AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523
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Batch 69

1. SUBJECT CLASSIFICATION
A. PRIMARY: Serials — Y-NS00-0000-G132
B. SECONDARY: Health--Tropical diseases--East Africa

2. TITLE AND SUBTITLE
Ecological studies on Aedes aegypti in East Africa preliminary to genetic control; semi-annual progress report,Jan.1974-June,1974

3. AUTHOR(S)
(101) Notre Dame Univ. Dept.of Biology

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1974	40p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Notre Dame

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)
(Research summary)

9. ABSTRACT

10. CONTROL NUMBER
PN-AAE-553

11. PRICE OF DOCUMENT

12. DESCRIPTORS
East Africa
Ecology
Mosquitoes

13. PROJECT NUMBER

14. CONTRACT NUMBER
CSD-3159 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

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CSD-3159 INC
Notre Dame
1/74-6/74 PN-AAL-553

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

SUBJECT: Annual Research Report

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:
Profs. George B. Craig, Jr., Theodore J. Crovello, and K. S. Rai
Department of Biology
University of Notre Dame
Notre Dame, Indiana 46556

3. Contract Period: 4 years, 1 July 1971 - 30 June 1975

4. Period Covered by Report: 2nd half of 3rd year, 1 January 1974 - 30 June 1974

5. Total A.I.D. Funding of Contract: $413,050

6. Expenditures - Previous Year: $99,224

7. Expenditures - Current Year: $82,482

8. Expenditures - Next Contract Year: $154,150

9. Narrative Summary - Accomplishments:

The first half of year 3 was covered by an earlier report (Jul-Dec 73) covering continuation and refinement of ecological studies. In the second half of year 3, reported herein, ecological studies were minimized and emphasis was on Phase II, a village-scale field release. We released males carrying chromosome translocation heterozygotes conferring about 67% sterility. The release extended over 10 weeks, with about 1000 males released each day. The released males were fully competitive and inseminated field females at the predicted rate. Egg hatchability in the village dropped from 90% + to 30-40%; when the release terminated, it slowly rose but had reached only 67% 10 weeks afterwards. When compared to a control village where no treatment was conducted, the release village showed a marked drop in adults collected while landing and biting. However, there was no reduction in rate of oviposition, perhaps because the experiment was too short. In a third village, treated by twice-weekly removal of larvae, the adult population declined virtually to zero. This initial experiment shows that translocations can be introduced into field populations and that they can survive long after introduction. This accomplishment is a first for any disease vector. These data further indicate that our original idea of population replacement with a translocation homozygote might be feasible. Therefore, release experiments in year 4 will attempt replacement rather than suppression of populations.

SEMIANNUAL RESEARCH REPORT

A. General Background

Aedes aegypti is well-known as a major vector of arbovirus disease over most of the tropical world. In East Africa, the species is widespread and abundant. Even minimal control is not economically feasible in most areas. To compound the problem, there are numerous behaviorally distinct populations within the species. In East Africa, one population is domestic, breeding inside houses and feeding on man, while another is sylvan, breeding in treeholes and feeding on other animals. The relative importance of these forms to disease transmission needs clarification.

In recent years, entomologists have been searching for biological methods to regulate vector species, in the hopes that such methods would avoid both insecticide resistance and environmental pollution. One biological approach is "genetic control", the use of genetic manipulation to turn a species against itself. While the use of radiation-sterilized males has attracted wide attention, this method has had little use for mosquitoes; reduced fitness of irradiated males makes the method impractical. However, there are many other ways of inducing sterility; sophisticated methods of genetic breeding allow production of males that confer sterility on their mating partners and yet are fit and competitive. Chromosome translocations conferring semi-sterility are particularly promising, both for *Aedes aegypti* and for many other pest and vector insects. This method requires field trials; the present project is so directed.

B. Project Objectives

Stated objectives of this contract include:

1. Develop a study area for intensive research on a population of *A. aegypti*.
   a. Determine absolute number and population fluctuation.
   b. Develop predictive life tables.
2. Establish 10-20 replicate sites for later use in release experiments. Monitor each population for at least one year.
3. Determine relationships between sylvan and domestic populations.
4. Develop chromosome translocations for use in genetic control.
5. Develop facilities for mass production of mosquitoes.
6. Make trial releases of lab-reared mosquitoes for methods development.
7. Conduct subsidiary research on bionomics of other *Stegomyia* mosquitoes.
8. Develop a major research component, the Mosquito Biology Unit (MBU) of the International Centre for Insect Physiology and Ecology (ICIPE).
9. Provide training opportunities for African scientists and technicians.
10. Serve as a mechanism to assist in strengthening the East African Community by affiliation with research institutes and universities.

C. Continued Relevance of Objectives

After three years of operation, it is evident that the original objectives (See Project Statement of March, 1971) were reasonable and can be obtained. The funding and the three year period were adequate for "Ecological studies...preliminary to genetic control". Additional time and funding is required to test the genetic control concept. AID has provided additional funding for a fourth year, in order to conduct a series of field releases. These field releases are the primary activity of the last half of year three, reported herein.

D. Accomplishments to Date

Facilities and personnel are given in Annex 1, Visitor's Guide to the Mosquito Biology Unit of ICIPE.

In the first half of our third year we terminated our pure ecological studies and the search for suitable translocation strains of *Aedes aegypti*. The accomplishments and findings of this period are covered by our semiannual report, July to December 1973. A final experiment on dispersal is reported in Annex 5.

Preparation for release experiments began in January of 1974. The production of release males started with the build-up in numbers of a marker strain and two strains heterozygous for single translocations. The production of the release male itself necessitated four subsidiary crosses between these three strains. An account of the translocation homozygotes and the genetical methods for preparing material for release is given in Annexes two and three.

Releases began in mid-March and reached soon a peak of about 1200 males per day. A further increase was not possible, partly because of space and working hour limitations and partly because of a chronic water shortage and repeated breakdown of the water supply due to the severe dry season.

Originally the release study was organized on the basis of two triplets of experimental villages. Each triplet was to contain one release village (see last semiannual report). The first triplet consisted of large villages of near or above 100 inhabitants, the second of small villages of around 50 inhabitants. Due to rearing difficulties our male production did not reach the envisaged 2000 males per day and we decided to concentrate our study on the triplet of large villages alone (Fig. 1, Table 1). Chibarani village was selected for the releases, Kwa Dzivo was used as village for source reduction and Majengo was designated as reference village receiving no treatment. All three villages were monitored the same way for population density, sterility, and survival of translocations and genetic markers (Table 2).

E. Findings

Due to our failure to recover in time a vigorous homozygous translocation strain, our original goal of population replacement had to be changed temporarily to population suppression by means of translocation heterozygotes. Our first extended release experiment to this effect produced the following results:

1. Our translocation males proved to be fully competitive and of equal survival value to native males (Fig. 2 and Table 3). Daily releases of between 300 and 1200 males maintained the ratio of release males to native males constantly above 10 : 1 and raised it occasionally to 16 : 1. The ratio of females to males dropped from an average of 60% to below 20% (Fig. 3).

2. After 8 weeks of daily releases of translocation males the egg hatchability rate dropped below the predicted 40%. The rate of recovery was very slow. Ten weeks after the releases were terminated, the hatch rate had only returned to 65% (Fig. 4). The oviposition rate (Fig. 5) was less encouraging. There was little difference between release and control village. However, the landing-biting catches (Fig. 6) showed two marked depression of adults in the treated village. After 8 weeks of release, the control village had 8 mosquitoes/man/hour, compared to 1.5 in the treated village. The difference was less extreme but still conspicuous in later weeks. the adult population recovered quickly when releases were stopped.

3. The killing of all larvae and pupae twice a week in Kwa Dzivo reduced the adult mosquito population rapidly and virtual eradication of the native population of domestic A. aegypti type form was achieved after 9 weeks. Immigration of domestic A. aegypti from neighbouring Chibarani and Majengo was minimal. A small amount of domestic oviposition by peridomestic A. aegypti ssp. formosus was observed, however, through the whole period. This intrusion of ssp. formosus into the domestic habitat is still taking place today after source reduction has been continued for 10 weeks beyond the originally determined date.

4. Migration between the release villages was during the whole release period negligibly small. Fluorescent dust marked males from Chibarani were recovered only once in Majengo and twice in Kwa Dzivo. A significant depression in fertility was observed only twice in Majengo, but regularly in Kwa Dzivo before the native population was removed. This low migration was not unexpected since we had dry season conditions for the whole duration of the releases, the rains being two months later than usual. See Annex 4.

5. Genetic analysis of egg collection and pupal collections done by Dr. N. Lorimer demonstrated an unexpectedly slow disappearance of the genetic markers introduced with the translocations and indicated so far a stabilisation of the M-linked translocation at around 50% (Fig. 7).

F. Relevance of Findings

We have demonstrated the ability to introduce a translocation into a population and to induce sterility long after the releases are over. We believe this is a major accomplishment.

The failure to maintain a significant reduction in adult population size does not prove a failure of genetic control by means of translocations. The double heterozygote release males introduced into the population the maximum level of sterility which could be expected. That the population size was not reduced as much as the sterility would lead one to expect confirms our earlier findings (semiannual report), that most of the mortality occurs in the larval stage and is density dependent. In domestic habitats egg mortality just reduces density dependent larval mortality.

The persistence of the male-linked translocation in the population for more than ten weeks after releases stopped indicates that selected translocations can be established in field populations for extended periods and suggests strongly that the original idea of population replacement is feasible once an appropriate homozygous translocation strain is available. This might very well be the case with the now available strain of Dr. N. Lorimer.

G. Modification in Research Design

In the report for the first part of year three, we indicated that no suitable strains with translocation homozygotes were available at that time. Therefore, we modified our release plans to use double heterozygotes, the aim being population suppression. The release experiment reported herein shows that our technology is satisfactory and that we can introduce chromosomes into populations. Dr. Nancy Lorimer, a new member of the MBU team, has now developed a translocation homozygote with satisfactory characteristics of vigor, competitiveness and potential sterility. This new mechanism was developed at the University of Notre Dame, in cooperation with Professor K. S. Rai.

We now return to original plan. During the next year (year 4), we will release the translocation homozygote, attempting population replacement. We believe that the long-term potential of this method is far greater. Ultimately, it should allow us to change populations by introducing genes for inability to transmit disease.

E. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. The following visitors were recorded in Jan-June 1974
   Leroy Williamson, Project Leader, Tsetse Res.Proj,, Tanga, Tanzania
   Danny B. Gates, Entomologist, TRP, Tanga, Tanz.
   Edgar A. Smith, Chief, Malaria Section, USAID, Washington, D.C.
   ICIPE Administrative Officers - J.H.Jivanjee, J.M.Ojal, J.Bar-Masai
   John A.Otogi, Parasitologist, Div.Vector-Borne Disease, Med.Dept., Nairobi
   Elizabeth O.Wangari, Entomologist, DVBD, Kenya Med. Dept., Nairobi
   Jaap van Esthen, ICIPE Res. Sci., Nairobi
   Paul Scheltes, ICIPE Res. Sci., Nairobi
   Gerold G. Masler, Biomathematician, E.A.Freshwater Fisheries Org., Uganda
   Grad. Students in Genetics - U.College Nairobi: Stephen Karanje, Douglas Ndivitu
   J. Hamon, Chief, Office of Vector Biology and Control, WHO, Geneva, Switz.
   J. Mouchet, Chief, Med.Entomology, O.R.S.T.O.M., Bondy, France
   Chris F. Curtis, Chief, WHO Unit for Genetic Control of Culicine Mosquitoes, New Delhi, India (U.Bristol, U.K.)

2. On 19-22 May, the ICIPE Advisory Committee for Technical Affairs met in Mombasa and reviewed the program of the Mosquito Biology Unit. This Committee consists of representatives of UNDP, FAO, IAEA, WHO, CIMMYT, ICRISAT, IITA, CIAT, CIP, IRRI and ILRAD. An account of their visit is given in the newspaper articles reproduced as part of Annex 1. The Committee spent a day plus evening reviewing the technical accomplishments of MBU and a second day touring the laboratory and the field site at the Rabai villages.
   This review by the Technical Advisory Committee represented the first annual "insect-oriented" review of the ICIPE scientific program. Next year, progress on ticks and tsetse will be reviewed and the year after, armyworm and termite. Mosquitoes were chosen for the first review because of a general feeling that MBU had made as much progress as any element in ICIPE. The Technical Advisory Committee Report on MBU was highly favorable; many members commented that the work was not only of high scientific caliber but also showed a good mix of laboratory and field, basic and applied research. It was hoped that other divisions of ICIPE could follow a similar model.

3. Another high-level review of ICIPE, including MBU, is expected in January of 1975. The Visiting Committee, representing the national academies of science which are represented on the ICIPE International Committee, consists of scientists at the highest level of international esteem. They are to review for quality of science in ICIPE. Included are:
   Dr. Harvey Brooks, Dean of Engineering, Harvard U.
   Sir Vincent Wigglesworth, Cambridge U., UK
   Dr. Perry Adkisson, President, Ent.Soc.America
   Dr. Douglas Waterhouse, Chief, CSIRO, Australia
   Dr. C.S.Holling, Canad.Dept. Agriculture
   Dr. Harry Hoogstraal, Chief, NAMRU 3, Cairo, Egypt
   Dr. D. Ekong

   AID might want to consult the report of this Committee concerning quality of work done by MBU.

F. STATEMENT OF EXPENDITURES

The budget for the third year of operation is as follows:

Expenditure	Budgeted 3 year Total	Year 3 (July 73-June 74 Proposed	Actual	Remainder
AT NOTRE DAME				
1. Salaries & benefits	98,960	34,000	29,504	+4,496
2. Supplies	9,404	145	76	+69
3. Bank charges	1,500	-	-	-
4. Travel - overseas	20,600	6,400	19,994	-13,694
5. Indirect costs-UND (14% of S&W)	12,935	4,760	3,852	+908
	143,400	45,305	53,426	
IN KENYA				
6. Salaries & benefits	21,000	12,000	6,759	+5,241
7. Housing allowance	10,800	4,500	3,741	+759
8. Rental - space	12,000	4,000	2,986	+1,014
9. Supplies	7,695	3,000	5,498	-2,498
10. Travel - local	12,000	5,200	3,065	+2,135
11. Capital	28,905	1,000	343	+657
12. Indirect costs-ICIPE (25% of direct, in Kenya)	23,100	7,425	5,862	+1,563
	115,500	37,125	28,254	
TOTAL:	258,900	82,430	81,254	+650

Notes:

1. Actual expenses, year 1 = 77,188; year 2 = 99,282; year 3 = 81680, total 258,140
   Amount budgeted for 3 years = 258,140
2. The high cost for travel was in part due to the fact that the team was being changed; earlier scientists and their families went home and new ones came out. Round trip tickets provided to the new ones. Also, the cost of travel by air has nearly doubled since this contract was devised in 1970.
3. The amount saved on salary at Notre Dame was due to the fact that Dr. Fanara left early in the year and was not replaced until much later, until we had word that the contract was renewed for a fourth year.
4. Note that salaries at Notre Dame are for the three scientists and the bookkeeper-administrative aide. Salaries in Kenya are for technicians, field aides and local labor. The large increase in funds for personnel for monitoring populations, as included in the budget, was not required. However, larger amounts will be required for day labor-collectors during the release programs planned for year 4.
5. The savings in rental were brought about by early payment of rent, in the previous year. This will not recur.
6. Inflation hit the project hard once again. Price rising seems constant on the Kenya coast.

G. WORK PLAN AND BUDGET FORCAST

1. The work plan for year 4 was given in ANNEX 18 of year 2 annual report. This was the PERT plan for for village-scale releases in field trials of genetic control. The general purpose is to carry out, analyze and document field releases of translocation strains (double heterozygote) in the East African village sites to establish the mechanisms and technological feasibility of this method of control. This will require, among others, completion of the baseline ecological studies; development of mass release strategies and techniques; and design, application and refinement of a theoretical, predictive computer model for use in planning and evaluating the field release. The following specific tasks are to be carried out in year 4.
   a. complete studies of village mosquito population fluctuations;
   b. complete work on life tables, including capture-mark-release-recapture experiments, associated computer modelling, and village de-faunating and re-invasion studies;
   c. complete work on migration between domestic and sylvan niches in both wet and dry season;
   d. complete studies on gene flow between indoor and sylvan populations;
   e. continue work as required on development and mass production of translocation to provide the type and quantity of the strain best suited for field testing;
   f. continue work on design and testing of release strategies, including computer modelling component;
   g. develop a probabalistic computer model as a basis for designing release strategies and predicting the impact of the releases on native populations - - and refine and upgrade the model through feed-back of results as the field trials proceed;
   h. conduct, monitor and thoroughly analyze mass releases of double-heterozygote A. aegypti in the Rabai District field sites near Mombasa to assess population suppression effect and reinfestation rates. The release experiment is to involve a minimum of two villages with no treatment, 2 villages where the populations have been suppressed by conventional treatment, and two villages where the males which are heterozygote for two translocations will be released;
   i. carry out a close, continuing interaction with the WHO A.aegypti project in India through the dissemination of progress reports and results and travel by Notre Dame scientists to the New Delhi project;
   j. prepare a comprehensive final technical report on the total, four-year project in a form and quality suitable for broad distribution throughout the international scientific community.

2. Anticipated budget for year 4 (1 July 1974- 30 June 1975)

Expenditure	Yearly average, years 1-3	Proposed year 4
AT NOTRE DAME (2620-25157)		
1. Salaries & Benefits	33,000	45,000
2. Supplies	3,500	1,000
3. Travel - Overseas	6,800	12,000
4. Indirect Costs-UND	4,500	6,300
(14% of S&B)		64,300
IN KENYA (2620-25165)		
5. Salaries & Benefits	7,000	12,000
6. Housing Allowance	3,600	6,000
7. Rental - Lab Space	4,000	6,000
8. Supplies	3,500	6,000
9. Travel-E.Afr.	4,000	7,000
10. Capital Equipment	9,000	8,000
11. Indirect Costs-ICIPE	7,400	11,250
(25% of direct)		56,250
TOTALS	86,300	120,550
AT NOTRE DAME - Computerized Systems Analysis - Crovello		
1. Salaries & Benefits		13,000
Systems Analyst - 12,000		
Crovello Summer Salary - 2,000		
Keypuncher-Typist, ½ time - 4,000		
2. Permanent Equipment (desk calc.)		500
3. Computer Supplies		500
4. Publication		100
5. Travel		1,000
	Direct costs	23,600
6. Indirect Costs (57% of S&B)		10,000
	TOTAL	33,600
GRAND TOTAL.....		154,150

Notes:

1. Salaries at Notre Dame, partl, include funds for Prpject Leader, Geneticist, Ecologist and part-time bookkeeper.
   Salaries in Kenya include funds for Laboratory Technicians and Field Aides.
2. The project has been hard hit by dollar devaluation and by galloping inflation in Kenya. Salaries and other budget items that were appropriate in 1970, at the time of original application, are now out of line.
3. Housing Allowance for each of the three scientists has been raised from $1,200 to $2,000 to reflect the staggering increase in rents on the Kenya coast - often 30% per year. Rental of lab space has also been increased, in accordance with demands of the landlord.
4. One additional vehicle is requested in capital equipment, to replace the Jeep Wagoneer, which is in a state of collapse after only two years of use.

Table 1. Description of release villages prior to release study.

W. Hausermann

Name of village	No. Inhabitants	No. houses	No. Containers	No. containers with larvae or pupae	L-B catch/man-hour	
					Total	♀♀
CHIBARANI (= CHI)	161	31	48	34	3.7	2.6
KWA DZIVO (= KDZ)	108	31	36	26	6.4	5.3
MAJENGO (= MAJ)	82	28	43	32	3.6	1.4
Average 15 other Rabai villages	87.9	21.9	35.2	19.7	3.4	2.6

Table 2. Treatments and population assessments of the three villages in release experiment.

W. Hausermann

Village	Treatment	Weekly population assessments for all three villages		Additional assessments
		Population size	Sterility	
CHI	Daily release of 300-1200 double hetero-zygote males from March 29 to June 9	1. Oviposition rates with 1 oviposition trap per house for 3 days per week	1. % hatchability of eggs collected in oviposition traps	1. Estimates for total adult populations at beginning and half time or release experiment
KDZ	Cleaning of domestic water pots every Wednesday and Saturday from March 30 till to date	2. Landing-biting rates with 3 men for 10 minutes per house once a week	2. % hatchability in egg batches of single females collected in L-B catch	2. Estimate for survival of release males and ratios of release males to native males for first half of release period
MAJ	No special treatment	3. Container index once a week		3. Assessment of competitiveness of release males in additional village
		4. Total pupal counts once a week		4. Assessment for survival of genetic marker and translocations from May 20 till to date, carried out by Dr. N. Lorimer

Table 3. Competitiveness of double heterozygote release males.

