PD-AAL-138

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
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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. negypti 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 1979
   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.Par-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 1979. 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	5,493	-2493
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 = 77,18[illegible]; year 2 = 99,282; year 3 = [illegible]1660, 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 (14% of S&B)	4,500	6,300
		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	6,000
11. Indirect Costs-ICIPE (25% of direct)	7,400	11,250
		56,250
TOTALS	99,300	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, 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 Project 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 soaring inflation in Kenya. Salaries and other budget items that were appropriate in 1970, at the time of original application, are now [illegible] of date.
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.

BEST AVAILABLE DOCUMENT

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: Population size	Weekly population assessments for all three villages: Sterility	Additional assessments
CHI	Daily release of 300-1200 double heterozygote 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

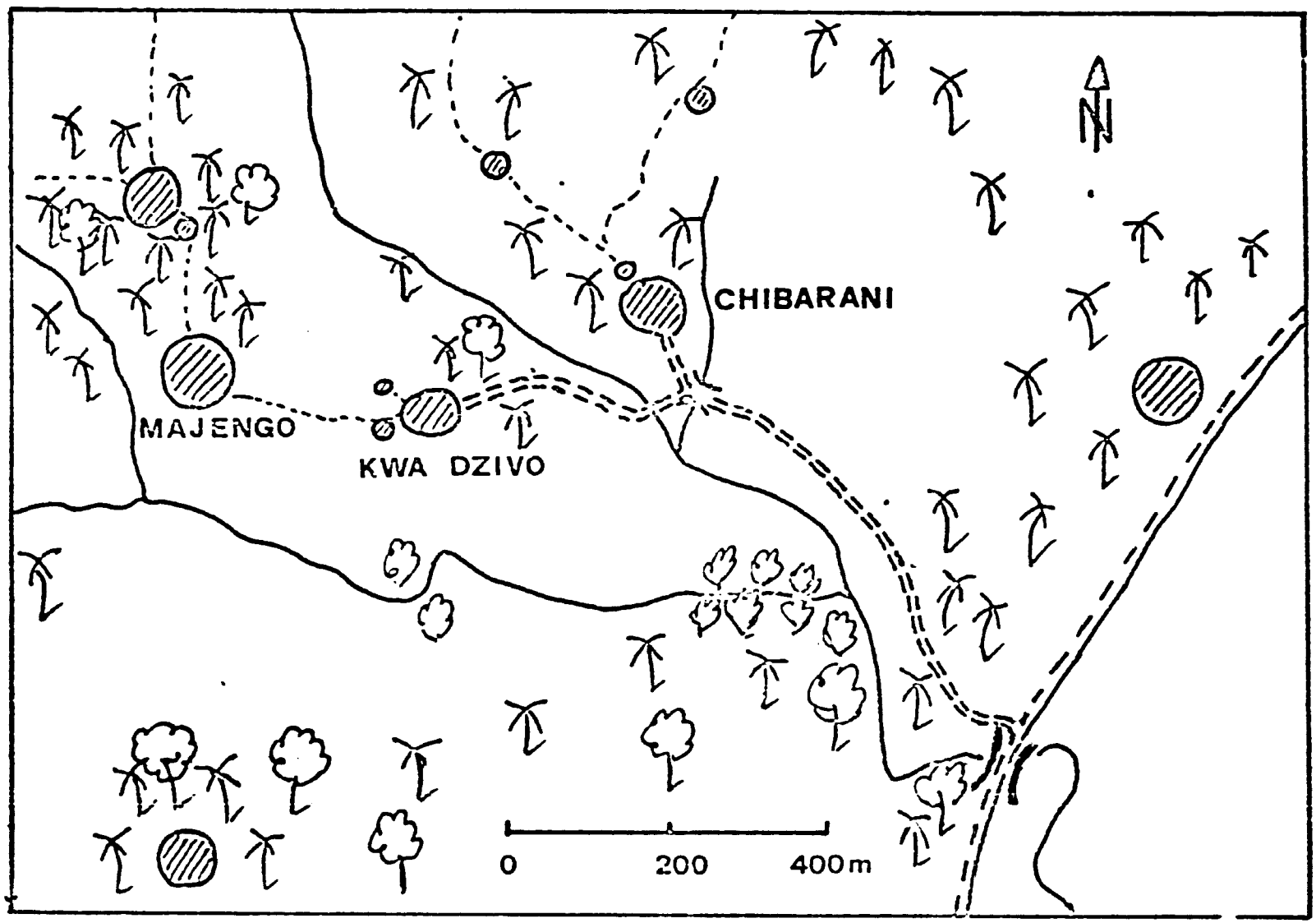


FIGURE 2

SURVIVORSHIP OF A.AEGYPTI MALES IN CHIBARANI

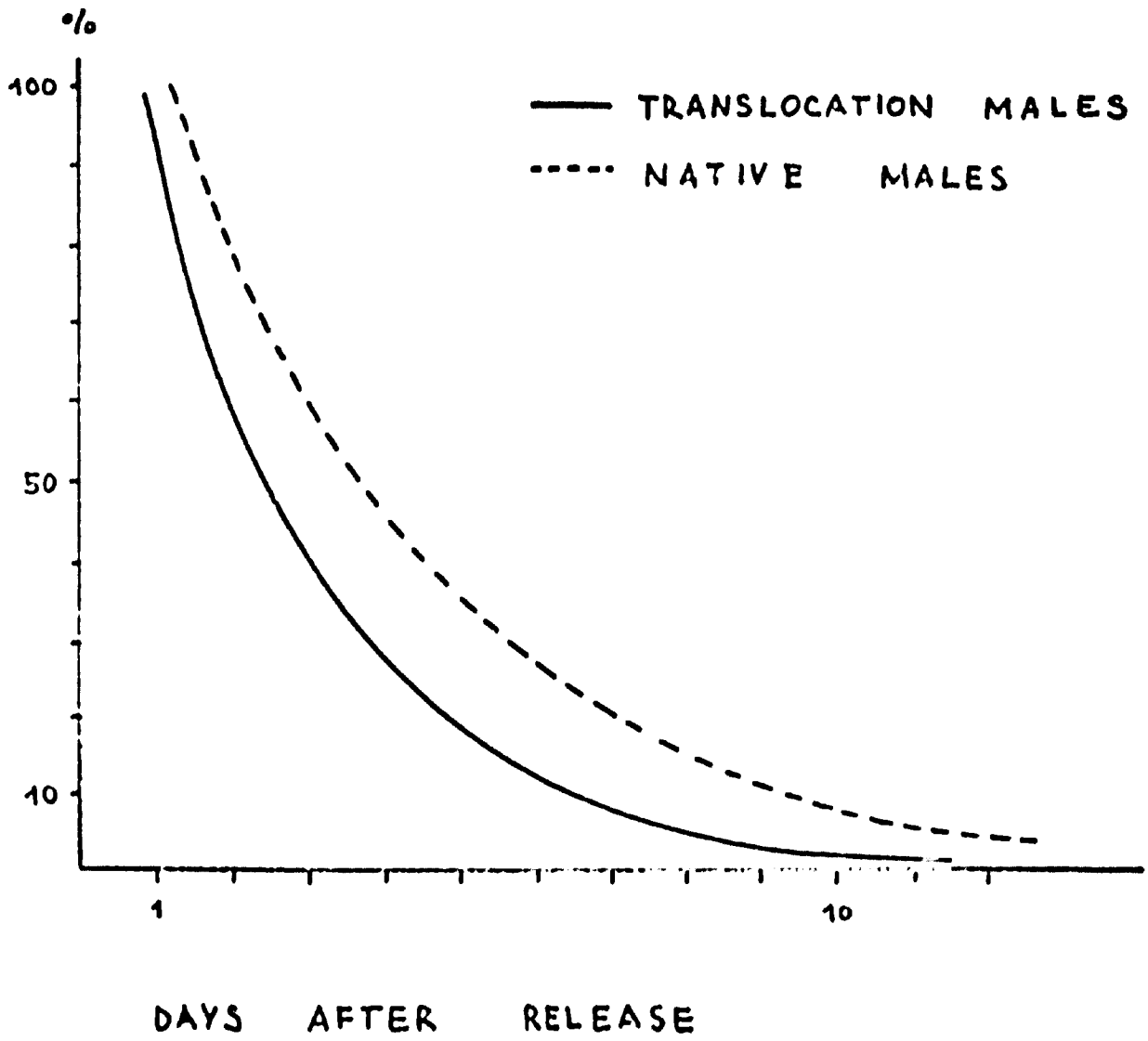


FIGURE 3

FEMALE RATIOS IN RELEASE AND REFERENCE VILLAGE

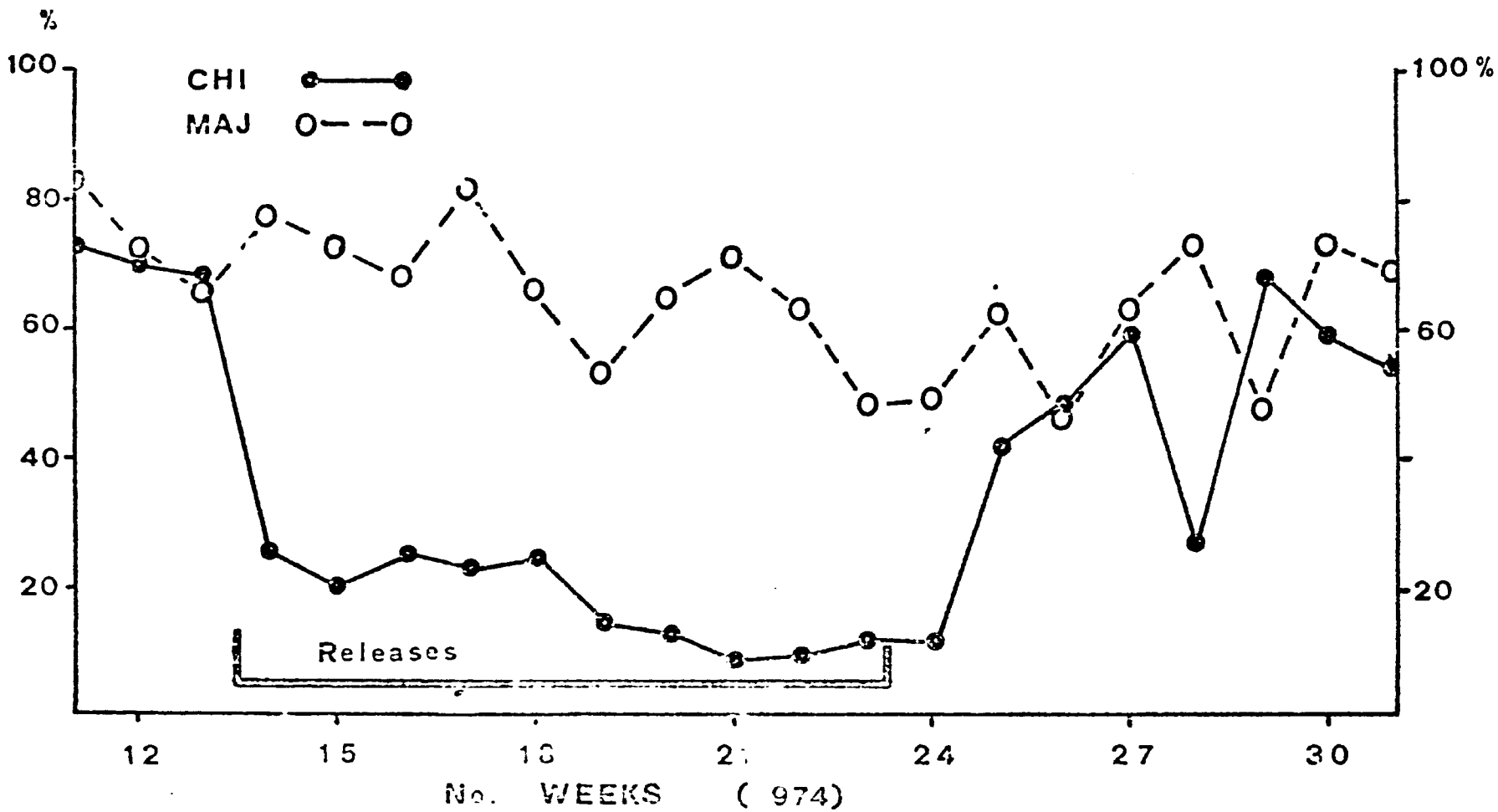


FIGURE 4

HATCHABILITY

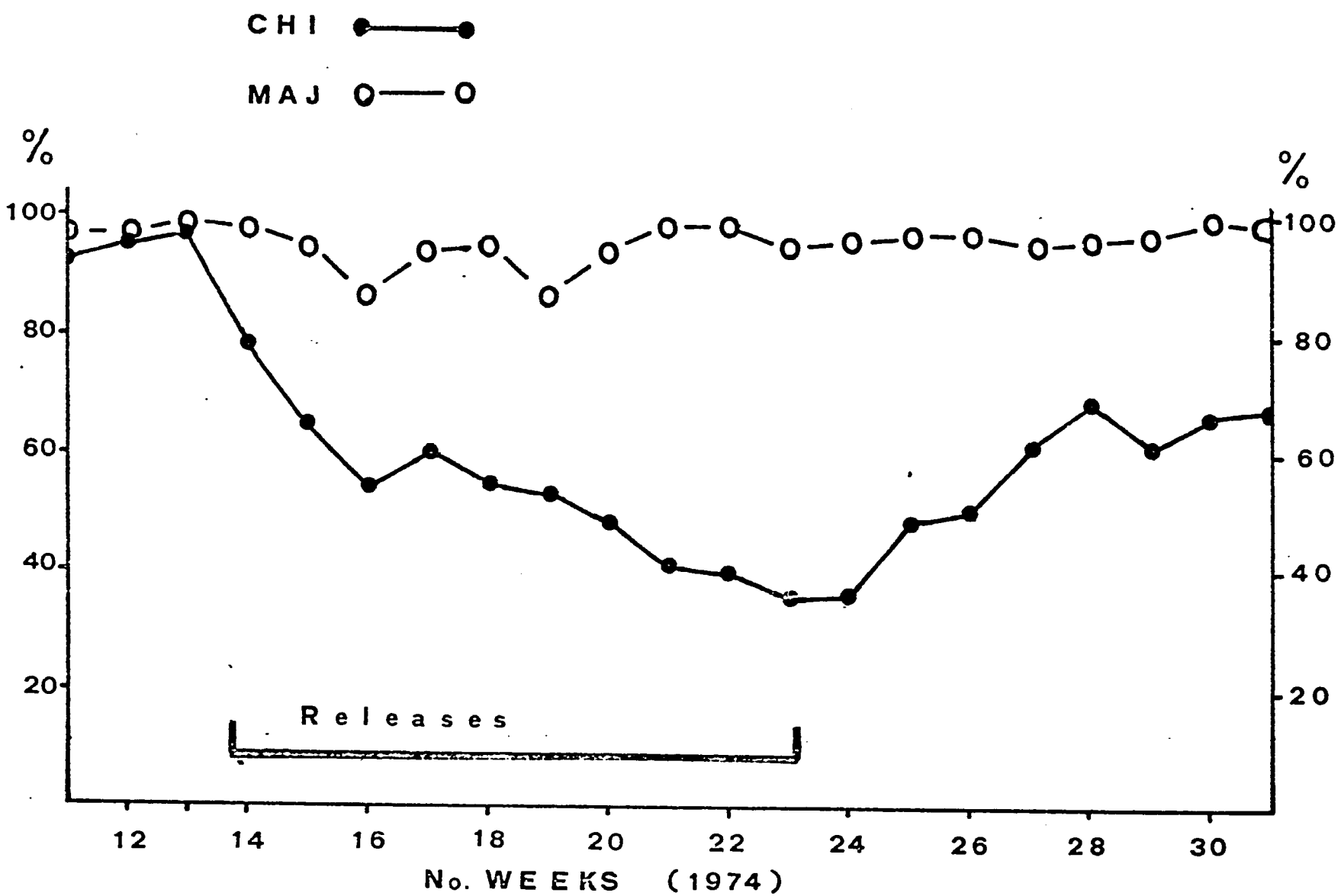
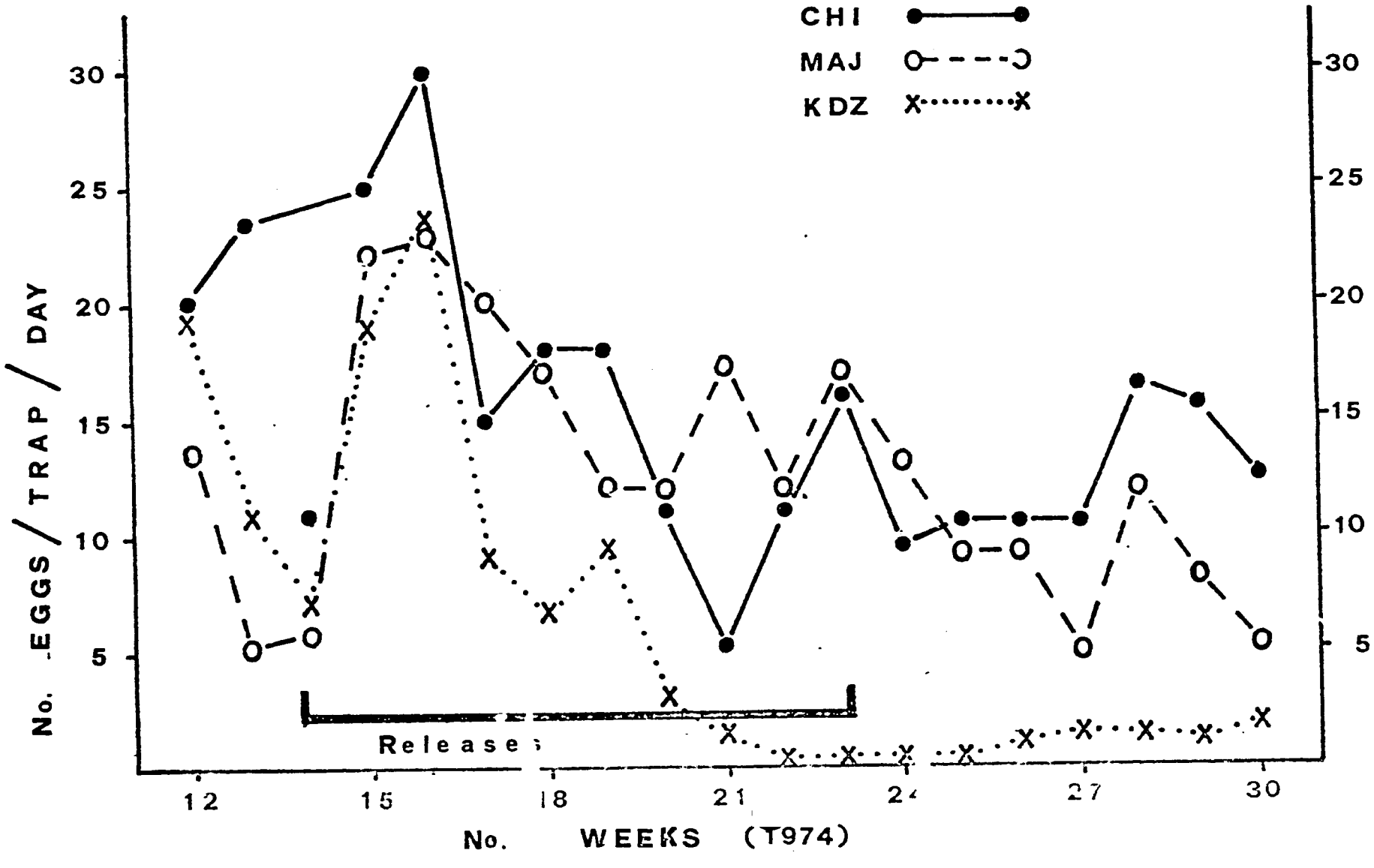


