differences in the susceptibility of Aedes aegypti strains to W. bancrofti are not due to environmental factors, but are genetic.

The numbers of infective larvae per mosquito was rather low (most positive mosquitoes showed only about two infective larvae). As Ramachandran et al. (1960) observed, "the optimum time for dissection was about the 9th - 11th days, for after that time there was a loss of larvae from mosquitoes the mortality of which also increased."

Moreover, Ramachandran (1966) also observed that "if infected mosquitoes are maintained after the larvae in them reach maturity a loss is likely to occur by the escape of infective larvae from the proboscis of the mosquitoes during the act of feeding on substances like apple, milk, sugar, water and raisins." Unfortunately in my experiments, the mosquitoes were held for 18 to 22 days. If Ramachandran's observations on Brugia malayi in Aedes aegypti also apply here, then larvae might have been lost and many mosquitoes may have died. The number of my recorded infective larvae is most probably short of the actual numbers, and also the percentage of infective mosquitoes may have decreased. Ramachandran (1966) could not find any infective larvae B. malayi, 18 days after an infectious blood meal. Because of this time limitation may be it is not proper to compare my results with those of previous workers.

MacDonald (1962a and 1962b) observed that 17.1 per cent of parental generation Aedes aegypti showed mature larvae of semi-periodic B. malayi. In the same experiments he found an average of four infective larvae per infected mosquito. My results are therefore in line with Macdonald's.

Jordan and Goatly (1962) studied the infectivity of W. bancrofti to Culex fatigans in coastal Tanzania. In their extensive experiments, the infection rate of mosquitoes 14½ days after an infectious blood meal ranged between 52 and 100 per cent. When their results are compared with mine, the obvious conclusion is the C.o fatigans is much more susceptible to W. bancrofti than any of the Aedes aegypti strains I tested. However the mean number of infective larvae per surviving C.o.fatigans compares faviourably with that of my infected Aedes aegypti. However because of the greater proportion of infected C.o. fatigans and the overwhelming (still increasing) numbers of C.o. fatigans in urban East Africa, it is most probably that C.o. fatigans is a much more effective vector of urban W. bancrofti than is Aedes aegypti. In most rural East Africa, Anopheles gambine complex and An. funestus are the major vectors. Since those two Anopheles species are also more susceptible to experimental infections than I have just shown for Aedes aegypti, then we may regard those Anopheles sp. to be more efficient vectors than Aedes aegypti.

Since Aedes aegypti is a diurnal feeder it would not be an efficient vector of nocturnal W. bancrofti. However, W. bancrofti periodicity in

in some parts of East Africa is relative, and E. Nnochiri (personal communication) maintains that it is subperiodic in certain areas of Uganda. Under such circumstances diurnal Aedes aegypti, that is relatively susceptible, would enhance opportunity of W. bancrofti transmission.

Epidemiologic investigations on W. bancrofti in East Africa (e.g. Nelson et al. 1962, Menu and Kilama 1972) have consistently shown higher infection rates and higher microfilarial densities in men than in women. There is not yet a fully plausible explanations for this observation; among the suggestions made is that hormones may influence the level of filaremia. However, if feral mosquitoes are proved to be efficient vectors, then this might account for the differences in exposure to infection among men and women. While men work in the 'forest', women stay at home; thus in addition to home acquired nocturnal infections, men would also acquire infections from diurnal biting exophagic Aedes aegypti.

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I am very thankful to Dr. and Mrs. Walter Hausermann for their help throughout this project, Miss Rosabela for mosquito rearing and maintenance, Mr. Nyiwade of the Division of Insect-Borne Diseases for his administrative assistance and Mr. Joel Sawe of Malindi District Hospital for locating an infected individual. Last but not least, I am most thankful to Mr. Jefa Wawara, without his participation this project would not have been possible.

SUMMARY

The susceptibility of four strains of Aedes aegypti to W. bancrofti was studied.

Preliminary results indicate that susceptibility /refractoriness is/are not restricted to any one subspecies of Aedes aegypti.

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TABLE I. THE STRAINS OF A. AEGYPTI USED FOR INFECTION WITH W. BANCROFTI

N A M E	SUBSPECIES, VARIETY, OR TYPE	STRAIN HISTORY AND COLLECTION SITE
MNAZI	FORMOSUS	Eggs collected with dirt samples from steps in coconut palms (Mnazi) in Rabai Location, August - November, 1971.
TREE-HOLE	FORMOSUS	Eggs collected with dirt samples from tree hole in mango tree at Mazeras, Rabai Location (3° 56'S, 39° 34'E), August 1971.
GANGA	QUEENSLANDENSIS	Adults caught in an indoor landing biting catch in various houses at Ganga, Rabai Location (3° 57' 10"S, 39° 33' 40"E), October 1971.
MKWAJA	TYPE FORM	Larvae collected from rain water drums at Mkwaja Village (Tanzania Coast) in backyards of several houses (5° 47' 30"S, 38° 51' 05"E) April 1971.