W. Hausermann

Days after first male release	Male recovery			Female recovery				Competitiveness
	Total	No. marked	% marked	No. marked	No. ovipositioning	No. with reduced hatch	% reduced hatch	
5	181	164	90.6	43	40	28	70.0	0.77
6	115	91	79.1	70	56	30	53.6	0.62
7	123	83	67.5	56	52	36	69.2	1.02
8	53	28	58.3	13	8	6	75.0	1.29
9	60	16	26.7	29	24	21	87.5	3.28
Totals	539	389	72.2	211	180	121	67.2	0.93

Release of translocation males: 500 per day on days 1-4

Release of virgin females: 600 per day on days 5 and 6

Recovery of males and females on days 5-9

$$\text{Competitiveness} = \frac{\text{\% ♀♀ with reduced hatch}}{\text{\% release males in total male catch}}$$

FIGURE 1

LOCATION OF VILLAGES IN RELEASE STUDY

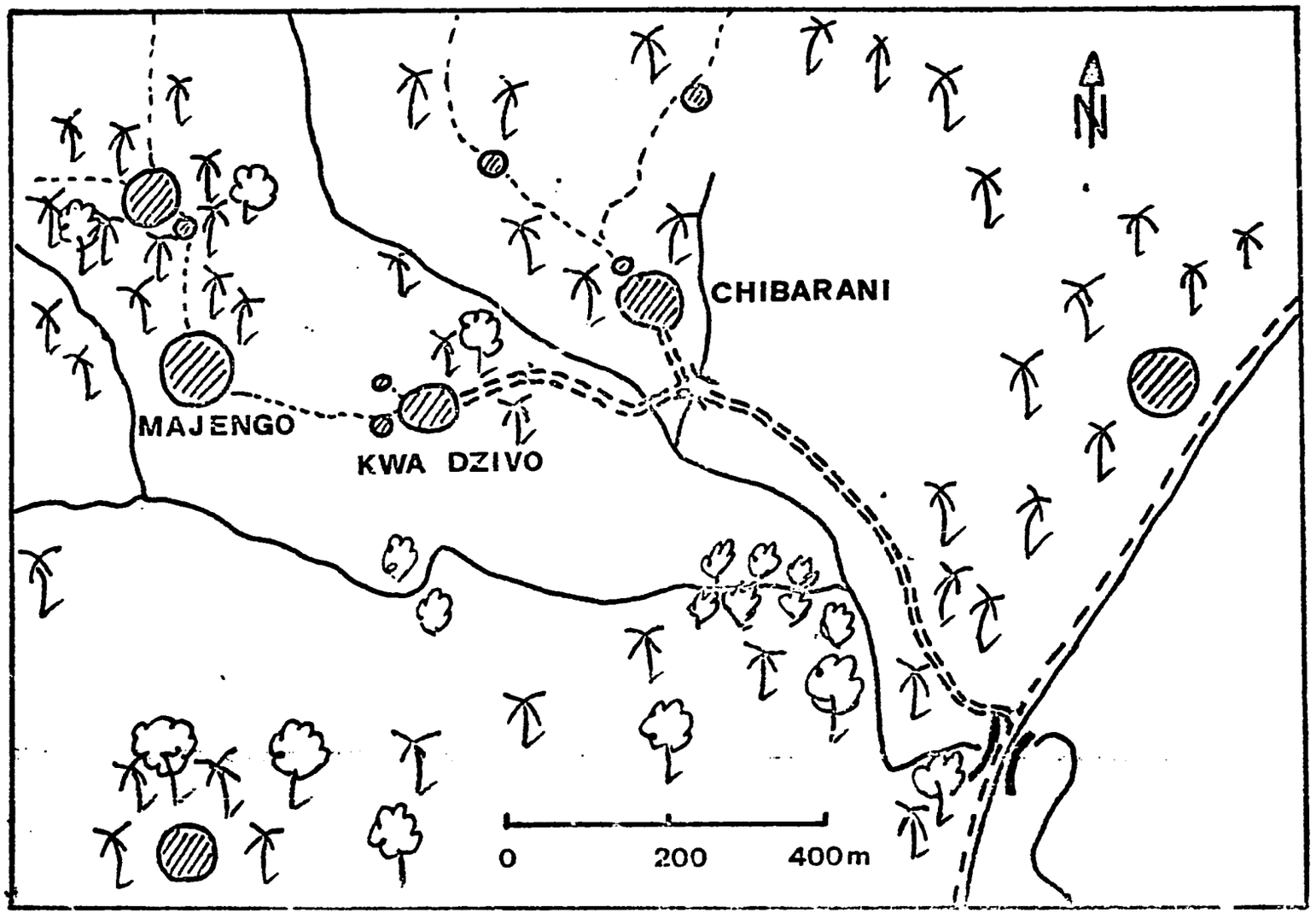


FIGURE 2

SURVIVORSHIP OF A.AEGYPTI MALES IN CHIBARANI

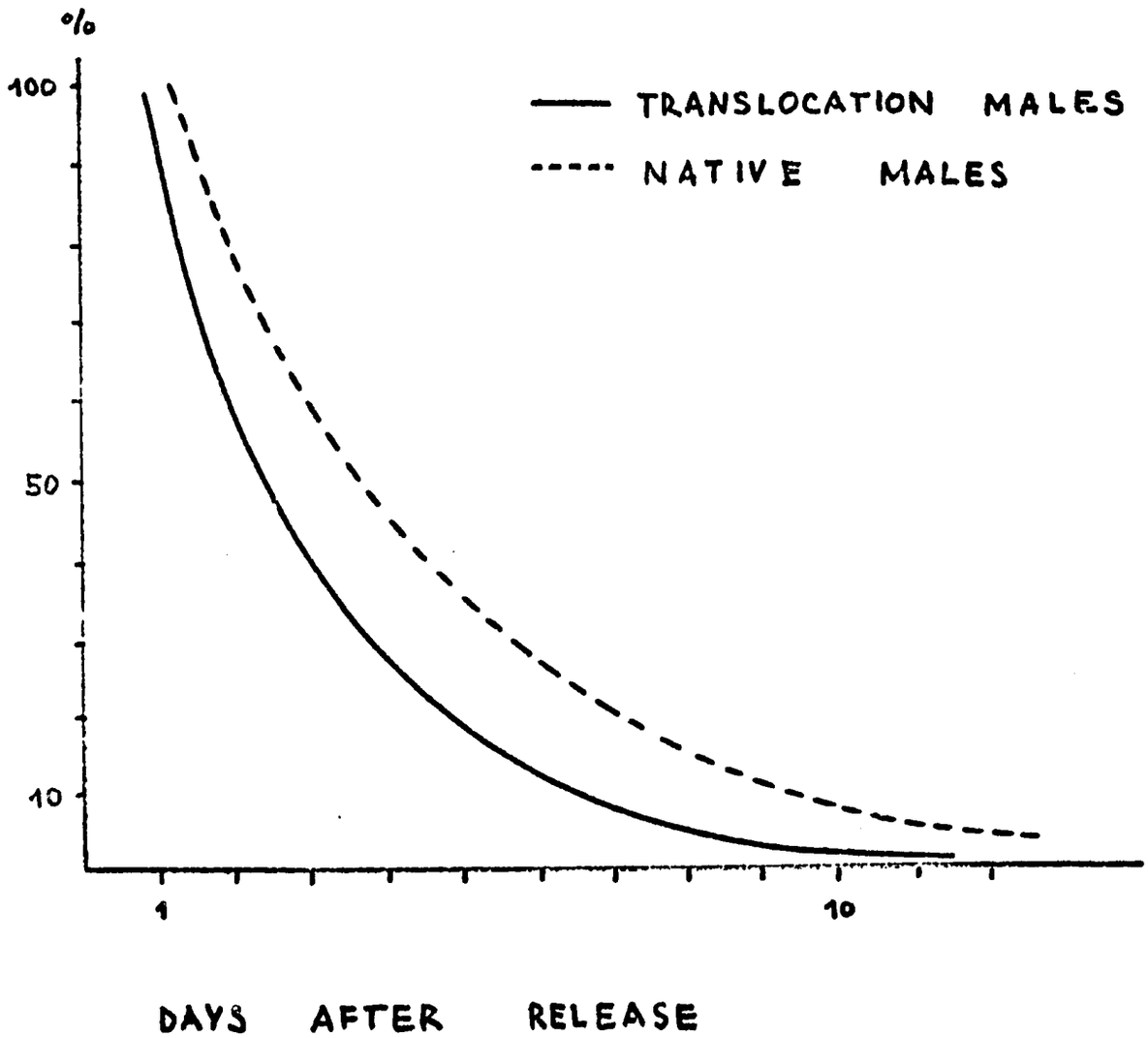


FIGURE 3

FEMALE RATIOS IN RELEASE AND REFERENCE VILLAGE

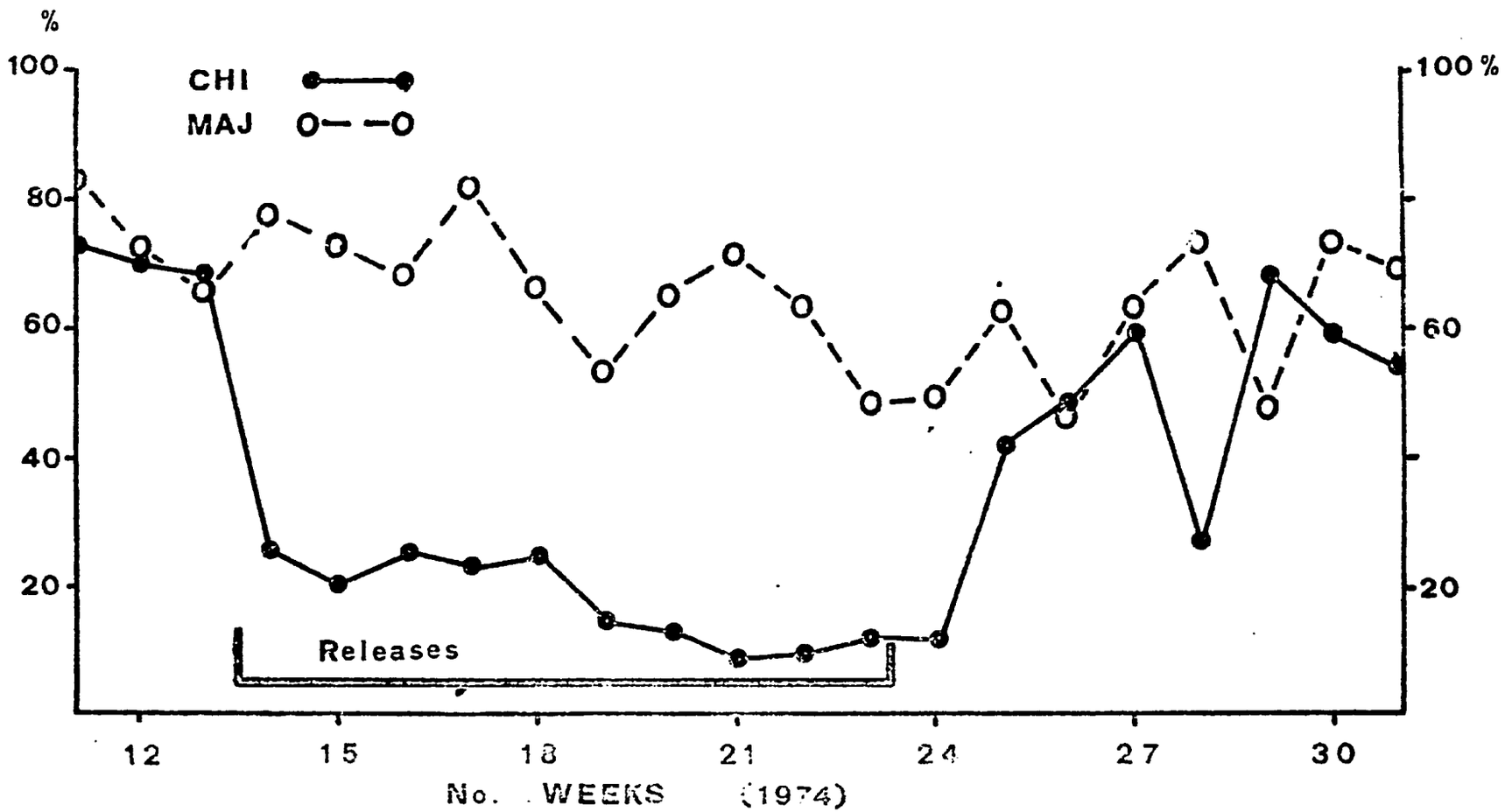


FIGURE 4

HATCHABILITY

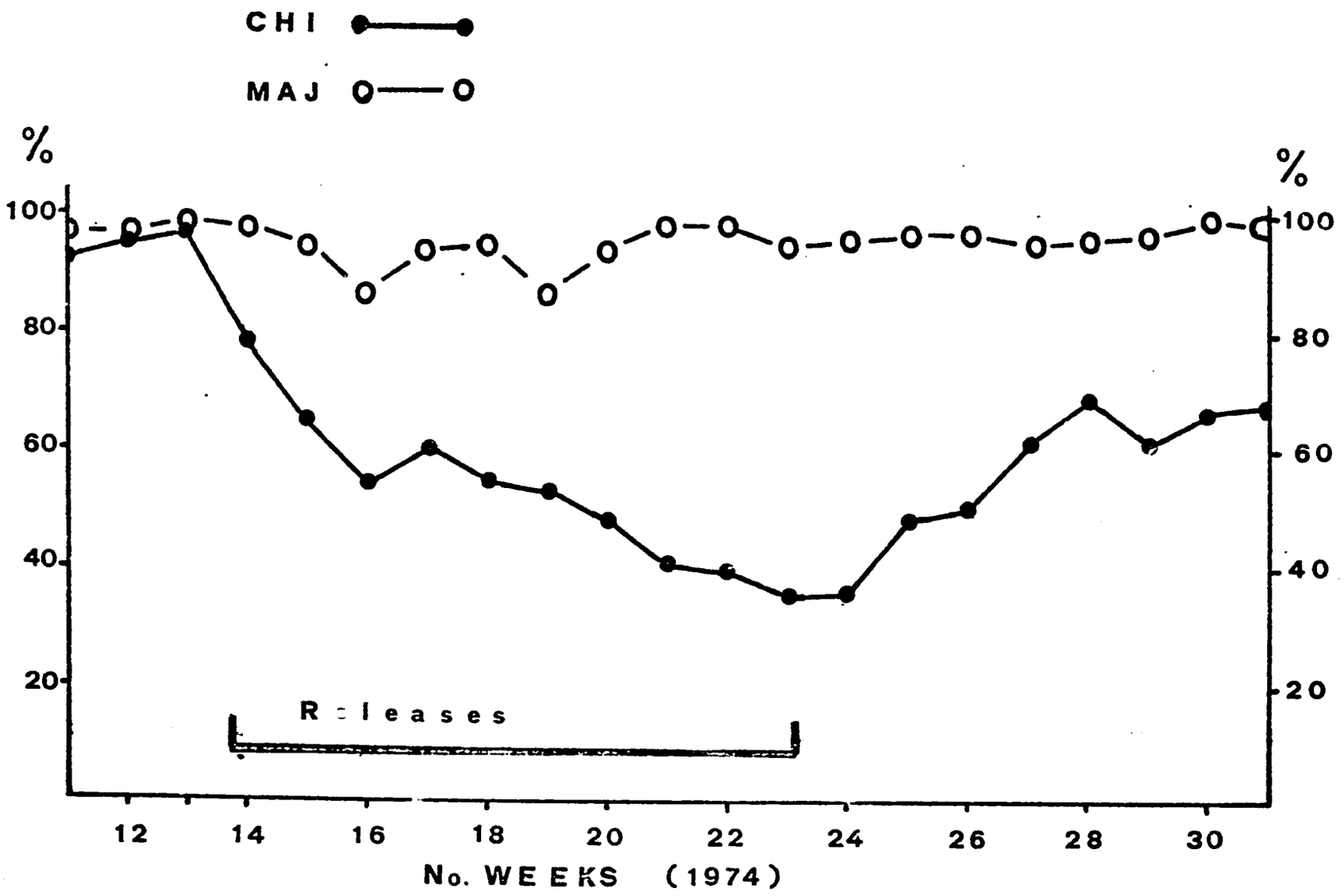
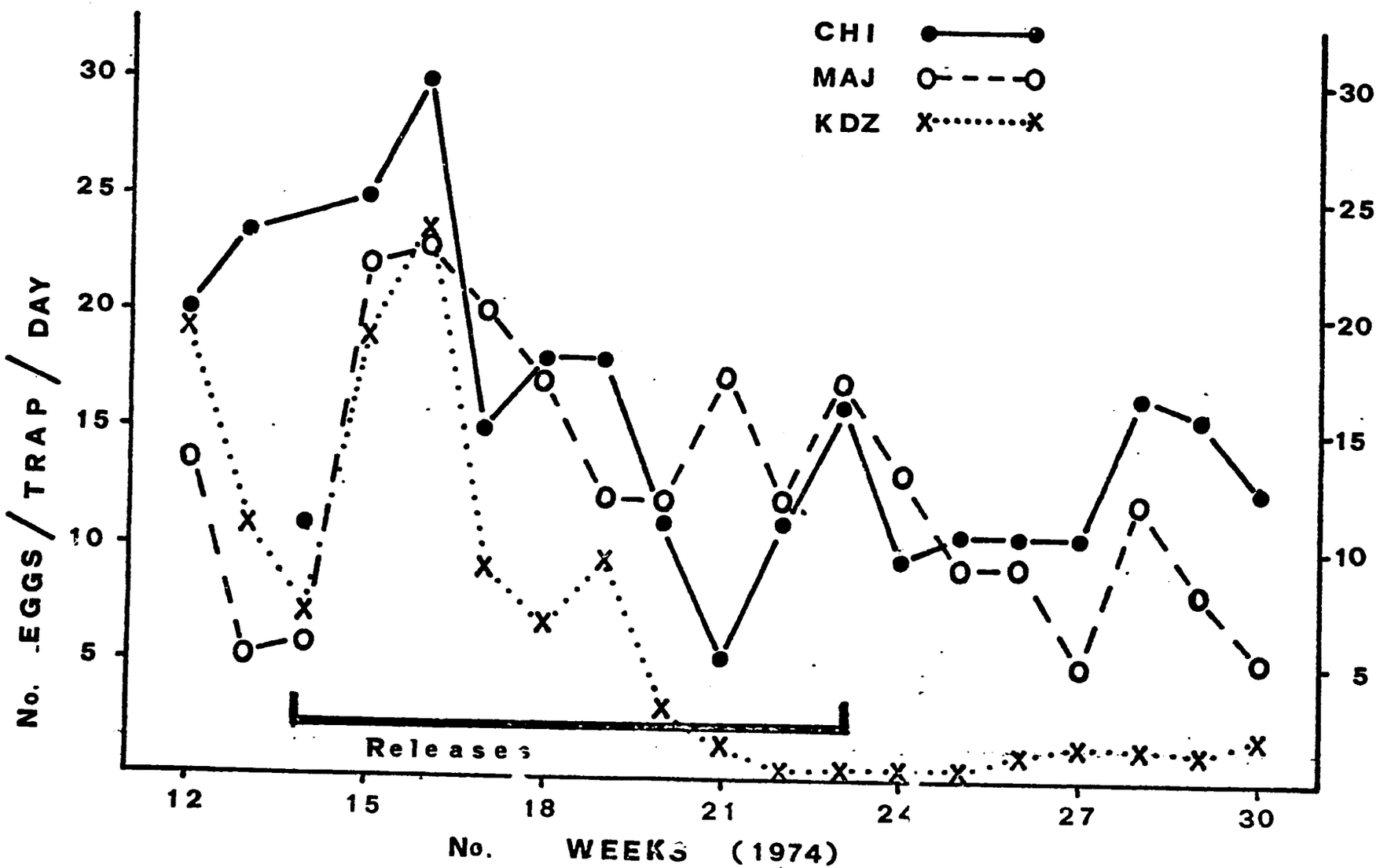