FIGURE 5

OVIPOSITION RATES



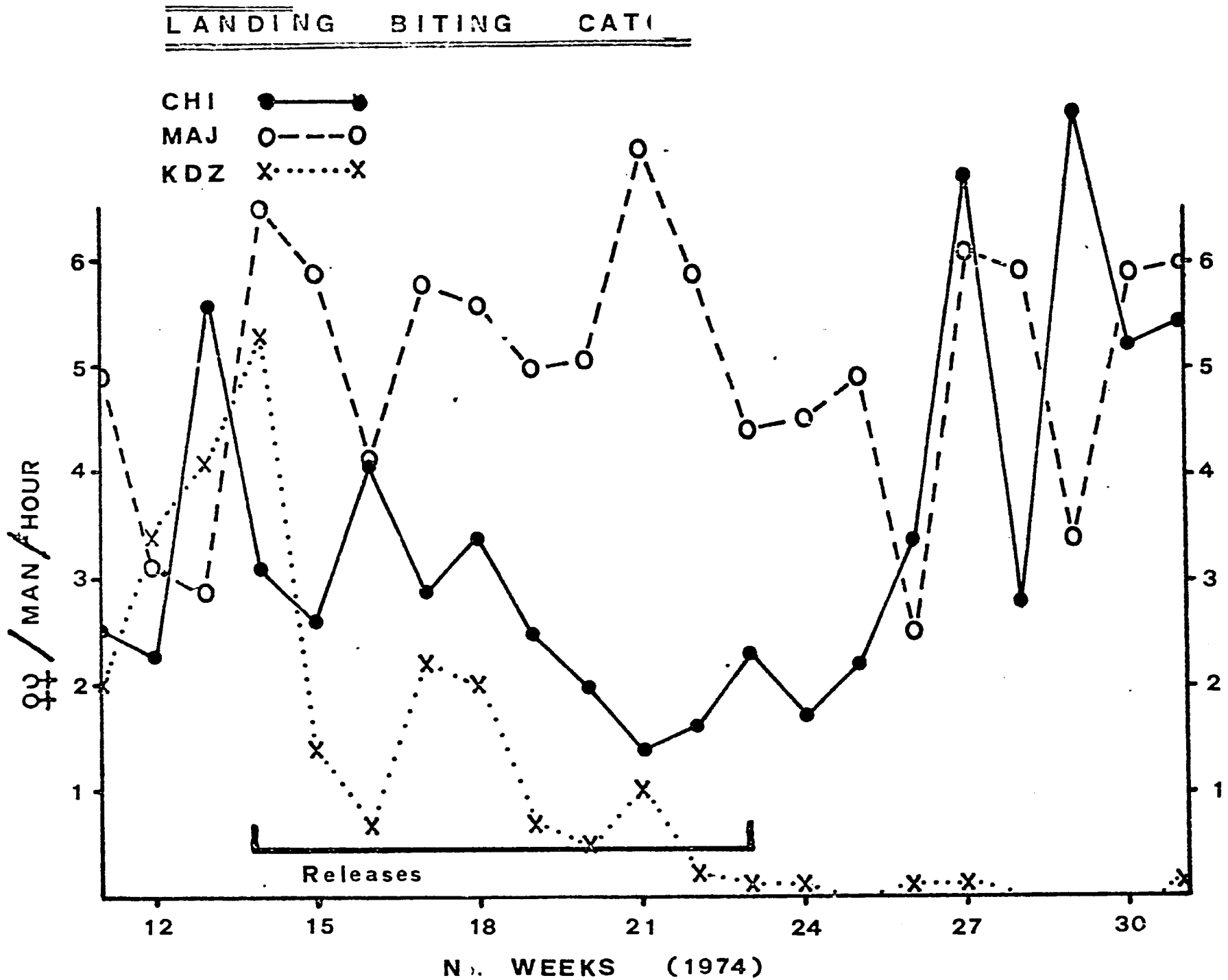

LANDING BITING CAT
CHI
MAJ
KDZ
♀♀ / MAN / HOUR
Releases
12
15
18
21
24
27
30
N. WEEKS (1974)

FIGURE 7 (by N. Lorimer)

GENETIC MONITORING IN CHIBARANI

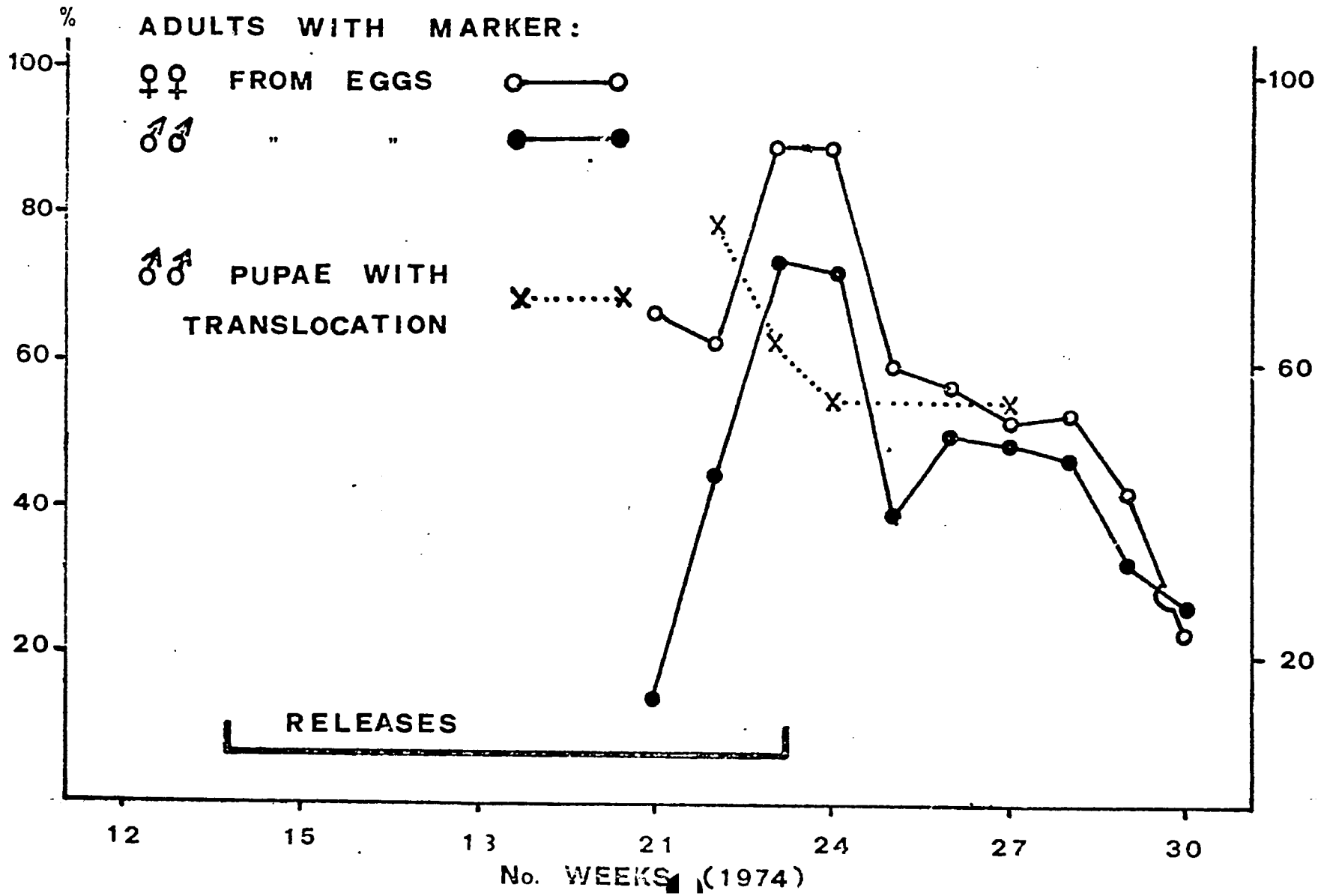


TABLE 1

TRANSMISSION OF TRANSLOCATION AT 1:2 t

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

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 th 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%
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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%