ANNEX 4

TABLE 2. THE SUSCEPTIBILITY OF FOUR STRAINS OF *AE. AEGYPTI* TO *W. BANCROFTI*

TIME (HRS) OF EXPT.	DAYS BEFORE DISSECTION	MOSQUITO STRAIN	NO. ♀♀ DISSECTED	NO. ♀♀ WITH INFECTIVE LARVAE	NO. ♀♀ UNINFECTED
21:30 - 21:40	22	GANGA	92	0	92
21:20 - 21:30	18	TREE HOLE*	85	0	85
21:30 - 21:40	22	MKWAJA*	117	15	102
22:00 - 21:10	19	MNAZI**	41	4	37
22:10 - 22:15	19	GANGA**	127	0	127

* FED JUNE 22ND, 1972
** FED JUNE 23RD, 1972

annex 5

REPORT ON SURVEY OF FERAL AND DOMESTIC POPULATIONS OF AEDES (ST.) AEGYPTI AND SOME OTHER STEGOMYA SPECIES IN UGANDA AND KENYA

M. Trpis

A. PURPOSE OF THE TRIP

1. The Mosquito Biology Unit of ICIPE is conducting basic research on ecology of Aedes aegypti in Kenya preliminary to a field trial for genetic control of this mosquito. In this respect mating and biting behaviour of Ae. aegypti are certainly two of the most important specific problems which should be analyzed in both field and the laboratory. Experiments which are in progress at the Vector Biology Laboratory, University of Notre Dame show that these behavioural phenomena can vary from population to population even in the same geographical area. VBL as the WHO International Reference Center for Aedes has about 60 "strains" of Ae. aegypti collected from various parts of the world. Some of them are fresh, some have been cultivated in this laboratory for several years. The first objective of this trip was to obtain freshly collected strains of Aedes aegypti for genetic and behavioral analysis in the laboratory.

2. Haddow (1945) and Lumsden (1951) reported that Ae. aegypti is not anthropophilic in Bwamba County, Uganda, in the area where yellow fever repeatedly occurred since 1935 (Sawyer and Whitman 1936). Similar observation on a non-anthropophilic population of Ae. aegypti was made by Garnham et al. (1946) in the Kaimosi Forest (SW Kenya). Ae. aegypti was scattered throughout the forest but rarely found in the native villages. From 185 huts searched for breeding in two years, 1943 and 1944 larvae were found only once. Breeding inside houses was not recorded. Our second objective on this trip was to study diversity of breeding habitats of Ae. aegypti in Uganda and Kenya.

3. Since these populations were needed for further analysis and behavioral studies in the laboratory it was our aim to bring reasonably large samples of live larvae of Ae. aegypti from various places from Uganda and Kenya to Notre Dame for further colonization.

4. Studies at MBU by Drs. Hausermann and Fanara show that there are three different populations of Ae. aegypti in the Rabai area, 16 miles North of Mombasa, Kenya. These are: 1. domestic, 2. peridomestic, 3. feral. We have found in our laboratory studies that the feral Ae. aegypti does not enter our olfactometer trap chambers as readily as the domestic one. This might indicate that the representatives of the feral populations are exophilic in their biting haviour. Six special baited traps made of clear plastic were constructed at VBL, UND and shipped to Mombasa to test the local populations of Ae. aegypti for this phenomenon.

5. I spent considerable time on discussion of various aspects of population dynamics with the three scientists (Drs. Hausermann, McDonald and Fanara) of the Mosquito Biology Unit at Mombasa.

B. FIELD STUDIES IN UGANDA

The aim of this six day trip (from 23 August to 28 August 1972) was to study the distribution of Ae. aegypti in domestic, peridomestic and feral biotopes and to collect live mosquitoes for further studies in the laboratory.

Bunono automobile scrap-heap located on Entebbe pennisula was the only place where Ae. aegypti was observed biting man in Uganda (McClelland, 1959). However, in the total catch of more than 9,000 specimens of various mosquito species Ae. aegypti represented only 2.5%. To our knowledge, there is no other reference on man biting population of Ae. aegypti from Uganda. Apparently Ae. aegypti in Uganda has a different biting behavior from that found in Tanzania (Lumsden, 195, Trpis et al. 1971) and Kenya (Teesdale, 1955). Ae. aegypti breeds in Tanzania predominantly in peridomestic and domestic habitats but outdoors. Little breeding can be found either in forests or indoors (Trpis, 1970). On the Kenya Coast, Ae. aegypti breeds at least in some areas equally domestic, peridomestic and forest sites (Teesdale, 1955, Hausermann, 1972, Fanara, 1972).

1. Entebbe

I arrived at Entebbe on 23 August as a guest of the Director of the East African Virus Research Institute, Dr. G. W. Kafuko to whom I express much gratitude for arrangement of my trip to Bwamba, Masaka, Lyatonde, Mbarara and vicinity of Entebbe. I am also thankful to Dr. L.G. Mukwaya, Mr. S.D.K. Sempala and Mr. Y. Ssenkubuge for their wonderful company, help at the field work and discussions on various aspects of ecology of Stegomyia and Toxorhynchites mosquitoes in Uganda.

First two days (23-24 August) I spent at the East African Virus Research Institute at Entebbe and made two short trips, first to Zika Forest and secondly to Peeba Forest, Kampala and Bwayise.

a. Zika Forest is part of the extensive lake-shore forest about 10 km from Entebbe. Many forest mosquito species occur in Zika Forest. Perhaps the most abundant is Ae. (St.) africanus known as a vector of yellow fever and Zika virus among monkeys. The less known Zika virus shares the same serological group as YF virus as well as the same host-vector system (forest monkeys - Aedes africanus). The scientists of the EAVRI have isolated this virus more than 30 times in Entebbe area. They believe that the immunity to Zika virus appears to reduce the level of viremia against VF in monkeys. The combine immunity to several of the group B viruses may produce immunity against YF.

b. Mpanga Forest is located about 40 km from Kampala on Masaka road about 16 km North of Lake Victoria. Forest Reserve of 450 hectares is in altitude 1,160 m. Extensive studies on ecology of forest mosquitoes were done here by EAVRI, Drs. Haddow, Gillett, Corbet and others in the late fifties. It is permanently flooded in lower parts having a character of swamp. The forest is thick in both swamp and slopes (Fig. 2).