FIGURE 5

OVIPOSITION RATES



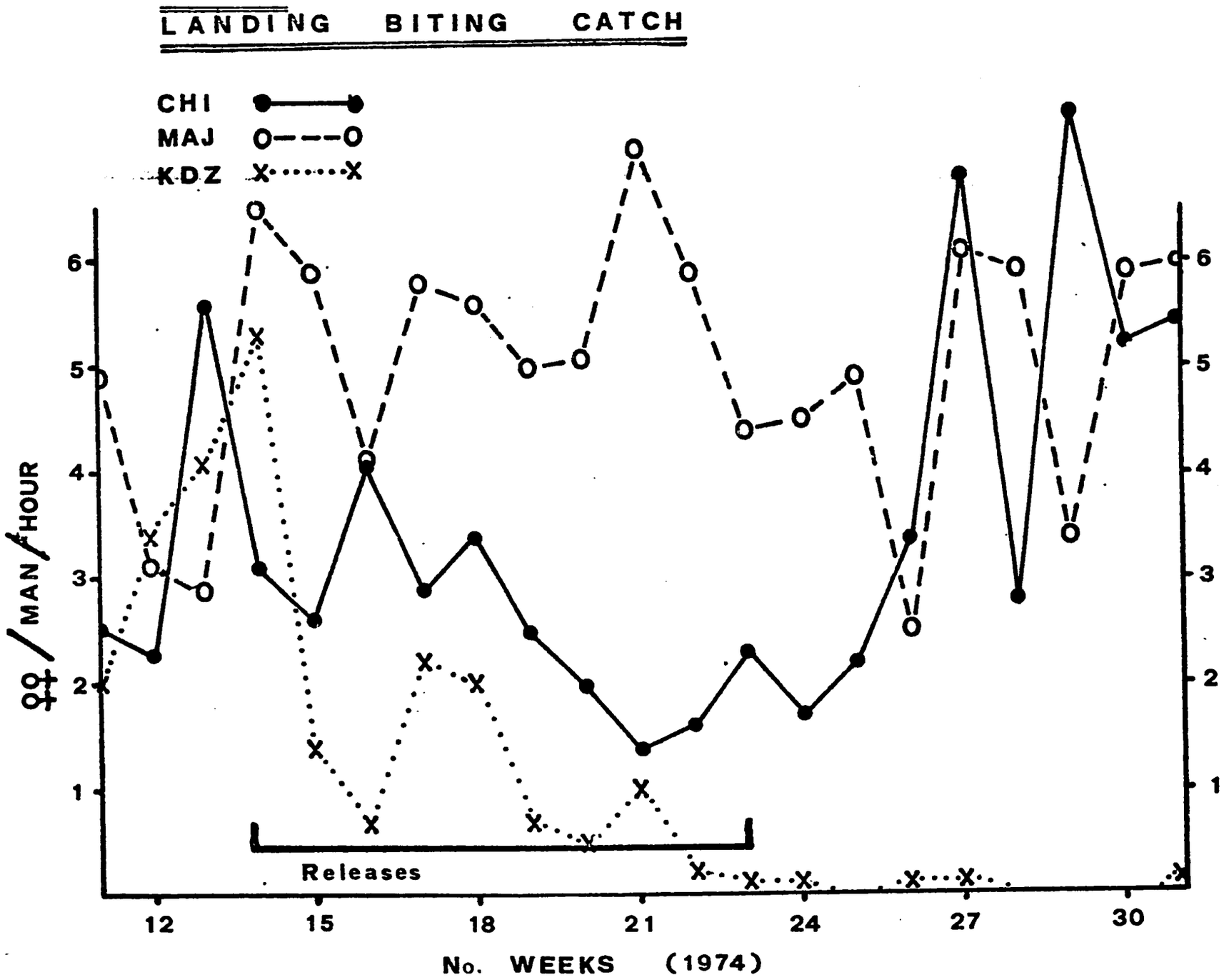
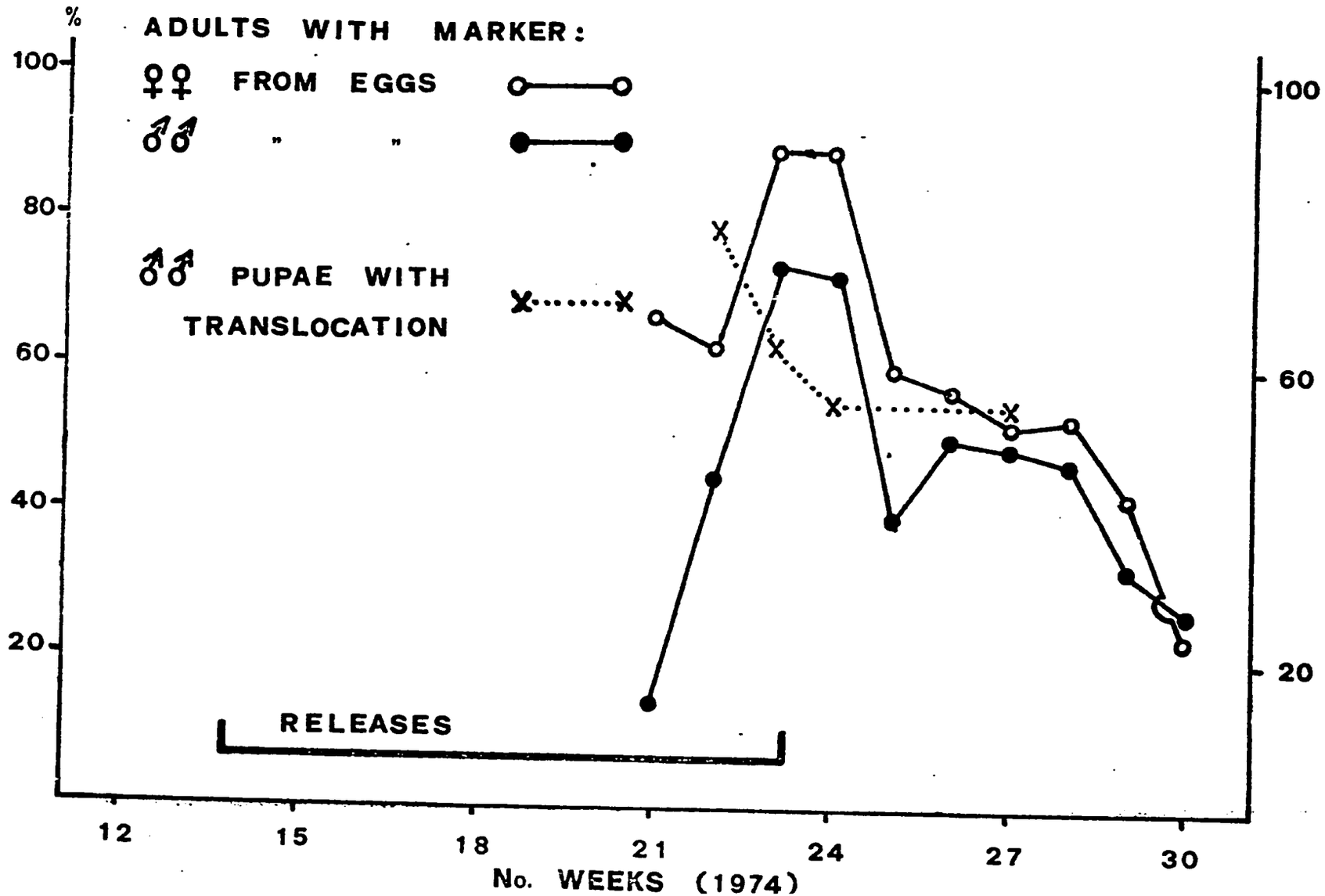

LANDING BITING CATCH
CHI
MAJ
KDZ
♀♀ / MAN / HOUR
Releases
No. WEEKS (1974)
12
15
18
21
24
27
30
1
2
3
4
5
6

FIGURE 7 (by N. Lorimer)

GENETIC MONITORING IN CHIBARANI



Visitor's Guide to Mosquito Biology Unit
MBU/ICIPE/Kenya

1. Sponsorship: The MBU is a part of the International Centre for Insect Physiology and Ecology, Nairobi, Kenya (PO Box 30772; Director - Prof. Thomas Odhiambo). MBU operates under ICIPE Research Director George B. Craig, Jr. (Director, Vector Biology Laboratory, University of Notre Dame, Notre Dame, Ind.46556, USA).

2. Funding: MBU operates on a 4-year research contract to the University of Notre Dame from the U.S.Agency for International Development. This contract terminates on 1 July 1975. Funds are also provided by the UNDP, via ICIPE, and by the U.S.Public Health Service.

3. Location: The laboratory is located in a 15 room home at Nyali Beach, north of Mombasa. P.O.Box 80804; Phone Mombasa 71653. A diagram is attached. Field studies are conducted in rural villages in Rabai District, west of Mombasa, a 30 minute drive from the laboratory.

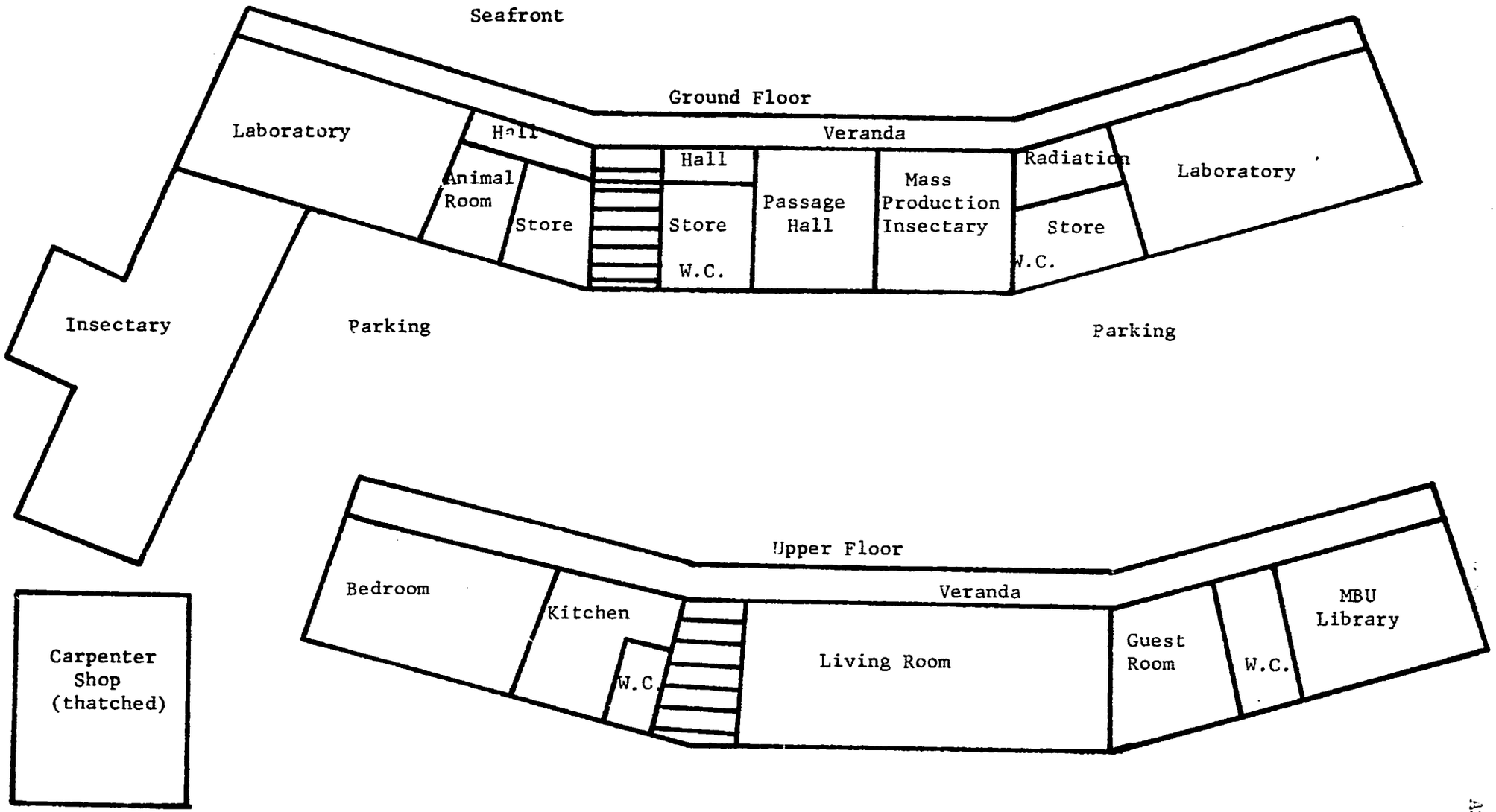
4. Personnel:
   - Scientists - 1st 3 years - Walter Hausermann,Project Leader(now Ciba-Geigy,Basel)
     - Paul McDonald, Geneticist (now U.Calif.,Berkeley)
     - Dean Fanara, Ecologist (now WHO, Indonesia)
   - Year 4 - L. Philip Lounibos, Project Leader (NIH PD Fellow)
     - Nancy Lorimer, Cytogeneticist
     - John Petersen, Ecologist
     - Fidelis Ogah, Population Geneticist (ICIPE Fellow)
   - Technicians: Rosbella Chesang - Laboratory management, microscopic work
     - David Maina - Special genetic strains, translocations
     - Samuel Mburu - Mass production
     - George Tsuma - Rearing of laboratory strains
     - Nicolas Juma - Electrophoresis
   - Field Assistants: Julius Mwandadu, Livingstone Ndenge
   - Workshop Assistants: James Rimba, Steve Wanjohi
   - Temporary Assts., Daily Basis:
     - Karim Iddi, Peter Kimani, Kenneth Saha,
     - Malaki Ralik, Eduard Mkuzi

5. Project Objectives:
   a. To study the ecology of the yellow fever mosquito, *Aedes aegypti*, in detail, to recognize the key environmental factors and to predict its responses to seasonal change and control operations.
   b. To attempt population suppression by genetic manipulation of populations, the so-called "genetic control".