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:

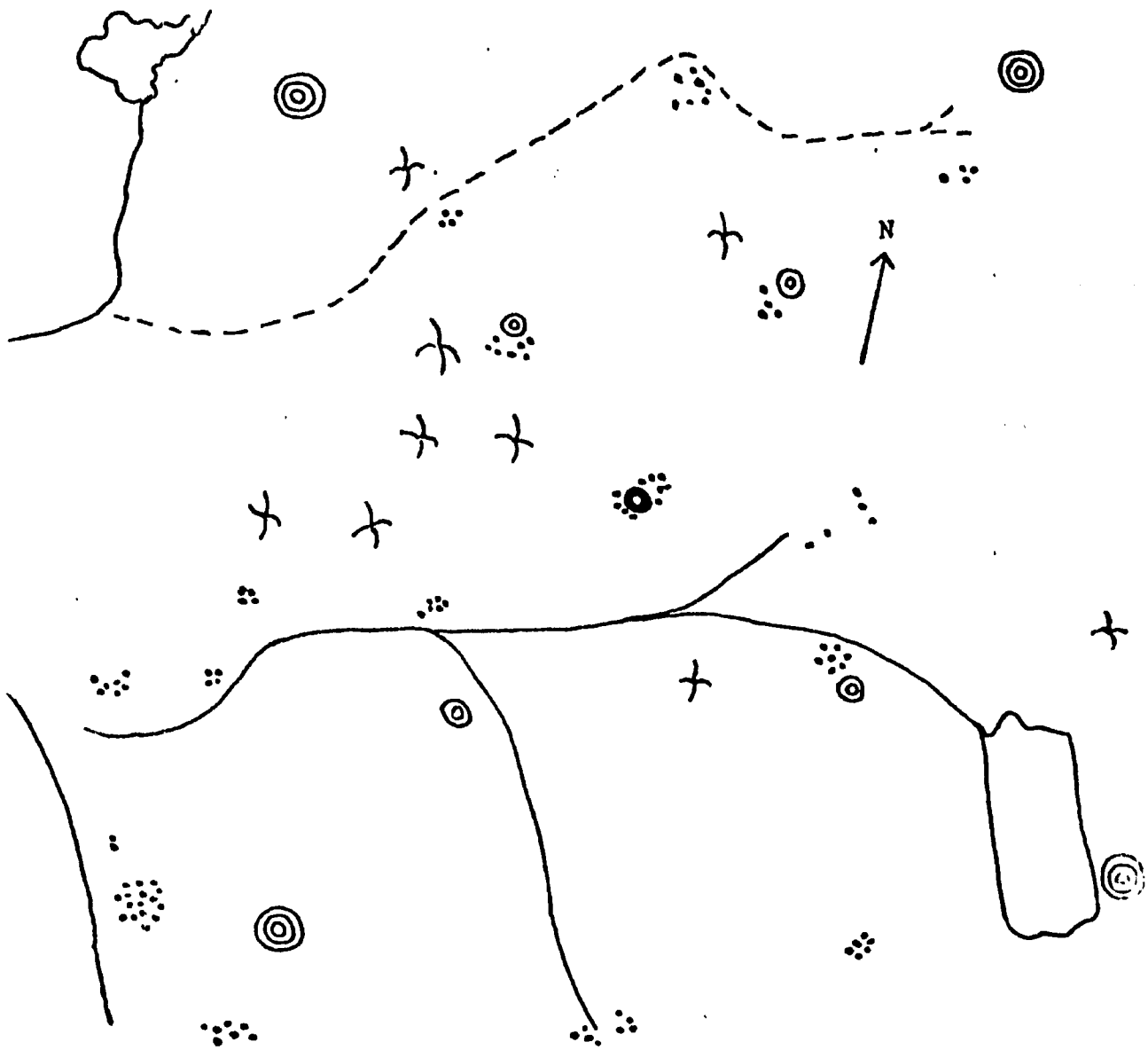
$$\text{Number Entering} = \frac{\text{No. Marked (Inside) Released}}{\text{No. Marked (Inside) Captured}} \times \text{No. Marked (Outside) Captured}$$

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

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

300 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	H HR	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

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.

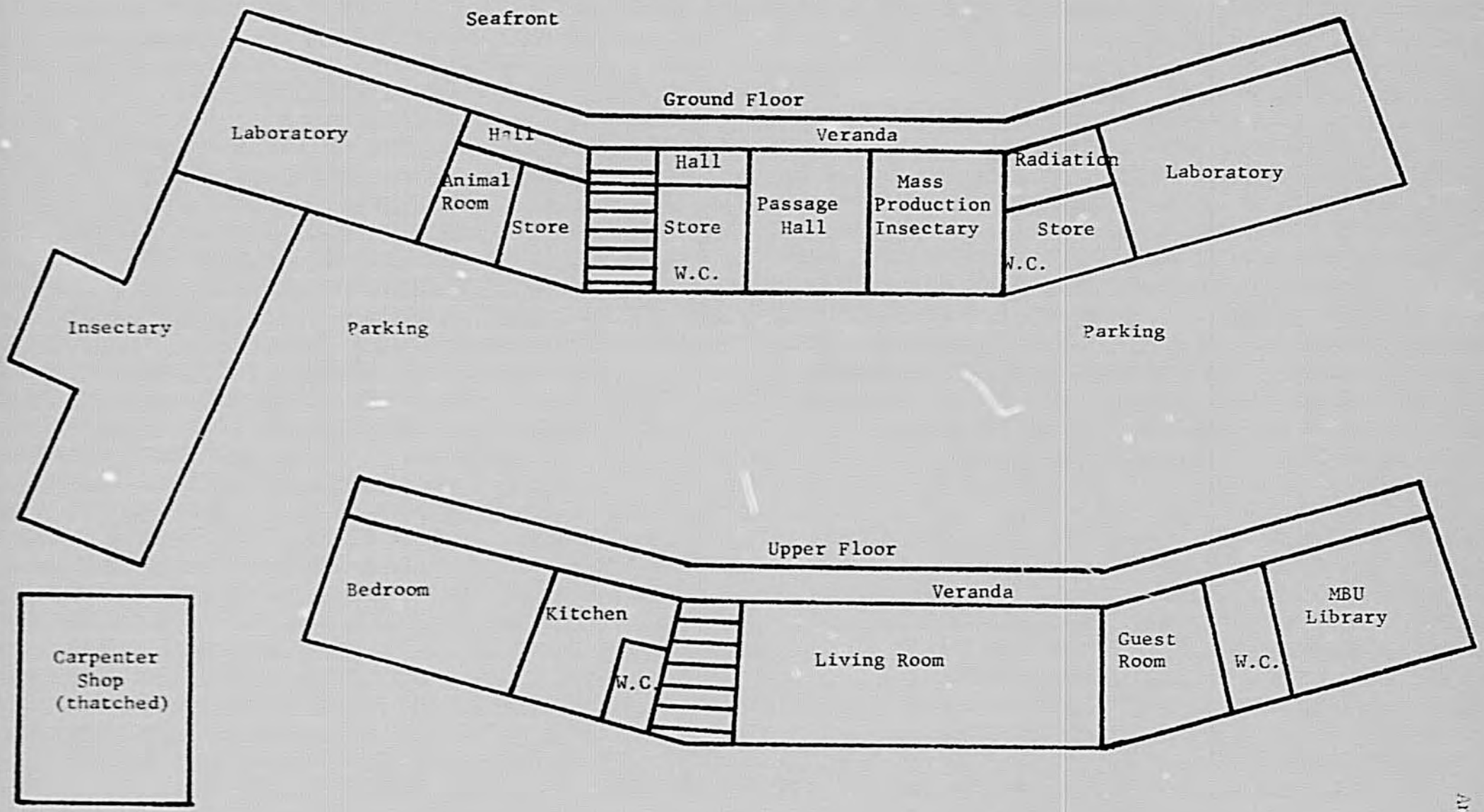
4. Personnel:
   - Scientists - 1st 3 years - Walter Hausermann, Project Leader (now Ciba-Geigy, Basel)
     - Paul McDonald, Geneticist (now U.Calif., Berkeley)
     - Dean Fanara, Ecologist (now WHO, Indonesia)
   - Year 4 - L. Philip Lounibos, Project Leader (NIH PD Fellow)
     - Nancy Lorimer, Cytogeneticist
     - 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 Tuma - 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 7[illegible]

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 laborstory, 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:

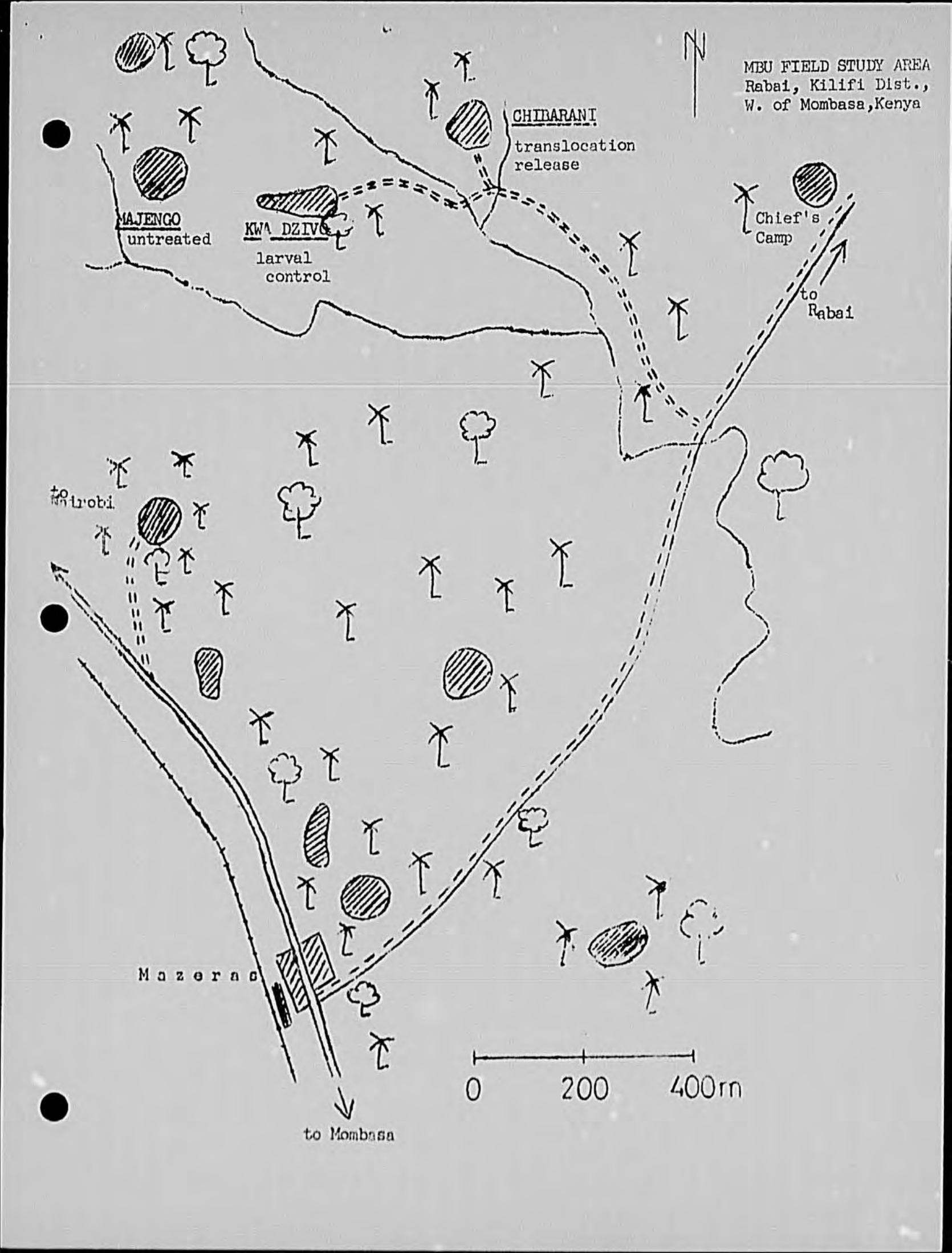
(a)Chibarani - Translocated males released, 1000/day for 8 weeks
(b) Kwa Dzivo - Larvae in domestic water containers (clay pots) removed twice weekly by cleaning pots
(c) Majengo - No control.

The distances between these villages are [illegible] 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

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 △	PROGENY ⏚	PROGENY N	PERCENT TRANSLOCATION IN PROGENY
⏚ x N; N x ⏚	25	-	101	98	25
⏚ x ⏚	50	65	401	74	49
⏚ x △	75	100	76	-	78

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:								
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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 (60) x MIXTURE (25)	322/1021 = 32%
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16 May	CHIBARINI (70) x CHIBARINI (25)	302/305 = 99%
16 May	CHIBARINI (50) x MIXTURE (25)	162/456 = 36%
16 May	CHIBARINI (50) x SINGLE HETEROZYGOTE	180/362 = 50%

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:

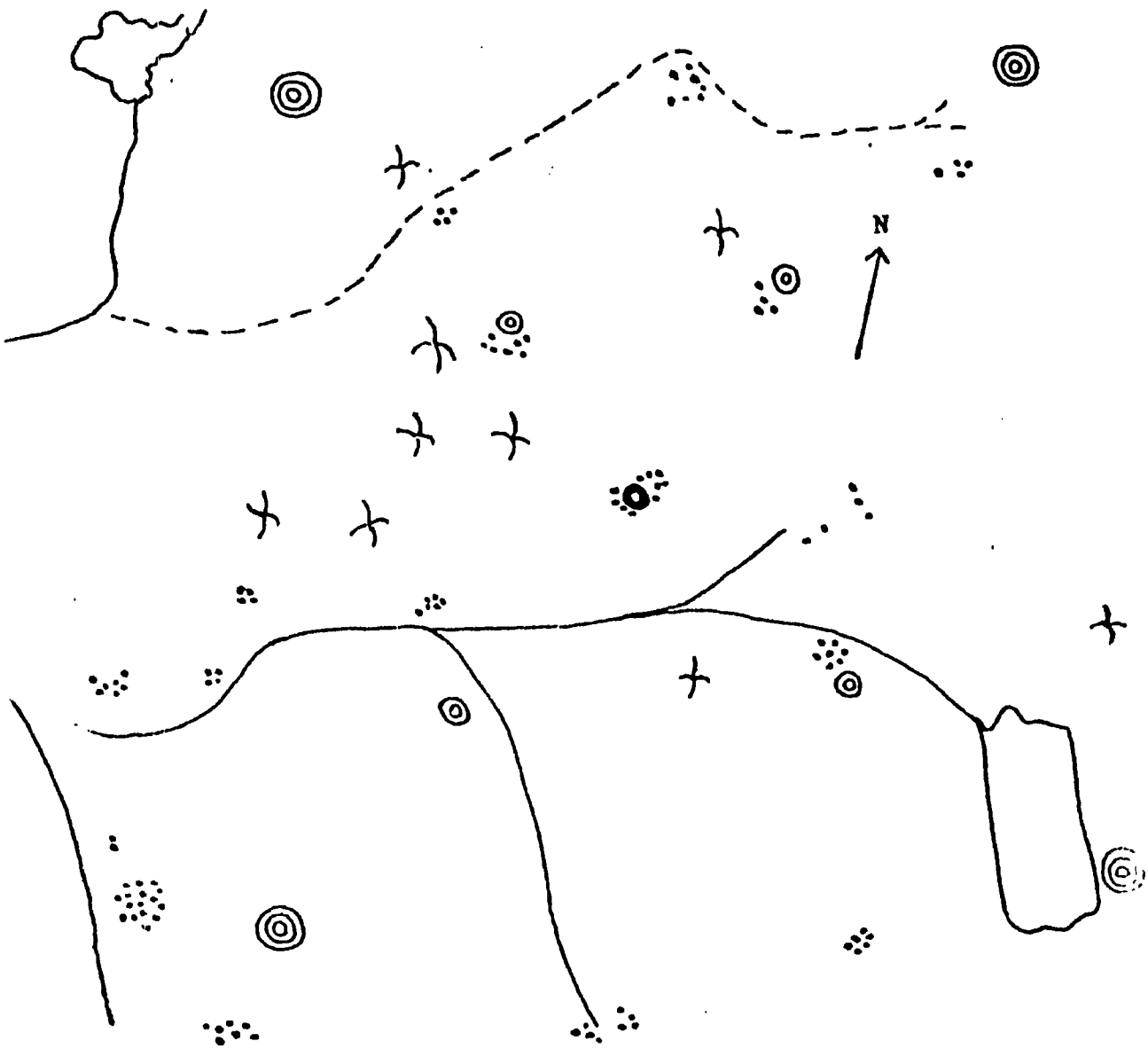
$$\text{Number Entering} = \frac{\text{No. Marked (Inside) Released}}{\text{No. Marked (Inside) Captured}} \times \text{No. Marked (Outside) Captured}$$

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

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

Semi-Annual Progress Report
Year 3 July 73- Dec 73

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

SUBJECT: Research Progress Report, Semi-Annual, AID/csd-3159

1. Project Title: Ecological Studies on *Aedes aegypti* in East Africa Preliminary to Genetic Control

2. Principal Investigator and Contractor:
Professors G.B.Craig, Jr., and K.S.Rai and T.J.Crovello
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 this Report:
3rd year, 1st half
1 July 1973-31 December 1973

5.-8. Fiscal Data: Not included in this report. See report for 3rd year, 2nd half.

9. Narrative Summary - Accomplishments:

The first half of year 3 involved continuation and refinement of ecological studies, emphasizing dispersal, reproductive potential and habitat selection in *Aedes aegypti*. A simple laboratory device was developed which allows behavioral segregation of domestic and sylvan strains. Marking studies showed that villages 400 meters or more apart have little significant exchange of populations. Our field study area, the rural villages in the Rabai area, continues to appear ideal, not only for genetic control tests but also as a field testing area for other control methods, i.e., hormones, mosquito pathogens, new insecticides. The villages are small, isolated, accessible and have a large year-round population of *A. aegypti*. Most important, the Rabai people are both hospitable and highly cooperative: they continue to assist our operations in every possible way.