We collected Ae. simpsoni from Dracaena and banana axils at the edge of the forest. A steel tower with several platforms for catching mosquitoes above the forest canopy (about 40 m) was built here by EARVI and later transfered to Zika Forest.

c. Kampala. A large automobile dump (Fig. 3), located in a suburban area of Kompala city was searched for breeding of Ae. aegypti and other species. Larvae of Ae. aegypti were collected from a steel box (100 x 60 x 120 cm) and live larvae sampled for further rearing and anlysis. Culex pipiens fatigans was more abundant than Ae. aegypti here.

d. Bwayise. Located at Kampala is a locality where the population of Ae. simpsoni is zoophilic. There is extensive breeding of Ae. Simpsoni in colocasia plant axils (Fig. 4). We have collected many larvae from axils (Fig. 5); however, no eggs were received from the females, which are strictly eurygamous (Mukwaya, 1971).

2. Trip to Fort Portal, Bwamba, Mbarara, Lyatonde and Masaka.

After two days work in Entebe area a round-trip of three days (from 25 to 27 August) was made through the SW part of Uganda. I was accompanied on this trip by Dr. L.G. Mukwaya, Mr. Y. Ssenkubuge and a driver. The service of laboratory technition, driver and VW-Combi vehicle was provided by the EAVRI.

Survey Methods

The short time available did not allow us to make detailed survey. In various physiographical areas the nature of the problem dictated survey predominantly in villages. Search for breeding of Ae. aegypti larvae was done in villages around human habitations, in plant axils, treeholes and inside houses in containers with drinking water such as water pots, buckets, metal drums etc. From all places where breeding of Ae. aegypti was found a sample of live larvae was collected for laboratory colonization and further studies.

a. Fort Portal. The trip from Entebbe to Fort Portal on 25 August lasted for 8 hours on not a quite good roads. Search for Ae. aegypti was made in and around Fort Portal. No breeding of Ae. aegypti was found in this area. Ae. simpsoni larvae were collected from colocasia axils in Nyakasula Village which is located close to foothills of the Ruwenzori Mountains. No breeding of Ae. simpsoni was found on slopes of the Ruwenzori Mountains. About 0.5 hectare pineapple plantation located on slopes of the Ruwenzori Mountains was searched for mosquito larva on 26 August. There was water in axils but no larvae (elevation of 1,500m).

b. Semlike basin and Bwamba area. The vegetation on N slopes of the Ruwenzori Mountains is thick, impossible for man to pass through. Black and white colobus (Colobus polykomos) monkeys are abundant in this area. Bwamba is a small area in the extreme west of Uganda, lying between the Ruwenzori

Mountains and the Semliki River. Yellow fever virus was isolated from a human case and from Ae. simpsoni in early forties. Haddow (1945) claimed that Ae. aegypti is non-anthropophilic in this area. However, it is not known whether or not Ae. aegypti is zoophilic here. It might be autogenous as well.

We have collected Ae. aegypti larvae from various manmade containers such as sink-water traps, motor tires, lories etc. (Fig. 6) in Bundibugyo Village located at the foothills of the Ruwenzori Mountains (Fig. 7). We collected also many larvae of Ae. simpsoni from colocasia axils in Bundibugyo Village. Haddow, Gillett and Mukwaya claim that this population is anthropophilic. Ae. africanus, Ae. argenteopunctatus and Toxorhynchites brevipalpis larvae were abundant in paw-paw treeholes in Sempaya Village.

On our return trip from Bwamba we stopped for overnight at Queen Elizabeth National Park on 26 August and continued in our trip to Mbarara the next day.

c. Mbarara, Ankole District. A survey for larvae of Ae. aegypti was done in Biharve Village in distance 15 km NE of Mbarara. We have found Ae. aegypti larvae in discarded tires located under roof of a house (Fig. 8). A sample of live larvae was taken for colonization and further investigation. This locality is in elevation of 1,500 m.

Further survey for breeding of Ae. aegypti was done on several places between Biharve and Lyatonde, which is the last village of the Ankole District.

d. Lyatonde. An extensive survey for outdoor and indoor breeding of ae. aegypti in Lyatonde village yielded many larvae from metal drums, clay water-pots and tires located at human dwellings outdoors. No larvae were collected from indoors. Banana plantations and corn fields are common in the village. They have patchy distribution, mostly in depressions between hills. The vegatation of the hills represents scattered trees and bushes (Fig. 9). Several samples of live Ae. aegypti larvae were collected for laboratory rearing.

e. Masaka, Masaka District. Masaka was the last locality where we made survey for indoor and outdoor breeding of Ae. aegypti in Uganda. We checked several houses in a suburban area of Masaka city. We found larvae in a metal drum placed under roofs to collect rain water for domestic use. A sample of live larvae was collected for colonization.

3. Laboratory Findings

Larvae of Ae. aegypti from five different localities, Kampala, Bundibugyo, Mbarara, Lyatonde and Masaka were successfully shipped to the Mosquito Biology Unit at Mombasa and reared to adult stage. Females were fed on man and mice to get eggs. Eggs were shipped to the Vector Biology Laboratory, UND. Rearing into next generation was also successful. The females from Bundibugyo (Bwamba) did not lay egg by the time when eggs of other four populations were shipped

to the United States. All mosquitoes from four above mentioned populations fit Mattingly's (1957) description of Aedes aegypti formosus, having deep black color of cuticle and scales.

Autogeny.

All four populations were tested for autogeny. No blood was offered to females for the first ten days, after emergence. Various degrees of autogeny was found in all of them. No autogenous development was reported in field populations of Ae. aegypti before. Lea (1964) was able to get eggs from a laboratory strain of Ae. aegypti which females were fed on egg albumin and 10% sucrose for 8 generations.

The writer found some degree of autogeny in Tanzanian field populations of Ae. aegypti and Ae. subargenteus (unpublished data). The "Autogeny Index" (AIx) for Tanzanian populations was AIx=0.17. The Uganda Ae. aegypti had more than ten times higher autogeny index, AIx= 2.50 than those from Tanzania.