6. Research Progress:

The research was organized in two phases. After a preliminary period devoted to site selection (Kenya coast has heavy year-round populations of *A. aegypti*), two years (June 71 to June 73) were devoted to studies of selected sylvan and domestic populations of *A. aegypti*. Simultaneously, strains with chromosome translocations were selected and developed in the laboratory. From July of 1973 onwards, primary emphasis shifted toward small-scale field trials of released males bearing translocations for genetic control. Results to date have been exciting. When males were released daily at a 10:1 ratio, a high level of sterility was established in a village. More important, sterility continued long after the releases were halted. By June of 1975, we expect to be able to make a well-documented recommendation on the feasibility of genetic control.

Phone Mombasa 71653
1 inch = 25 feet (————)



7. Summary - Ecological Research:

a. Population composition - On the Kenya coast, A. aegypti occurs in two distinct populations, domestic and sylvan. These populations have very little genetic exchange, although their habitats overlap. The populations differ in their dependence on rainfall and in their preference for oviposition sites, hunting sites and host species. They can also be distinguished on the basis of coloration of both larvae and adults. The domestic form, subspecies aegypti, (a) has adults that are brownish and creamy white, with some pale mutants of the queenslandensis variety, (b) has a high frequency of yellow larva, (c) lives inside houses, with larvae breeding in clay pots and other water containers, (d) prefers man as host. The sylvan form, subspecies formosus, (a.) has adults that are black and silvery white, (b) has no yellow larva, (c) lives in forests, with larvae breeding in treeholes, (d) prefers other animals, not man, as host. The numbers of the domestic form are reasonably constant throughout the year, whereas the sylvan form is abundant only during the rainy season. Also during the rainy season a third form, peridomestic, appears, breeding in outdoor habitats such as old tires, discarded containers, etc. The peridomestic form results from hybridization of the other two forms.

b. Many experiments have been conducted on marking and releasing mosquitoes for later recapture, in studies of population size and dispersal. Mosquitoes are either dusted with fluorescent dyes or individually painted with dots in a distinctive pattern. Recovery rates on these experiments have been in the vicinity of 30-50%, exceptionally high for this sort of work. We find that in a typical village of the Rabai District, with 15-20 houses, there is an ongoing population of about 1000 mosquitoes, with 50-100 new adults emerging each day. Both sexes move at random through the village, from house to house. Relatively few adults leave the village, the number depending on the distance to the adjacent village. Less than 1% will move as far as 800 meters away from the village of origin. These characteristics make the Rabai area an excellent study arena for experiments on genetic control; each village may be considered as an ecological island.

8. Summary - Genetic Research

a. In the laboratory, chromosome translocations have been induced by radiation and bred and analysed with genetic marker stocks. In two years, 78 translocation heterozygotes have been isolated. Initially, we had hoped to obtain translocation homozygotes and to use them in experiments on genetic manipulation by replacement of populations. However, we were unable to obtain homozygotes in the time available. Therefore, we have developed a system of double heterozygotes which confer high sterility. Field females mated by males carrying this system have 20-30% egg hatch; moreover, almost all resulting progeny also carry the system. The population will continue to be affected long after the initial release.

b. A field experiment on genetic control was initiated in April, 1974. Three villages were used; one was for the release of translocated males, the second was for control by removal of larvae and the third was untreated. All villages were monitored weekly for landing-biting adults, pupal output, oviposition rate and sterility. Males were released at the rate of 1000 per day, every day for 60 days. This gave a ratio of better than 10:1 of release males over native males.

c. Results of the first field experiment are still coming in at the time this is written. However, there is no doubt that the high sterility was established in the experimental population. The villages all started with94-98% egg hatch. In the translocation release village, the egg hatch rate declined steadily to 40% hatch after six weeks and remained at this level for 10 additional weeks (to the present). Note that releases were halted after the first 8 weeks.

9. Visit to the Field Site:

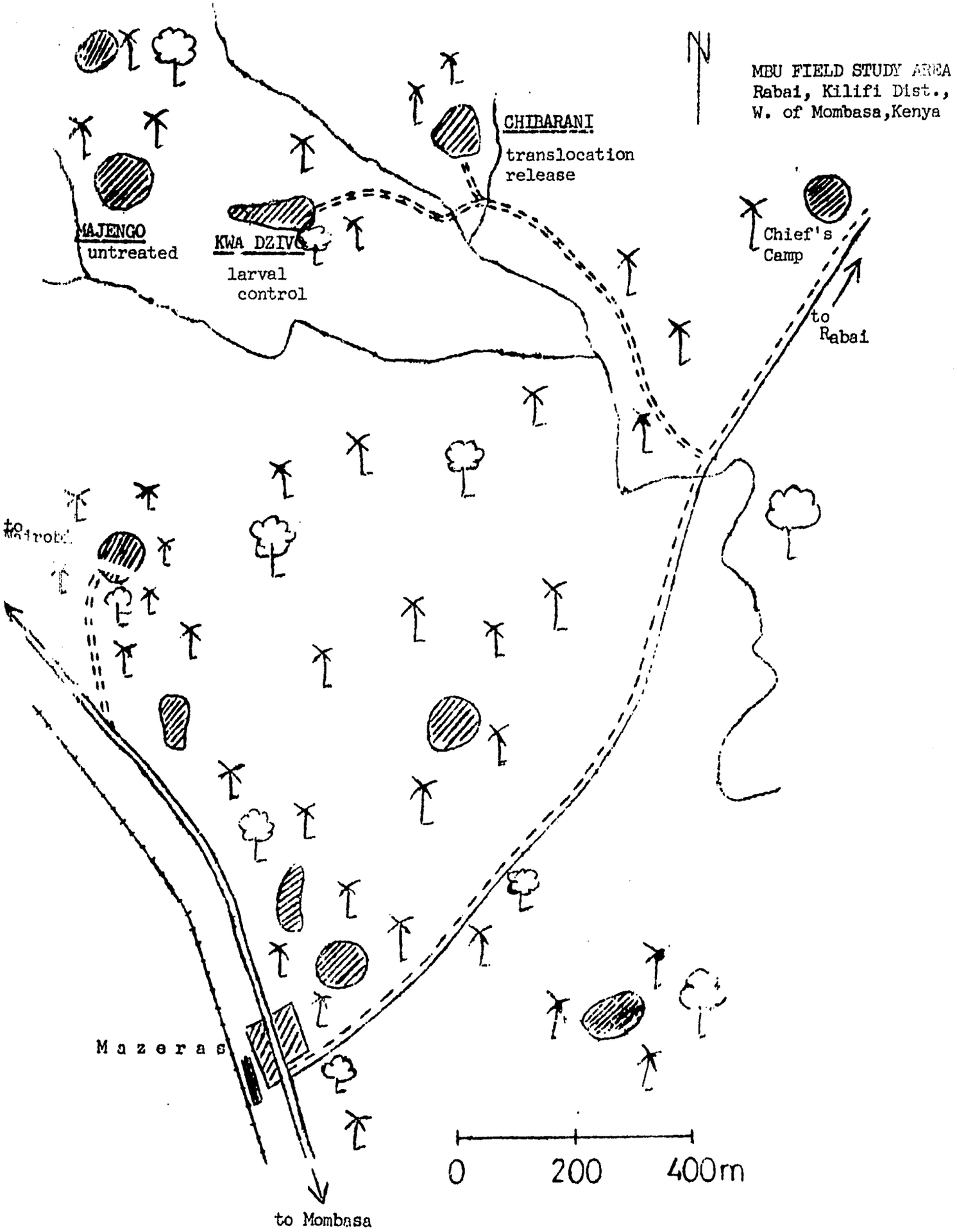
a. All of MBU's experimental field sites are situated in the area of the Rabai people in Rabai and Ruruma of Kilifi District, just west of Mombasa. The reasons for selecting sites away from the immediate coast and outside the municipal boundaries of Mombasa are twofold:
   a. Mombasa exercises within its boundaries a vigourous mosquito control program which prohibits long range field observations. No mosquito control(or other insect control) is conducted in Rabai.
   b. The people in the mosquito-producing areas of Mombasa and the coastal tourist belt are heterogenous, mobile and highly individualistic. Full cooperation of all people, which is essential for our kind of work, is very difficult to obtain on the basis of friendly persuasion. In Rabai, the population is homogeneous and extensive cooperation with the villagers can be obtained almost everywhere after proper introduction and acquaintance with the village elders. In practice, the people have given us a warm and friendly welcome. We are deeply grateful to them.

b. The field sites are reached after a 30-40 minute drive from the MBU laboratory. The approach leads through Mombasa Island and then follows the road to Nairobi for about 10 miles. After climbing the coastal foothills in Mazeras, the Rabai area lies to the Northeast of the Nairobi road, in the coconut belt. Please see the attached sketch map. In the Chief's Camp, we have located an ICIPE Mobile Laboratory; testing for egg hatch and microscopic examination for translocations are done here. The three experimental villages are typical of hundreds of others in the coastal belt. They were treated as follows:
   (a)Chibarani - Translocated males released, 1000/day for 8 weeks
   (b) Kwa Dzivo - Larvae in domestic water containers (clay pots) removed twice weekly by cleaning pots
   (c) Majengo - No control.

   The distances between these villages are 2[illegible] m and 20[illegible] m, respectively. Biting and domestic breeding in all villages monitored weekly.

c. Subsequent experiments will be done in villages that are further apart. More effort to prevent immigration will be made. Two more releases are planned, one in October of 1974 (short rains) and one in January of 1975 (long rains).