A major change in our strategy for genetic control was undertaken, specifically in the chromosome mechanism to be used for releases. Although we have isolated more than 70 chromosome translocations, we have been unable to breed a stock that has the translocation homozygous and is also viable and competitive. (This possibility was anticipated in earlier reports.) Therefore, our genetic control program will be based on release of double heterozygotes which cause high sterility plus effects in subsequent generations. Thus, we are now aiming at population suppression instead of replacement. Stocks with double heterozygotes were constructed and put into mass production. A village-scale field release will be started in the second half of year 3.

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 forbiological 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 conferrring 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 2½ 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. Ideally, an additional three years at the same level of support should be provided. However, some progress could be made with one more year at higher funding.

D. Accomplishments to date

1. Description of Facilities

No significant change since last report.

2. Research Findings - Ecology

In the past two years of ecological research, we have learned enough about the situation of A. aegypti on the Kenya Coast in order to embark on genetic control experiments on a village and even location scale (Annex 10). Several important and interesting ecological problems still remain:

a. Estimates of larval survivorship and speed of development
b. Estimates of fecundity and fertility
c. Quantitative rather than qualitative description of dispersal
d. Quantitative estimate of gene flow between domestic-peridomestic and peridomestic-sylvan A. aegypti.

In the past six months, experiments were conducted on all four of these problems.

Attempts to estimate survival rates and speed of development of domestic A. aegypti larvae were not as successful and informative as desired (Annex 1). However, they demonstrated that intraspecific competition was the major factor accounting for larval mortality and a key factor in in the life table budget of domestic A. aegypti populations.

Fecundity of domestic A. aegypti in the field was measured on the basis of number of eggs per female and per day (Annex 2). Combined with estimates of the absolute number of females per village, accurate estimates of the total daily egg production in a vilage are feasible. A second method to estimate the egg production of the same village directly was also developed. This permits an independent check on the validity of the of the estimates of absolute numbers of adults made by mark-release-recapture methods (Annex 2).

New experiments on the amount of dispersal and the amount of immigration into a given village have established a fact that we previously suspected (Annex 3). Dispersal activity depends very much on the local setting, vegetation cover, the proximity to other villages and also wind speed and direction. If there are other villages close by, i.e., within about 200 meters, the exchange of the domestic A. aegypti between villages may amount to up to 50% of the respective village populations. This exchange drops steeply if the distances go beyond 200 meters. However, we feel a strong need for additional dispersal studies in different settings.

Attempts to measure the amount of gene flow between the domestic and peridomestic forms and between peridomestic and feral or sylvan forms had to be abandoned. It was planned to uses different genetic markers for these studies; Africanization of the marker stocks was more difficult and time-consuming than originally estimated.

Host preference studies initiated by Consultant Dr. Milan Trpis in

an olfactometer and by Dr. Fanara in a field cage were continued (Annex 4). Again, the dark A. aegypti type of peridomestic origin was the most avid man biter in the field cage as well as in the domestic and peridomestic habitats. The black A. aegypti subspecies formosus of sylvan origin was reluctant to bite man, while the domestic A. aegypti of type form was intermediate in its aggressiveness. It was remarkable that a second strain of A. aegypti formosus from a forest in Ethiopia showed essentially the same reluctance to bite as our local sylvan strain.

The studies on habitat selection initiated by Dr. Trpis were continued in a newly developed cage device simulating the conditions of the peridomestic and domestic habitat and forcing the mosquito to select one of the two habitats. Many different strains of domestic and sylvan origin were tested in this cage (Annex 5); results showed a clearcut difference, thus allowing behavioral fractionation of these biological forms in the laboratory. The F_1 hybrids, appearing type form in color, did not show a clear preference for one habitat over the other. An analysis of the environmental cues and a genetic analysis of habitat selection will be carried out after a modified version of our "Mosquito Behavior Fractionater" is completed.

3. Research Findings - Genetics

During the past six months, we have concluded that a significant change in MBJ's genetic control strategy had to be made. For reasons that are not entirely clear, it proved to be more difficult to obtain translocations in the homozygous condition than we initially expected. Although we had no difficulty in producing and isolating many heterozygous chromosome translocations, we could breed only a few of them to the homozygous condition and none of these is fit enough to establish itself against a field population when released. This discovery, although disappointing, was not altogether unexpected. In earlier reports, we had indicated alternative approaches if homozygotes could not be obtained. We still believe the approach is highly meritorious and deserves further research. Getting the correct chromosome combination is probably a function of time of research; considering the theoretical value of such a mechanism, the short amount of time applied in this project (2 man-years) is grossly inadequate.

The program is now directed toward population suppression, via double heterozygotes, rather than population replacement as could be done with a homozygote. Males carrying the double heterozygote system could theoretically induce up to 75% sterility in the field population, provided they are competitive and are release in adequate numbers.

Two schemes for the production of double heterozygote males have been developed. One of these uses our best homozygote translocation strain plus a strain which is heterozygous for a sex-linked translocation as parent strains. The other uses two different sex-linked translocation-heterozygous strains. Both methods require a complex series of several generations of crosses before the final product is ready for release. The method of production is somewhat comparable to that for hybrid corn.

As in previous report periods, considerable time was again spent on maintaining translocation lines and testing them for homozygosity. No additional homozygous lines were recovered.

Several lines with translocations involving the sex chromosomes are still maintained and are presently being tested for M-linkage, i.e., linked closely to the male-determining allele. If such an M-linked translocation heterozygote is found, it will be used for the production of release males (Annex 6).

The mass production facility is in final stages of preparation. Our release experiments are aimed at continuous daily release of comparatively small numbers of adults into fairly isolated populations. The continuous daily production of about 5000 adults of both sexes has been envisaged. Such amounts are not true mass production, but they are within the capabilities of MBU. Preliminary trials of mass production of adults of a normal strain have shown no great difficulties. However, the production of double heterozygote males for extended periods will require maintenance of three additional strains, two different parental strains and one strain for test crosses. Since the fertility of the two parental strains and of the cross producing our release males will be reduced, it is not at the moment certain whether the production goal of 5000 adults per day can be achieved. Experiments toward this end will be carried out as soon as the parental strains are available.(Annex 7, 8)

4. Relevance of Research Findings

Among the numerous potential methods for genetic control of insect vectors, the use of chromosome translocations proposed by Serebrowski (1940) and Curtis (1968) is the most elegant. The results of our research on A. aegypti have advanced substantially the understanding of the control potential of translocations in mosquitoes.

a. The failure to produce a single homozygous translocation strain of sufficient fertility for releases has made evident that we still do not know enough about the real nature of translocations. On theoretical grounds, the effort made should have been sufficient to produce at least some useful homozygotes. No generally acceptable hypothesis on the nature of chromosome translocations can be advanced today to explain why our efforts failed. In all probability, an entire new approach to the production of chromosome translocation homozygotes in mosquitoes is required.

b. Mattingly's repeated suggestions (1957, 1958, 1967) that more efforts be made to study the genetic makeup of field populations, especially with regard to behavioral traits, have been followed. As a result, the environmental and genetic conditions responsible for the evolution of taxonomically and behaviorally different populations are today much better understood.

c. Our investigations into the ecology of A. aegypti on the Kenya Coast have demonstrated the presence of a wide range of habitats known to be exploited by this species. Correspondingly, the species is fragmented into populations and subpopulations of all its infraspecific categories. The Kenya Coast constitutes an ideal area to study evolutionary trends and strategies of A. aegypti. Such studies would seem increasingly important since the rapid changes in the developing world will constantly create new situations with new opportunities for exploitation by A. aegypti. The great genetic adaptability of this species has already been shown in many papers by Grab and others.

d. The presence of numerous distinct populations of domestic A. aegypti makes the Kenya Coast an excellent testing ground for novel methods of mosquito control such as parasites, pathogens and juvenile hormones.

5. Modification in research design

Since no suitable strains with translocation homozygotes have become available for releases so far, our release plans have changed to the release of double heterozygotes (Annex 6). With this our aim has changed from population replacement to population suppression, which warrants the continuous release of large numbers of males instead of smaller numbers of both sexes. We expect that our facilities for continuous rearing will suffice for the daily production of about 5000 adults or 2500 males. This means that we will be able to cover at most two villages with releases. The releases are planned to begin in March and to continue until the beginning of the rains expected for late April.

Our original release plan called for releases in four villages. In two of these villages the native populations would have been removed by conventional control and substituted by homozygous translocation populations. Since this will not be possible with doublt heterozygotes, our release and monitoring program will extend over six villages (schedule attached).

Time schedule for double heterozygote male releases for population suppression

Month	February	March			April			May
Week No.	7 8	9	10	11	12	13	14	15 16 17 18
	Pre-Release	Release						Post-Release
Village Group I :								
SHAURI MOYO	Monitoring of oviposition rates and fertility once a week	Daily releases of 1000 males Weekly monitoring of oviposition and fertility						Weekly monitoring of oviposition and fertility (come back)
KIBARANI		Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility						Weekly monitoring of oviposition and fertility (come back)
MWELE		Weekly monitoring of oviposition and fertility						Weekly monitoring of oviposition and fertility
Village Group II :								
MGANDINI I	Monitoring of oviposition rates and fertility once a week	Daily releases of 1500 males (bigger village) Weekly monitoring of oviposition and fertility						Weekly monitoring of oviposition and fertility (come back)
MGANDINI II		Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility						Weekly monitoring of oviposition and fertility (come back)
KWA DENA		Weekly monitoring of oviposition and fertility						Weekly monitoring of oviposition and fertility

Village Group I : All villages of group I are fairly isolated. Immigration accounts for less than 20 % of population

Village Group II : Villages are not well isolated. Immigration may at times account for up to 50 % of population

6. Outline of activities of the last half year

The last half year will be devoted entirely to the one extended release of double heterozygote males and the writing of publications. The preparation for the release will take until the end of February. The release will be carried out continuously through March into April until the rainy season starts. No field experiments will be made after the end of April unless the project is continued for a fourth year.