Since nothing is known on autogeny in field populations of Ae. aegypti it is premature to make any conclusions on feeding, autogeny, relation to hosts (if any) and vectorial capacity in Bwamba and other Uganda populations of Ae. aegypti. Perhaps autogeny is responsible for the fact that Ae. aegypti is non-anthropophilic and does not function as a vector of yellow fever in Uganda.

Ten females of the Mbarara population were dissected after ten day feeding on carbohydrate diet. All of them had fully developed eggs (IV - V stage Christophers, 1911) in their ovaries. The number of fully developed eggs per female was between 1-19 average 10.0. The problem of autogeny is being subjected to further investigation at the Vector Biology Laboratory, UND.

C. Field and Laboratory Work in Kenya

I left Entebee for Nairobi on 28 August and spent one day at the International Center of Insect Physiology and Ecology discussing various scientific problems with the Director of ICIPE, Prof. Thomas Odhiambo and with Drs. R.H. Leuthold, D. Denlinger and T. Dadhialla. I met and had talks also with Mr. Ojal, the Administrative chief of ICIPE. I left Nairobi for Mombasa on 29 August and spent 12 days in field and the MBU laboratory, discussing various aspects of population dynamics, dispersion, movements, host choice and other ecological aspects of inland and coastal populations of Ae. aegypti in Kenya with Drs. W. Hausermann, P. McDonald and D.M. Fanara. I was very well impressed with effort and amount of data collected by all of these three scientists during a period of one year, which is for this type of work a short period of time. Since they reported their results in the 1972 Annual Report and further data are reported in the present report I will not repeat them here.

1. Field test for exophilic-endophilic behaviour of Ae. aegypti in Rabai area

Six plastic traps for testing exophilic-endophilic behaviour of mosquitoes were constructed at VBL. The traps were 150 x 150 x 600 mm in size. Three circular openings were made on each side of the trap for conically shaped entrances of traps made of metal screen and a plastic rim. Six traps were shipped from Notre Dame to Mombasa by air-freight; however, by the seventh day of my staying at MBU the traps did not arrive. Dr. W. Hausermann offered to buy a sheet of clear plastic and make another set of traps. We made a new set of traps in three days, so we could test them at least once before my departure.

Problem

Most of the feral populations of Ae. aegypti do not feed on men inside houses. Being exophilic in their biting behaviour, they feed on their hosts in open. The writer tested several feral and domestic populations for exophilism and endophilism in an olfactometer in the laboratory. Five different hosts (man, rat, chicken, turtle and frog) as representative of mammals, birds, reptiles and amphibians were tested. There was significantly lower response of all feral populations to the all tested hosts. We feel that there must be a behavioural barrier which prevents Ae. aegypti formosus from entering human dwellings.

In our field experiment at Rabai, it was supposed that Ae. aegypti in the Bejuma stream forest are exophilic so they will not enter the baited traps. If they enter the trap they may be endophilic. In our field experiment we used only three kinds of hosts, mice, chicken and frogs. The traps were hung on tree branches in the Bejuma Forest and left overnight. No mosquitoes were found in traps next morning. It is premature to draw conclusions from this experiment because it should be done in many replicates and on various places in forests, peridomestic and domestic habitats using various kinds of bait.

2. Trip to Wasini Island

On 7 September 1972 I made a trip together with Drs. Hausermann, Fanara, McDonald, Mrs. Hausermann, Mrs. Fanara and a laboratory technician, Mr. George Tsuna to a coastal area south of Mombasa and to Wasini Island.

Purpose of the trip

1) To find out whether or not there is breeding of Ae. simpsoni in the coastal area of Kenya and what is the extent of its breeding.

2) To find out whether or not there is breeding of Ae. aegypti in an inhabited offshore coral island.

3) To find out the degree of domesticity in case there is Ae. aegypti present on Wasini Island.

4) What other mosquito species are present on Wasini Island and in villages of the Kenya seashore.

a. Kiwambale village is located about ten miles from the Ocean. Thatched roof houses are scattered through vegetation of the coastal plane. Mango and paw-paw trees are the most abundant there. The ground between clusters of houses is covered with grass and small bush. Pineapples are quite common and are grown in the form of patches in grass among bush. Several hundred of pineapple axils were checked for breeding of Ae. simpsoni. Most of those with water were positive for Ae. simpsoni larvae. About 50 treeholes and coconut steps with water were sampled for mosquito larvae. Some of the flooded holes were positive for Ae. aegypti; other species were Aedes calceatus, A. simpsoni, A. soleatus. Larvae of Ae. aegypti, A. calcatus and Culex fatigans were found in a big metal drum located under a palm tree. A branch of palm tree was tightened to the trunk of the palm and the base of the branch was inserted into the drum. This is a method used by local tribes to collect rain water for domestic use including cooking and drinking. These water drums also become suitable breeding sites for Ae. aegypti larvae.

b. Pemba Channel Fishing Club - Five discarded motor tires located near bush in the Pemba Channel Fishing Club garden yielded about 50 Toxorhynchites brevipalpis larvae (Fig. 10). In some tires Ae. aegypti and Eretmapodites larvae were found.

c. Shimoni Village - This is a small fishing village with few old arabic type houses at the Indian Ocean. Aedes aegypti and Eretmapodites quinquerittatus were found both in treeholes and in junked motor parts.

d. Wasini Island is located one mile offshore, south of Shimoni Village. It is irregular in shape and inhabited on three places (Northeast, Northwest and Southwest). Baobab trees (Adansonia diggitata) and variety of succulent plants are common (Fig. 11). This coral island is built in the shape of an atoll. The central portion is flooded at high tides (Fig. 13). There are several more but smaller and uninhabited coral islands south of Wasini, which were not searched at this time. These islands might be useful for genetic control experiments.