10. Outreach: MBU has working contacts with the following agencies:
   a. University College, Nairobi - Entomol.Dept.-Prof.Odhiambo,Botany Dept. - Prof. Olembo, ICIPE home laboratory
   b. Division of Vector-Borne Disease, Kenya Medical Dept., Nairobi
      Director - Dr. J.D.Roberts; Entomologist - V.J.Prashar
   c. East African Institute of Malaria & Vector-Borne Disease, Amani, Tanzania
      Director - Philip Wegesa; Entomologist - John Raybold
   d. Dept. Preventive Medicine, School of Medicine, Univ. Dar-es-Salaam,Tanz.
      Senior Lecturer - Wenceslaus Kilama, Filariasis
   e. East African Inst. of Virus Research, Entebbe, Uganda
      Entomologists - Louis Mukwaya, S. Sempala


MBU FIELD STUDY AREA
Rabai, Kilifi Dist.,
W. of Mombasa, Kenya
N
CHIBARANI
translocation
release
MAJENGO
untreated
KWA DZIVO
larval
control
Chief's
Camp
to
Rabai
to
Nairobi
Mazeras
to Mombasa
0
200
400m

ENOUGH TO GIVE YOU THE CREEPS

What makes insects tick?

Adrian Grimwood's COASTLINE

VISIONS of the *Hellstrom Chronicle*; a score of the world's leading insect scientists and United Nations' agency representatives are bottled up this weekend in a Coast hotel discussing the possible genetic control of ticks, army worm, tsetse fly, termites and mosquitos.

Officially designated as the second meeting of the International Centre of Insect Physiology and Ecology (ICIPE) Policy Advisory Committee, this top-draw dialogue has brought in the best brains from Africa, America, Europe and Asia.

While discussions on some subjects tend to be generalised, the scientists have been able to lay special emphasis on preliminary research being conducted locally into controlling yellow fever mosquitos.

For two years now a team of a dozen scientists and technical assistants, led by United States Prof. George B Craig, have successfully established a small research laboratory on the North Coast's Nyali suburb.

Here they have exclusively concerned themselves with trying to understand the life cycle, breeding habits and population movements of one *aedes aegypti* — the yellow fever vector.

(Nyali residents need have no fear about living in a "fever-prone" area — the unit was established there because it was conveniently close to nearby field research centres).

Part of subsequent scientific field experiments from Nyali have involved the process of "chromosome translocation" (desexing) as a means to genetic control.

Processed mosquitos (genetically altered) have been released around the test villages in the coastal hinterland.

Having studied the local mosquito habitat, the scientists have released the genetically altered male mosquitos with which they hope to suppress and manipulate the local mosquito population.

Although it is probably still too early to draw any definite conclusions from this rather exotic-sounding line of research, initial results do seem to please the wary scientists.

Meanwhile, back at Bamburi's Kenya Beach Hotel the meeting, presided over by ICIPE's Governing Board chairman, Prof. J. W. S. Pringle, is also discussing the work of other research projects now under way within Kenya.

Headed by its director, Dr. Thomas Odhiambo, the Nairobi-based ICIPE now has more than 30 local and overseas scientists examining equally fascinating aspects of insect research.

It's also interesting to note that while all this formidable international effort is being marshalled against such pests, the ICIPE, also conducts intensive research into the ecological implications of a too-effective approach to insect control.

22 April 1974

East African Standard

Insect seminar ends

Standard Reporter, Mombasa.

Sixty insect scientists and representatives of international groups concerned with the health and welfare of both man and animals ended their two-day Mombasa seminar on Saturday.

The focal point of the delegates' discussion was progress in pest research, with special emphasis on the preliminary advance made towards the genetic control of yellow-fever mosquitoes.

The meeting was organised by the International Centre of Insect Physiology and Ecology (I.C.I.P.E.).

The delegates who attended are members of I.C.I.P.E. policy advisory committee, whose membership includes the centre's directors of research as well as representatives of various international agricultural research institutes.

Topics discussed included research policy and the relevance of the centre's programmes to African and world needs.

An in-depth examination of the work accomplished by I.C.I.P.E.'s Mosquito Biology Unit, whose field director is Dr. Walter Hausermann, featured a tour of the unit's facility at Nyali and visits to mosquito monitoring stations in the field around Mombasa.

The unit is well-known to Mombasa residents. It was established two years ago by I.C.I.P.E. with support from U.S. and the University of Notre Dame, U.S.A., and has conducted many field experiments with the co-operation of Coastal residents and the Kenya Government.

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Annex 2

Translocation Homozygosity

Paul T. McDonald

A translocation homozygote strain has been isolated and is being established. The AT 1:2 t translocation derives from the irradiated domestic strain GANGA. The translocation heterozygote is characterized by a fertility of 30 percent and a pseudolinkage of 637/653 or 0.98. In the first trials for homozygosity, the four females tested negative. In the next trial, four females tested negative and one female positive. In a subsequent trial, one of four females tested positive for homozygosity.

Once it was established that the homozygous state was viable for the female, the next step was the production of males homozygous for the translocation. Since the translocation was an m-linked one, the appropriate male crossover was isolated and from him the M-linked line was derived.

Once both sexes were produced as homozygotes, they were mated together and a homozygous pure breeding line established. Because of difficulties in the fitness (fertility and fecundity) several lines are in process of being established and these will be mixed together in order to incorporate as much genic heterozygosity as possible into the translocation homozygous stock. At present 40 homozygous parents have produced only 55 homozygous progeny in establishing the strain.

There is reason for great hope that this strain can be made into a fit strain through proper manipulation. The fecundity of some of the females is comparable to that of the multiple marker strain (86 eggs) and in some cases the fertility of the homozygous females has been 95 percent when outcrossed and 83 percent when bred with homozygous males.

The transmission of the translocation has been determined from various types of mating combinations. The results to date are given in Table 1. This indicates that the transmission characteristics are favorable for the possible use of this translocation in a control program.

The figures on recovery of homozygotes from the mating of two heterozygotes (Table 1) are taken from the strain after it had passed through the bottleneck of the homozygote. Once this initial selection had been passed, it was considerably easier to recover homozygotes. Fecundity and fertility of the homozygotes have, in general, increased, although they are not yet at an acceptable standard. Colonies of the homozygote strain are being maintained at Notre Dame and will be sent to Mombasa once they are well established.

TABLE 1

TRANSMISSION OF TRANSLOCATION AT 1:2 t

PARENTAL CROSS	PERCENT TRANSLOCATION IN PARENT	PROGENY			PERCENT TRANSLOCATION IN PROGENY
		Δ	♠	N	
♠ x N; N x ♠	25	-	101	98	25
♠ x ♠	50	65	401	74	49
♠ x Δ	75	100	76	-	78

Annex 3

Releases of Translocations

Paul T. McDonald

As reported in the previous Annual Report, the decision to raise and release a mixture of 50 percent males heterozygous for a single male-linked translocation (AT 1:2 n @ M) and 50 percent males heterozygous for two different sex-linked translocations (AT 1:2 n @ M/AT 1:3 f @ m) was reached in December. The stocks of both translocation lines were multiplied as quickly as possible and the multiple marker Africanized (MMA) stock was also expanded.

The translocations for release were both tightly linked to the sex-linked marker wild type for rust eye. In the case of the AT 1:2 n M translocation, the pseudolinkage between the markers for the translocation (wild for rust eye and wild for spot abdomen) was 376/376 = 1.00. Accordingly, less than 0.27 percent of wild eye male progeny of a known heterozygote would be crossovers and possibly non translocated. Furthermore, less than 0.81 percent of wild-eye-male progeny of wild-eye sons of a known heterozygote would be crossovers and possibly non-translocated. This level of possible non-translocation introduction was considered tolerable. Further analysis of the pseudolinkage of this translocation will permit a more accurate assessment of the non-translocation introduction in the releases.

In the case of the 1:3f @ m translocations, the pseudolinkage between wild type eye and wild type black-tarsi is 185/190 = 0.97. For genetic crosses, the presence of both pseudolinked markers is necessary to guarantee the presence of the translocation.

Based on larval survival and egg hatchability data collected over the preceeding six months, the scheme for production of the release males was devised and space allocated in the genetics room and mass production room. Table 1 lists the types of crosses set. To control the effect of any contamination, every cross was set in a series of gallon cages instead of a single population cage. For ease of handling, plastic basins for rearing 200 to 300 larvae were used.

Genetic selection from the progeny of each of the four preparatory crosses was required (see Table 2). For the pupal selection the staff was quite experienced and new members readily were trained. The selection of adults was more exacting. The wild type black-tarsi and the mutant black-tarsi phenotypes were often difficult to distinguish, especially in females. The females of the multiple marker stock were all black-tarsi mutants, yet the expression had come to resemble the wild type, illustrating the probable occurrence of an evolution of dominance.

The genetic crosses were either set by the geneticist or under his direct supervision. The adult progeny of each day's crosses 1, 2, and 3 were examined and checked for possible contamination. Two instances of contaminated groups were found, and in both cases the contamination was eliminated before they could be used in other crosses. The checking of adult phenotypes daily provided the critical control point in the entire production operation, in the effort to avoid and contain any contamination in the series of genetic crosses necessary for the releases.

Pupal selection alone was applied to progeny from crosses 4 and 5, and this was done in the interests of managing all the other work carefully with the staff available. (Table 2) By avoiding the adult selections, only a slight amount (less than 0.81 percent) of non translocation males would be included among the release males.

As a final check on the quality of individuals taken for release in the field, a sample was taken at approximately ten-day intervals, and these males were mated with wild type virgin females of the strain collected in the release village before any releases. The results are entered in Table 3.

On two occasions the phenotypes of the release males were checked, and on both occasions, the mixture was approximately 50 percent of each type of male.

	Single Heterozygote	Double Heterozygote
19 March	87	93
- June	29	24

Furthermore, a sample containing 13 single and 11 double heterozygotes was mated with 40 virgin multiple marker females. The results indicate transmission from both types of males in equal frequency with 95 percent of males carrying the M-linked translocation and 33 percent of females carrying the m-linked translocation, based on 101 progeny.

During the course of the releases, a problem developed with the feeding of the larvae, and, until new food was available, a considerable loss of the stocks occurred. In order to augment the release males of the mixture, a second line was initiated for release, and this line was the expanded stock of the single heterozygote AT 1:2 n @ M. This stock was released only when the number of males of the mixture was below 1000.

Dusted males were released during the first six weeks in order that they could be identified in collections in the field. Each day the group was dusted with a different color or combination of colors, for a period of ten days in a color cycle.

The objective of the rearing scheme was the provision of approximately 1000 males daily on a long term basis. The actual numbers of males released and their colors are given in Table 4 for the period 29 March-14 May.