E. Dissemination of Research Results

1. Visitors

Among MBU's visitors of the past six months the following were particularly beneficial and stimulating to MBU:

a. Prof. Theodore Crovello, a biostatistician from the Department of Biology at the University of Notre Dame. He advised us with the planning of the last year of the project and has taken on the task to develop various release strategies and to test them by computer simulation.

b. Dr. Louis G. Mukwaya, entomologist of the East African Virus Research Institute in Entebbe, Uganda. Dr. Mukwaya stayed for more than a week, to familiarize himself with the A. aegypti situation of the Coast, which is different from anywhere in Uganda.

c. Dr. Richard H. Baker, mosquito geneticist from the Pakistan Medical Research Centre in Lahore, Pakistan. Dr. Baker stayed for two days and accompanied us on field trips. Dr. Baker expressed his hopes to embark one day on genetic control of Culex tritaeniorhynchus, a major vector of Japanese encephalitis in West Pakistan.

d. Prof. Franz Huber, ICIPE Director of Research from the Max Planck Institute in Seewiesen, Western Germany, stayed with our Unit for three days and also visited our field sites. He has expressed his interest in our studies on habitat selection by A. aegypti and agreed to advise and assist us with his expertise on insect orientation.

e. Dr. Leroy Williamson, the newly arrived Director of the USDA-USAID sponsored tsetse research project in neighbouring Tanga, visited us one brief afternoon. We discussed briefly the goals of our projects and compared working conditions and concepts. Dr. Williamson insepcted our radiation source and informed himself about our arrangements concerning safety and monitoring. We hope that closer relationships with the Tanga Unit will develop in the near future.

2. Travel of MBU Scientists

In the past six months all three MBU scientists spent some time visiting other research insitutitions and attending scientific conferences.

In August Dr. McDonald combined his visit to the United States with an invitational visit to the WHO / ICMR Unit on genetic control of mosquitoes in New Delhi. His report is attached as Annex 9. As a major result of this visit our release plans have changed from a first release of translocation homozygotes to double heterozygotes. It was further agreed that the two projects would exchange their reports and share closely the information and experience gathered by the two projects. We now hope to learn from the outcome of the releases of translocation heterozygotes in the New Delhi field sites before we start with our releases in March.

Once in United States Dr. McDonald attended the 13th International Congress of Genetics in Berkeley. He took part with a slide show in a discussion group on teaching and research in genetics in developing countries. Discussion leader of the group was Prof. Reuben J. Olembo of the University of Nairobi, who had taken interest in our Unit since its beginnings. Dr. McDonald also spent one week at the University of Notre Dame, where he gave a seminar on the research achievements of MBU.

In August Dr. Fanara visited the research detachement of the US Naval Medical Research Unit No. 3 in Ethiopia. Contacts were made with the medical entomologists P. Neri and L. Sholdt and valuable information gathered for comparison of the behaviour of domestic and feral populations of A. aegypti in Ethiopia and Kenya.

In October Dr. Hausermann attended the 9th International Congress on Tropical Medicine and Malaria in Athens. He contributed an invitational paper in the section vector control. The topic of the paper was: Population dynamics of Aedes aegypti in East Africa in view of genetic control. The paper is attached as Annex 10. With great satisfaction it was noted that one of the congress resolutions expressed the need for in depth studies in ecology and population genetics of the most important vectors of disease. Even if the ideal of genetic control of pests and vectors has so far not many successful campaigns to its credit, it has at least provided strong stimuli to study the ecology and genetics of the major disease vectors on the population level. The visit to Athens was followed by a brief visit to the section Vector Biology and Control at WHO headquarters in Geneva. Unfortunately Dr. Pal, who originally had suggested this visit was absent in India. However, a very fruitful and stimulating discussion took place with Dr. Arata, the ecologist of VBC. As a consequence of this discussion Dr. Hausermann was asked to contribute his comments to the WHO Stegomyia survey program (Annex 11).

3. List of visitors to MBU July-December 1973

Prof. Th. J. Crovello,	Department of Biology, University of Notre Dame Notre Dame, Indiana, USA
Prof. Jan de Wilde,	Department of Entomology, University of Wageningen, Wageningen, Holland
Prof. S. M. Smith,	Department of Biology, University of Waterloo, Waterloo, Canada
Prof. D. Schneider,	Max Planck Institute, Seewiesen, Western Germany
Ms. Phoebe Omamo,	Division of Vector Borne Diseases, Nairobi, Kenya
Dr. L. G. Mukwaya,	E. A. Virus Research Institute, Entebbe, Uganda
Mr. R. Lubega,	Tropical Pesticides Research Institute, Arusha, Tanzania
Mr. W. H. Obudho,	Tropical Pesticides Research Institute, Arusha, Tanzania
Mr. E. Chipanga,	Port Health Office, Mombasa, Kenya
Ms. N. Donovan,	ICIPE, Nairobi, Kenya
Mr. B. Berry,	ICIPE, Nairobi, Kenya
Prof. S. Takahashi,	ICIPE, Nairobi, Kenya
Dr. R. Baker,	Pakistan Medical Research Centre, Lahore, Pakistan
Prof. Th. R. Odhiambo,	ICIPE, Nairobi, Kenya
Mr. M. Atashili,	ICIPE, Nairobi, Kenya
Mr. P. Orawo,	ICIPE, Nairobi, Kenya
Prof. and Mrs. F. Huber,	Max Planck Institute, Seewiesen, Western Germany
Dr. and Mrs. M. Kaib,	ICIPE, Nairobi, Kenya
Dr. Fidelis Ogah,	ICIPE, Nairobi, Kenya
Dr. and Mrs. L. Williamson	Tsetse Research Project, Tanga, Tanzania
Dr. H. P. Striebel,	CIBA - GEIGY LTD., Basel, Switzerland
Mr. R. Russo,	Department of Biology, University of Notre Dame, Notre Dame, Indiana, USA

F. Major Events

Staff

October 1973 saw the departure of Dr. Dean M. Fanara, the MBU ecologist. Dr. Fanara took up an appointment with the WHO Vector and Rodent Control Research Unit in Djakarta, Indonesia. Since this project is approaching the end of its present contract period, no new appointment was made for the remaining 8 months.

In November MBU was joined by UNDP-ICIPE sponsored Dr. Fidelis Ogah from Nigeria. Dr. Ogah is a population geneticist with a Ph.D. from Cornell University in Ithaca, USA. He will apply electrophoretic techniques to study the genetic diversity of the Aedes aegypti populations in our study areas before, during and after releases of genetically altered mosquitoes.

Following a suggestion of Professor Odhiambo an exchange program for ancillary staff was initiated between ICIPE Nairobi and MBU Mombasa. The aim of this program is to extend the experience and knowledge of the MBU ancillary staff to other ICIPE target insects and to further the cohesion between MBU and ICIPE staff. As first participant in this program Miss R. Chesang spent one month at ICIPE. During this time she familiarized herself with rearing techniques for tsetse flies and ticks, but had soon to replace a regular ICIPE insectary assistant who was absent on leave. In future more effort should be made that participants of this exchange program are also assisting in actual research work of selected research scientists.

From May onwards Mr. J. Mwandandu, one of our field assistants was used additionally as driver of one of our field vehicles. This proved to be a great relief for the scientists since some of the transporting and collecting trips could be delegated to him. However, on September 25, we nearly lost our assistant cum driver, while our oldest Landrover was completely destroyed by fire. Mr. Mwandandu suffered serious second degree burns and had to be hospitalized. Luckily he has recovered totally and is back at work since the middle of November. The cause of the fire was the apparently spontaneous ignition of a bottle of ether which was brought out to our field laboratory. The bottle was transported under our routine precautions, placed in a plastic coutainer on the front seat next to the driver. The cash refund for the actual value of the lost car from our insurance is expected in the near future.

Equipment

No large equipment purchases were made.

G. Not included in this report.

II. Annexes

1. The population dynamics of larval *Aedes aegypti* - Raymond J. Russo
2. Fecundity of domestic *A. aegypti* populations - Hausermann
3. Intervillage movement of mosquitoes - McDonald
4. Progress report - Fanara
5. Studies on habitat selection - Hausermann
6. Translocations - McDonald
7. Mass Production - I - McDonald
8. Mass Production - II - McDonald
9. Visit as temporary advisor to WHO/ICMR Unit, New Delhi, India - McDonald
10. Population dynamics of *Aedes aegypti* in East Africa in view of genetic control - Paper given by Hausermann at Int. Congr. Trop. Med., Athens
11. Comments to WHO computer survey of *Stegomyia* mosquitoes - Hausermann

IMMEDIATE OPENING

PhD - ECOLOGICAL SYSTEMS ANALYST

AN EXCITING OPPORTUNITY FOR A DYNAMIC ECOSYSTEMS ANALYST WHO WISHES TO COMBINE THEORETICAL AND ACTUAL FIELD APPROACHES.