On the atoll rims of the Wasini Island we found breeding of Aedes (St.) vittatus in coral rock-holes (Fig. 12). This population is partly autogenous. We found larvae of Ae. aegypti in both outdoor and indoor containers. A sample of Ae. aegypti was taken from two old "Dugout" types of boats located on the Wasini seashore. In one of them more than 60% were yellow larvae and in the other boat which was only 3 m in distance from the first one only dark larvae occurred. In the village populations

of Ae. aegypti spot abdomen (s) and pale abdomen (pa) mutants occurred in high frequency. In the central part of the island we found larvae of Ae. aegypti formosus in treeholes. On the Southeast side of the island is a palm-tree plantation about two hectares in size. The undertree vegetation represents short (15-25 cm) grass with scattered (30-40 cm) bush (Fig. 14). Coconut shells unseparated from husks were found in piles on many places. Most of the shells with water were positive for larvae of Ae. aegypti (Fig. 15). Adults are dark ssp. formosus.

We left Wasini Island in the late afternoon. On our way back to Mombasa we found a permanent swamp with patches of Cyprus grandis. This is the plant in which Aedes (St.) woodi breeds in its axils. We were not able to get enough specimens of the plant to find whether or not Ae. woodi breeds in this swamp. Water level of the swamp was very high at this period of year and our experience from Tanzania is that populations of Ae. woodi in permanent swamps are always higher in the dry season. Sampling is also easier during the dry season than the rainy one. It is suggested that this locality should be checked for breeding of Ae. woodi between June-August next year.

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Illustrations

Fig. 1. Headquarters of MBU, located on shores of Indian Ocean just north of Mombasa, Kenya. Building is rented for three years; contains research laboratories, insectaries, library, carpenter shop, radiation source plus living quarters of Dr. Hausermann, MBU Field Director.

2. Mpanga Forest located 40 kw SW of Kampala and 16 kw North of Lake Victoria. Thick forest harboring many mosquito species including Ae. africanus, the vector of yellow fever among forest monkeys.

3. Kampala, Uganda; an automobile scrap-heap where Ae. aegypti and Culex pipiens fatigans breed in scrap iron.

4. Colocasia plantation at Bwayise, the habitat with non-anthropophilic population of Ae. simpsoni breeding in plant axils.

5. Colocasia axils as breeding sites of Ae. simpsoni.

6. A habitat in Bundibugyo village where a non-anthropophilic population of Ae. aegypti breeds in man-made containers.

7. Bundibugyo village with the Ruwenzori Mountains in background.

8. The habitat in Biharve village (15 km NE of Mbarara) where larvae of Ae. aegypti were collected from a discarded motor tire.

9. Lyatonde village, breeding habitat of Ae. aegypti outside houses.

10. Discarded motor tires in the Pemba Channel Fishing Club near Shimony Village, Kenya are suitable breeding sites for Toxorhynchites brevipalpis, Ae. aegypti and Eretmapodites quinquevittatus larvae.

11. Baobab trees and variety of succulent vegetation on coral island of Wasini.

12. Coral rock-holes filled with rain water on the atoll rim of Wasini Island breeding site of Aedes (St.) vittatus.

13. Central part of the atoll-shaped coral island of Wasini.

14. Coconut palm-tree plantation of SE side of the coral atoll of Wasini Island. Shells unseparated from husks scattered in grass are suitable breeding sites of Ae. aegypti larvae.