TABLE 1

Scheme of crosses necessary for continuous daily production of 1,000 males heterozygous for two translocations ⇑M/ ⇑m
1,000 males heterozygous for one translocation ⇑M

Cross	Size ♀♀ x ♂♂	No. cages per day (60♀♀ x 20♂♂)	Expected yield of eggs	Adults required	Expected yield of adults	No. adults needed	No. 1st instars set	No. pans per day
Preparatory crosses in genetics room:								
1. MMA x MMA	120 x 40	2	7,200	MMA ♀	1,940	540	2,000	8
2. MMA x M⇑	120 x 40	2	7,200	⇑M ♂	580	440	1,750	7
3. ⇑ m x MMA	60 x 20	1	3,600	⇑m ♂ ⇑m ♀	140 140	100 ♂ 100 ♀	750	3
4. MMA x ⇑m	300 x 100	5	18,000	⇑m ♀	1,440	1,200	4,800	16
Production cross in mass production room:								
5. ⇑m x ⇑M	1200 x 400	20	72,000	⇑M/ ⇑m ♂ ⇑M	2,160	2,000	8,100	27

TABLE 2

Genetic Marker Selection For Crosses

Cross	Translocation	Pupal Selection	Adult Selection	
		eye	abdomen	hind tarsi
1.	Marker Stock (♂♂+♀♀)	rust	spot	black-tarsi
2.	1:2n @ M(♂♂)*	wild	wild	black-tarsi
	1:2n @ M(♂♂)**	wild	----	
3.	1:3f @ m(♂♂)	wild	spot	black-tarsi
	1:3f @ m(♀♀)	wild	spot	black-tarsi
4.	1:3f @ m(♀♀)**	wild	----	----------
5.	1:2n @ M(♂♂)	wild	----	----------
	1:2n @ M/1:3f m(♂♂)	wild	----	----------

* To renew cross 2

** For use in cross 5; excess for release if necessary.

TABLE 3

Quality Control of Release Males

DATE	CROSS ♀♀ X ♂♂	HATCHABILITY
11 April	CHIBARINI (60) x CHIBARINI (25)	969/1046 = 93%
11 April	CHIBARINI x MIXTURE (60) (25)	322/1021 = 32%
22 April	CHIBARINI x CHIBARINI (34) (17)	608/631 = 96%
22 April	CHIBARINI x MIXTURE (37) (19)	53/158 = 34%
1 May	CHIBARINI x CHIBARINI (70) (25)	756/829 = 91%
1 May	CHIBARINI x MIXTURE (50) (25)	72/113 = 64%
16 May	CHIBARINI x CHIBARINI (70) (25)	302/305 = 99%
16 May	CHIBARINI x MIXTURE (50) (25)	162/456 = 36%
16 May	CHIBARINI x SINGLE (50) HETEROZYGOTE	180/362 = 50%

Annex 4

Movement of Mosquitoes Between Villages

Paul T. McDonald

Studies on movement of domestic Aedes aegypti between villages have been completed since the last Annual Report. A total of nine releases were made. Newly-emerged adult males and females of the RABAI DOMESTIC strain were released in various numbers within and at measured distances from the center of Kwa Dena village. A sketch map of the area including the village is included as Figure 1. The release points located at 400 meters and 800 meters from the center of the village are also indicated on the map. The daily handing-biting catches were done by two men, each spending thirty minutes in each of the 14 houses of the village.

The recoveries of the mosquitoes released within the village in the L-B catches of the nine days subsequent to release are given in Table 1. Generally the recovery was quite good except for 1 December's release. This release has been excluded in analysis of movements into the village.

The estimate of immigration was done as follows:

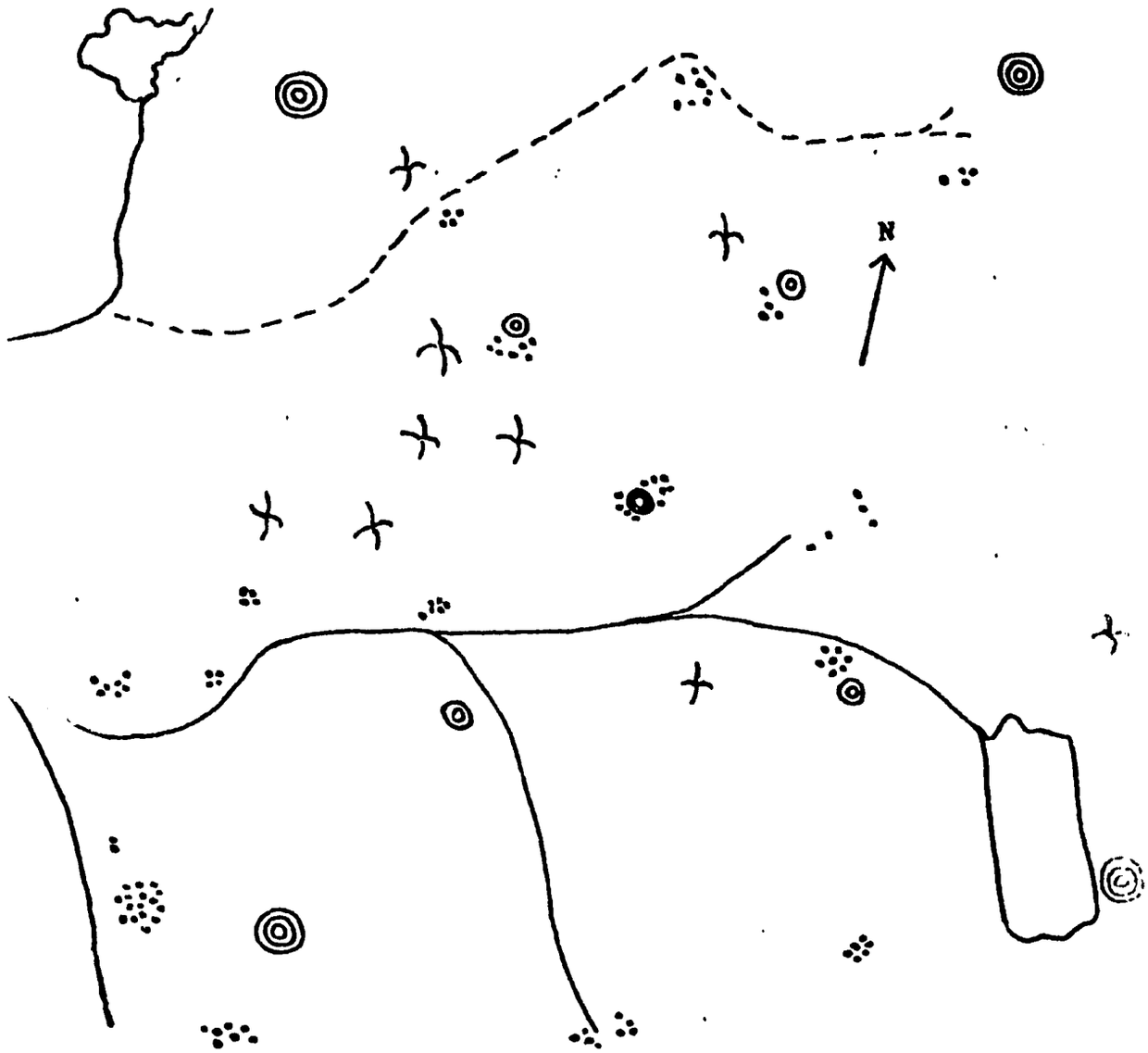
$$\text{Number Entering} = \frac{\text{No. Marked (Inside) Released}}{\text{No. Marked (Inside) Captured}} \times \text{No. Marked (Outside) Captured}$$

$$\text{Percent Released (Outside) Entering} = \frac{\text{No. Marked (Outside) Entering}}{\text{No. Marked (Outside) Released}}$$

On the basis of the foregoing calculations, the percent released entering from various distances from Kwa Dena have been determined for distances of 200m, 400, 800m for both males and females (Table 2). According to this, immigration drops off sharply with distance, and 800m should be an effective distance for a barrier between villages.

The different directions of immigration has been associated with varying levels of immigration, with the predominate immigration from the downwind direction - (Table 3). The movement into the village was mostly from the SW, opposite to the predominating monsoon from the NE during the period of the release experiments.

FIGURE 1



LEGEND

KWA DENA

400 m

800 m

Houses

Trees

Road, Quarry

TABLE 1

RECOVERY OF MARKED MOSQUITOES WITHIN KWA DENA

RELEASE DATE	MALES	FEMALES
9 NOVEMBER 73	49%	21%
16 NOVEMBER 73	52%	32%
1 DECEMBER 73	10%	24%
12 DECEMBER 73	41%	32%
29 DECEMBER 73	28%	21%
11 JANUARY 74	53%	36%
28 JANUARY 74	55%	37%
5 FEBRUARY 74	43%	34%
14 FEBRUARY 74	59%	35%

TABLE 2

MARKED MOSQUITOES ENTERING KWA DENA

	MALES			FEMALES		
	200 m	400 m	800 m	200 m	400 m	800 m
IV	47%			42%		
V	55%			43%		
I	32%	1%		45%	6%	
II	24%	1%		39%	10%	
VI		2%			6%	
IX		0.3%			2%	
VII			0%			0.9%
VIII			0.2%			0.4%
AVERAGE	40%	1%	0.1%	42%	6%	0.7%

TABLE 3

MARKED MOSQUITOES ENTERING KWA DENA DURING NE - MONSOON

200 m		400 m		800 m	
19.8%	10.3%	2.8%	0.5%	0.5%	0%
82 %	62.3%	4.3%	3.3%	0.8%	0.5%

TABLE 4

Release Males at Chibarani

Date	Color	Number*	Death	Released
29 March	HR	729	7	
30 "	HY	900	2	
31 "	DR	621	5	
1 April	DG	1086	12	
2	DO	1275	13	
3	DG, DR	777	7	SH only
4	DG, HR	420	4	
5	HG, HR	228	0	
6	HG	708	4	
7	DY	795	35	
8 April	HR	903	15	
9	HY	1000	5	(1/2 D.H. mix)
10	DR	1000	3	(1/2 D.H. mix)
11	DG	297	25	
12	DO	444	5	Mix
13	DGDR	288	1	
14	DGHR	417	5	
15	HGHR	1278	0	
16	HG	1047	1	
17	DY	963	5	
18 April	HR	633	3	
19	HY	0	0	
20	DR	420	5	
21	DG	624	4	
22	DO	291	3	
23	DGDR	402	4	
24	DGHR	657	8	
25	HGHR	1326	92	
26	HG	1005	47	
27	DY	384	2	

TABLE 4 (con't.)

Date	Color	Number*	Death	Released
28 April	HR	858	16	
29	HY	1005	4	
30	DR	1005	27	
1 May	DG	834	6	
2	DO	804	29	
3	DGDR	1000	147	
4	DGHR	1059	29	
5	HGHR	1140	9	
6	HG	1200	3	
7	DY	1002	8	
8	HR	1005	9	
9	HY	1590	9	
10	DR	1200	4	
11	DG	1020	25	
12	DO & DY	450	4	
13	---	1050	0	
14	---	1325	3	

* 29 March - 8 April	Single M-linked heterozygotes only
9, 10 April	Mixture 75%-25% Single and Double
11 April - 14 May	Mixture of 50%-50% Single and Double