15. Piles of coconut shells in husks as breeding habitats of Ae. aegypti on Wasini Island.

16. Field station of MBU in Rabai study area near Mombasa.

17. Isolated village, Rabai area, where population dynamics of Aedes aegypti is under study.

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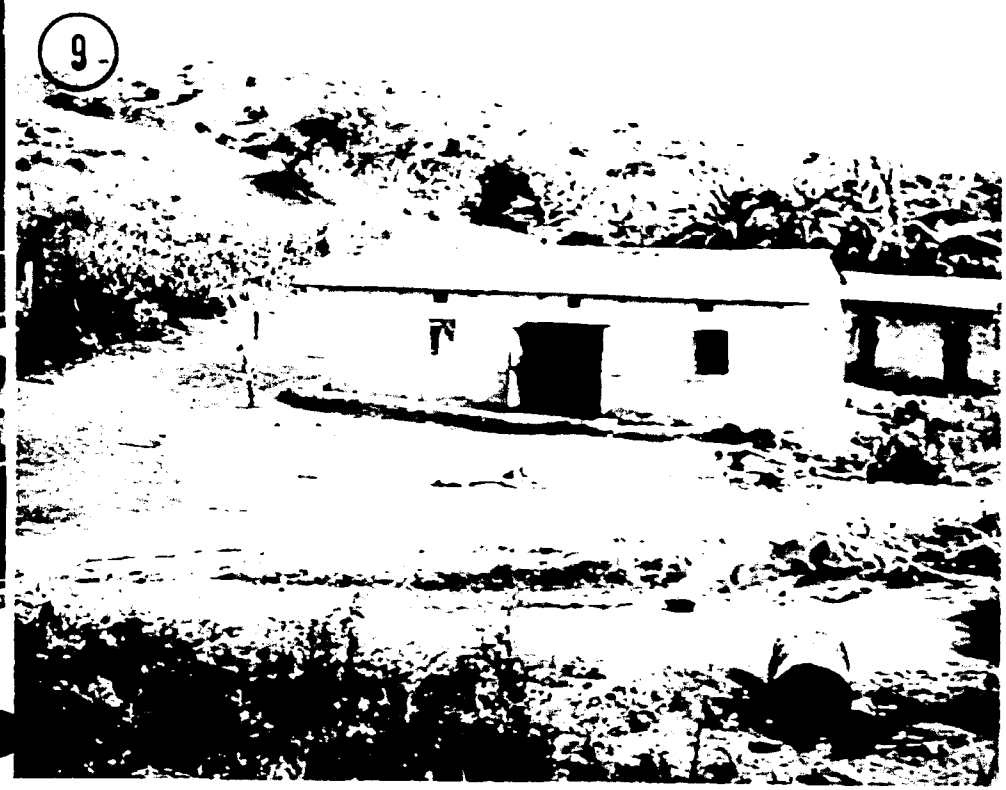
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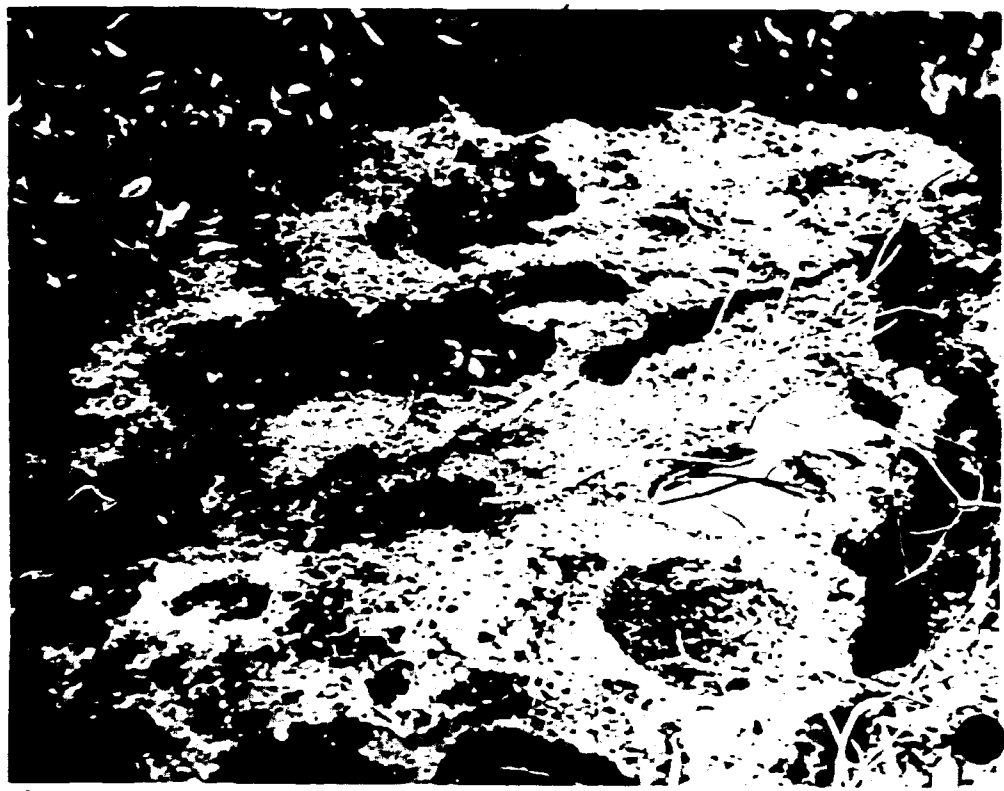

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annex 6

Report on Possible Relationships Between Reptilian Malaria and Aedes aegypti (L)*

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Refractoriness to Plasmodium gallinaceum has been found by Kilama (1970) to be genetically transmitted as a monofactorial gene, and of eight strains from around the world, this gene was found only in feral strains of African Aedes aegypti. Because P. gallinaceum has a deleterious effect on A. aegypti in lowering its fecundity (Hacker, 1971), this gene will therefore appear to offer an advantage to the mosquito in the face of possible malarial infection. Garnham (1966) states however that P. gallinaceum is found to occur naturally only in Asia, and therefore it is unlikely that this gene is maintained in the African A. aegypti populations through interaction with this malaria. Using precipitin testing of blood from wild-caught A. aegypti, McClelland and Weitz (1963) found that nearly 50% of the females from Ganda, near Malindi, Kenya, contained blood from reptiles. Therefore, Craig (1971) suggested that the selection for this gene occurred possibly as a protection against a reptilian malaria. Several species of lizard malaria are known from East Africa, including Plasmodium pitmani from skinks and P. acuminatum and P. fischeri from chameleons (Garnham, 1966).

In the light of these observations, it was felt that exploration of possible malaria-reptile-aegypti relationships would be useful. Therefore the following general lines of investigation were followed. First, a survey of reptilian populations for malarious individuals in selected areas was carried out. Secondly, a number of A. aegypti strains were tested for their ability to feed on these reptiles in the laboratory. The third step was to determine if the reptilian malariae can develop in individual mosquitoes blood-fed on the malarious lizards. If and when these factors can be established positively, then the relationship of the malaria and Kilama's susceptibility gene can be systematically investigated and compared to Plasmodium gallinaceum.

I. Collection of Lizards and Blood Smears

Materials and Methods

The lizards were collected over a three-month summer period from 1 June to 24 August from five areas in Kenya.

1. Mombasa. In and near the city of Mombasa on the southeast coast of Kenya.

2. Rabai. The Rabai area is 15-20 miles inland from Mombasa and is a comparatively hilly, farming area adjacent to an untilled forest area.

3. Malindi. This is a rolling farming area between the coastal city of Malindi and Ganda village, and is largely a cashew and coconut production area.

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4. Kodera. It is known alternately as Kodera Forest or Kodera Valley and is the site of a seedling nursery and a large, planted pine forest. It is located in South Nyanza Province about 25 miles from Lake Victoria.

5. Gembe Hills. This is an area of rocky hills and grass on the shores of Lake Victoria south and west of where Kivorongo Gulf opens into the lake. The collection area was a less rugged terrain between Gembe Hills and the lake shore.

The method of collection was either by stunning with a 1.5 x 30 cm rubber band "shooter" or by hand with the help of local people. The size range of the specimens was limited to those over 5 cm nose to anus length. Blood smears were occasionally taken on the spot, but usually were transported to the lab and caged before taking blood samples. Initially, the two blood smears were taken from each lizard by cutting the end of the tail. However, this method proved unsatisfactory and later mosquitoes were used as micropuncture needles. Since the bloodmeal coagulated within 5-10 minutes after ingestion by the mosquito, the smear was made immediately. The wings and legs were removed from the etherized mosquito, the thorax severed from the abdomen with a pair of minuten mounted on orangewood sticks, the bloodmeal forced from the abdomen on to the surface of the slide, and a thin smear was made in the normal manner.

All dried blood smears were fixed in methanol for three minutes, rinsed in distilled water and stained with Giemsa (George T. Gurr-R66) in 4% dilution for 30 minutes. Examinations of slides was with oil immersion at 1000x. The specimen was classified as negative if no parasites were found after four lengthwise sweeps.

Incidental to the blood smears, an inspection of the lizards for mite and tick ectoparasites was carried out. Those found were stored in 70% ethanol and later mounted in polyvinyl lactophenol or Turtox non-resinous mounting medium.

Results:

Tentative identifications of the lizards were made with the help of Dr. Alan McKay, at the Kenya National Museum, Nairobi and verified by Mr. Robert C. Drewes of the California Academy of Sciences. The total number of lizard specimens (n=97) and the number from which blood slides were collected (n=89) are listed in Table I. One of the Gekkonids collected in Mombasa, Lygodactylus angularis Gunther was found far north of its normal range--south of Southern Tanzania--and was probably brought in by the trading boats (dhows). The Latastia johnstoni Boul from Rabai was also found somewhat north of its recorded range (Drewes, pers. communic.).

With respect to the blood parasitism, only the lizards from Kodera were found to be positive. Among Mabuya striata striata Peters, a peridomestic skink, were found individuals infected with Plasmodium pitmani Hoare and Trypanosoma boueti Martin. Two of eleven specimens were found infected with the malaria in an initial collection, and in a subsequent collection, four malarious and one with trypanosomiasis was found among twenty individuals.

The blood parasites and ectoparasites found, along with the specific designation of the hosts are recorded in Table II. Identifications to genus of the mites were based on Zumpt (1961) and the tick on Hoogstraal (1956). The Plasmodium was identified based on the descriptions of Garnham (1966) who had collected lizard blood smears in the same area (Kodera) in 1942 and again in 1962. The trypanosome was tentatively identified as Trypanosoma boueti Martin by Dr. F. G. Wallace at the University of Minnesota.

Table II. Blood and ectoparasites obtained from lizards in Kenya.

Lizard Host	Parasite	Frequency
	Blood Parasites	
Mabuya striata	Plasmodium pitmani	5/31
Mabuya striata	Trypanosoma boueti	1/31
	Ectoparasites	
Agama agricullis	Pterygosomid mites (Pterygosoma sp.)	1/1
Hemidactylus mabouia	Pterygosomid mites (Geckobia sp.)	1/11
Mabuya striata	Laelapid mites (Sauronyssus sp.)	several
Mabuya planifrons	Laelapid mites (Sauronyssus sp.)	2/18
Mabuya planifrons	Ixodid ticks (Aponomma sp.)	1/18

II. Bloodfeeding and Infection of Aedes aegypti

Materials and Methods:

A. First the ability of the mosquito to feed on lizards was checked. Examples from four families of lizards were chosen for the trials: Agamidae, Chameleontidae, Gekkonidae and Scincidae. All were collected in the Mombasa and Rabai area. The strains of Aedes aegypti tested were from laboratory colonies established for less than two years at the Mosquito Biology Unit/ International Centre for Insect Physiology and Ecology, Mombasa. The GANGA strain is a domestic, pale form of A. aegypti, TREEHOLE and MNAZI strains are feral dark forms, and MKWAJA an intermediate, peridomestic form.

The lizard was first immobilized by wrapping in a wire mesh and placed in the plastic "gallon" cage containing the mosquitoes. The mosquitoes had all been starved 24-36 hours previous to the trial. At termination of the trial, the number of mosquitoes which had blood-fed was recorded.

B. Infection of Aedes aegypti with malarious lizard blood was the final step attempted. The specimen (Mabuya striata) with the heaviest malarial infection was used in all trials. Even so, the level of infection was low, and counts made from smears taken on the infection trial days consistantly showed 0.8-1.0% of the red blood cells parasitized. As is apparently typical for Plasmodium pitmani, 45-50% of these cells contained mature gametocytes, while the remainder had all stages of trophozooites and schizonts represented.

Table I. Species identifications and numbers of specimens of lizards collected at five sites in Kenya.

Site	Lizard Family	Species Identification	Number Collected	Number Blood Smeared
MOMBASA	AGAMIDAE	*Agama agama lionotus* Boul	7	7
	GEKKONIDAE	*Lygodactylus angularis* Gunther	1	0
	GEKKONIDAE	*Lygodactylus picturatus mombasicus* Loveridge	5	4
	GEKKONIDAE	*Hemidactylus mabouia* (Jonnes)	8	8
	SCINCIDAE	*Mabuya maculilabris comorensis* (Peters)	2	2
	SCINCIDAE	*Mabuya planifrons* (Peters)	11	11
			34	32
RABAI	CHAMELEONTIDAE	*Chameleo dilepis* Leach	3	3
	CHAMELEONTIDAE	*Rhompholeon* sp.	1	1
	GEKKONIDAE	*Hemidactylus mabouia* (Jonnes)	1	1
	GEKKONIDAE	*Lygodactylus picturatus mombasicus* Loveridge	1	1
	LACERTIDAE	*Latastia johnstoni* Boul	1	0
	SCINCIDAE	*Mabuya striata striata* (Peters)	1	1
	SCINCIDAE	*Mabuya varia varia* (Peters)	2	1
			10	8
KODERA	GERRHOSAURIDAE	*Gerrhosaurus nigrolineatus nigrolineatus* Hallowell	2	1
	LACERTIDAE	*Lacerta johnstoni* Boul	3	0
	SCINCIDAE	*Mabuya striata striata* (Peters)	31	31
			36	32
GEMBE HILLS	AGAMIDAE	*Agama agricollis* (cf) *gregorii* Gunther	1	1
	SCINCIDAE	*Mabuya striata striata* (Peters)	3	3
			4	4
MALINDI	LACERTIDAE	*Eremias spekii sextaeniata* Stejneger	1	0
	SCINCIDAE	*Mabuya maculilabris comorensis* (Peters)	5	5
	SCINCIDAE	*Mabuya planifrons* (Peters)	7	7
	SCINCIDAE	*Riopa afer* (Peters)	1	1
			14	13

Preliminary trials were run on two strains of A. aegypti, MBALAMWENI and MOMSABU, but there was very high mortality due to the age of the mosquitoes when blood-fed. A second lot of 34, four-day-old adults of a marker stock (Si, s, blt) was then infected with very little mortality.

Eight days post-infection, the midguts of the surviving MBALAMWENI and MOMSABU strains were dissected out in a drop of saline and mounted permanently in polyvinyl lacto-phenol medium. The midguts of the marker stock were dissected five days post-infection and were similarly mounted. All were examined immediately after dissection for presence of oocysts at 250x with phase contrast microscopy.

Results:

The mechanical obstacles offered by the lizard can be overcome, although in the case of the skinks (Scincidae) the scales are an effective barrier. In these lizards, almost all blood-feeding occurred in the soft membranes around the eyes, with additional biting at the leg-body juncture and occasionally under the larger scales dorsal and posterior to the hind legs. Although all the lizards tested proved to be susceptible to blood-feeding, the Agama agama and the Chameleo dilepis were particularly so.

(Table III).

Table III. Blood-feeding ability of A. aegypti strains among four families of lizards.

Lizard Family	Lizard species	Strain	No. of aegypti tested	Exposure time (min.)	Percent blood-fed
AGA	A. agama	GANGA	20	75	85
CHA	C. dilepis	GANGA	16	45	81
CHA	C. dilepis	TREEHOLE	10	30	20
CHA	C. dilepis	MNAZI	20	30	85
GEK	H. mabouia	MKWAJA	8	45	50
GEK	L. picturatus	GANGA	15	120	20
SCI	M. planifrons	GANGA	20	90	25
SCI	M. planifrons	GANGA	110	20	15
SCI	M. planifrons	MNAZI	20	45	40

In the 39 midguts dissected and inspected, no oocysts development was seen. The mortality among blood-fed individuals in the first three trials was 63-92%. The A. aegypti used in these trials were relatively old (18-20 days). However mortality among the marker stock which was four days old at the time of infection was much lower, around 6% (Table V).

Table V. Infection of Aedes aegypti with Plasmodium pitmani from the skink lizard, Mabuya striata.

Mosquito Strain	Strain Age (days)	No. Infected	Days After Infec.	Mortality	No. Dissected
MBALAMWENI	18	8	8	5 (63%)	3
MOMSABU	18	24	8	22 (92%)	2
MOMSABU	20	9	8	7 (78%)	2
SI-S-BLT	4	34	5	2 (6%)	32
		Total dissections:	39		
		Total with oocysts:	0		

Discussion:

The data collected here do not provide definitive answers to the questions asked, but at least they begin to put the problem in a working perspective. The lizard collections in each of the five areas provide a rough index of species commonly seen and most easily caught. The choice of Kodera and Gembe Hills as collection sites was not random. Garnham (1966) had found Plasmodium pitmani in Kodera twice previously, as well as at other locations along the shores of Lake Victoria. However, he noted that the malarial distribution was spotty and highly localized. In view of this, it is perhaps not surprising that infections were not found in areas other than Kodera since 1) the other localities were chosen as a matter of convenience rather than as known sites of lizard malaria occurrence, and 2) the number of lizard specimens per species collected was small.

The relative susceptibilities to A. aegypti blood-feeding of the four families of lizards indicate that the more heavily scaled Scincidae are more resistant to mosquito feeding than the others. Further investigations comparing preferences of the biting abilities of domestic versus feral strains on these lizards are also indicated.

The failure to produce oocysts in the mosquito gut walls under the conditions described herein, while certainly not precluding its possibility, may have been expected for the following reasons. First of all, the blood parasitemia was fairly low and the infection not new (as reticulocytes were common in the smears). At least in Plasmodium gallinaceum these factors have a large effect on the infectivity of blood to the mosquito (Kilama, 1970). Secondly, the number of mosquitoes blood-fed was far too few, and it is possible that the time allowed for oocyst development before dissection (5 days) may have been too short. Thirdly, attempts to infect A. aegypti with lizard malariae have been positive only in the case of the presence of a single oocyst after infection with Plasmodium agamae (Bray, 1959). Jordan (1964), though unable to infect A. aegypti found as many as 70 oocysts in Culex pipiens after infection with Plasmodium floridense. Finally, the discovery of a phlebotomine vector for Plasmodium mexicanum in California (Ayala and Lee, 1970; Ayala, 1971) gives the first convincing evidence that at least some lizard malariae are not vectored by mosquitoes at all.

Summary:

1) The striped skink, Mabuya striata, in Kodera Valley, Kenya, are found to be frequently infected with Plasmodium pitmani.

2) Aedes aegypti are found capable of feeding on selected members of four commonly found, East African families of lizards, with the chameleons and agamids being most frequently bitten.

3) Positive infections of Aedes aegypti with the lizard malaria, Plasmodium pitmani were not found.

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