CONSTRUCT AND EXERCISE A COMPUTER MODEL OF THE POPULATION DYNAMICS OF THE MOSQUITO, AEDES AEGYPTI, AS IT OCCURS IN COASTAL VILLAGES IN KENYA. EVALUATE DIFFERENT INTEGRATED CONTROL STRATEGIES ON THE COMPUTER TO HELP FIELD WORKERS TO DETERMINE OPTIMAL CONTROL METHODS.

PROJECT BACKGROUND: For the past three years, the United States Agency for International Development (AID) has supported basic research in Kenya and at the University of Notre Dame entitled, ECOLOGICAL STUDIES ON AEDES AEGYPTI IN EAST AFRICA PRELIMINARY TO GENETIC CONTROL. This project (called MBU, the Swahili word for mosquito) is one of several carried out with the cooperation of the International Center For Insect Physiology And Ecology (Nairobi) and the Vector Biology Laboratory (Notre Dame). Sufficient field experience and data have now accumulated to warrant controlled releases of genetically defective mosquitoes whose effect remains in the population for more than one generation (e.g., by the release of translocation heterozygotes). Preliminary results indicate that population fertility has been reduced from 98% to 50%. Currently, MBU involves four PhD's, both American and African, and eight laboratory and field assistants. Support personnel at Notre Dame include Professors Craig (mosquito biology), Crovello (computing, systems analysis) and Rai (cytogenetics and release of chromosomally modified mosquitoes). MODABUND, the 34,000 computerized mosquito reference bank was created at Notre Dame and is available to MBU personnel for searching. Notre Dame has excellent computing facilities, centered around an IBM 370/158 virtual memory configuration. Dedicated keypunching and secretarial service are available to the project at Notre Dame, as are simulation compilers. If not, we will buy them.

JOB RESPONSIBILITIES: The main task is to complete a systems analysis of the population dynamics of village populations of Aedes aegypti before control AND to design a "release module" to add to it to determine optimum release strategies. He (or she) also would consult with PhD personnel in Kenya on the statistical design and analysis of releases, of their monitoring and of related experiments.

JOB LOCATION: After an orientation at Notre Dame, the successful applicant will locate at MBU headquarters (a beautiful old estate on the Indian Ocean) in Mombasa for several weeks. Here she (or he) will gain familiarity with the project, with the way data are collected, and with the ecosystem to be modeled. You would take part

in ongoing releases and suggest changes in monitoring, data accumulation, etc. In short, the SYSTEMS DESCRIPTION, up through the systems diagrams and equations (but not the evaluation of coefficients) is scheduled to be done in Kenya. The programming, exercising, etc. will be done at Notre Dame. The total time spent in Kenya is somewhat flexible. A valuable byproduct for the successful applicant will be discussions with World Health Organization personnel at WHO headquarters in Geneva, etc. Such meetings can serve as an excellent introduction to health-related ecosystems activity. Publication of results is expected.

JOB QUALIFICATIONS: A PhD completed by September 1974 is absolutely essential. The appointee will be given formal status at Notre Dame as a Postdoctoral Fellow. Preference will be given to applicants with actual computerized ecosystems analysis experience in all phases, including programming (applicants will be responsible for their own programming). We prefer experience in population dynamics (versus nutrient cycling, etc.) and basic biostatistics. Less important, but desirable is experience with Diptera, pest control and an IBM 360 or 370.

If you are interested and are a dynamo, please send curriculum vitae, transcripts, three letters of reference, phone number, etc. to

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[redacted]

Notre Dame is an affirmative action, equal opportunity employer.

NOTE: The position is for at least one year, beginning on 1 September 1974, or as soon thereafter as possible. If the proper person cannot be found immediately, the job will be kept vacant until we succeed. Crovello will be in Venezuela, hence unavailable for discussion, from 20 July to 25 August.

PEACE ON EARTH

VECTOR BIOLOGY LABORATORY

DEPARTMENT OF BIOLOGY, UNIVERSITY OF NOTRE DAME

The 35 people at Notre Dame who work on mosquitoes are organized into the Vector Biology Laboratory. First initiated in 1957, the VBL contains people having widely diverse interests, from molecular biology to population biology, yet with all concentrating on the same group of organisms. This multidisciplinary approach to mosquitoes has been synergistic.

Components of the VBL:

1. World Health Organization International Reference Centre for *Aedes*

   Over 30 species and 150 strains of mosquitoes are maintained. Emphasis is on *Aedes aegypti*, with standard strains, geographic variants, inbred lines and strains with up to 8 genetic markers. This material is available on request to interested research workers.

2. MODABUND

   In the Mosquito Data Bank, the recent literature on mosquitoes, has been computerized. 34,000 references on mosquitoes have been taped, covering 1940 to the present. Computerized key word searches allow rapid preparation of bibliographies.

3. Training Program in Mosquito Biology

   An NIH-sponsored Training Program is devoted to the production of new PhD's able to apply aspects of modern biology to the traditional fields of medical entomology and parasitology. Mosquito-borne pathogens maintained include *Plasmodium gallinaceum*, *Crithidia fasciculata*, *Brugia pahangi*, *Dirofilaria immitis* and *Foleyella seasonalis*.

4. Laboratory Research Programs

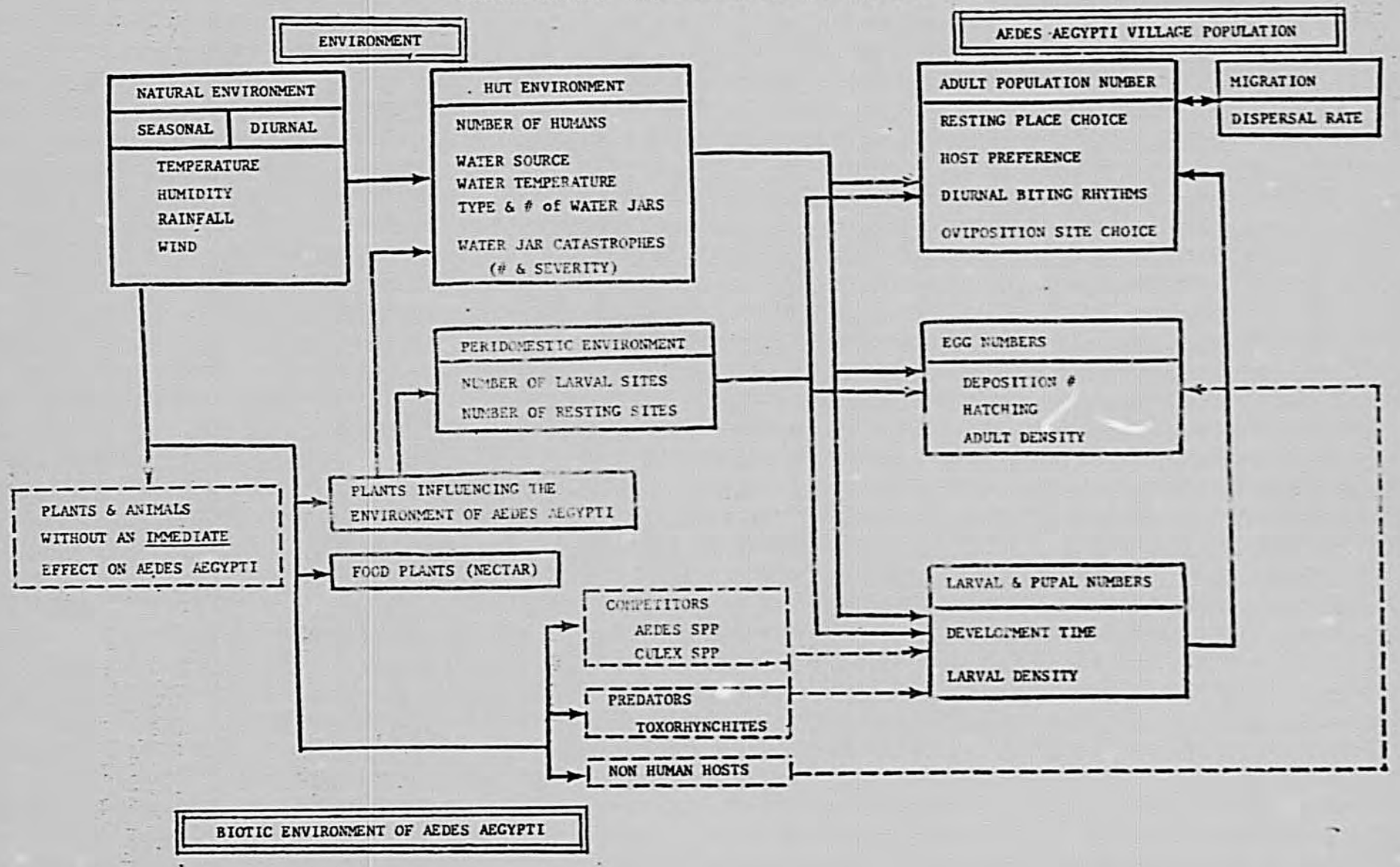
   a. Formal Genetics - Craig
   b. Cytogenetics - Rai
   c. Reproductive Biology - Fuchs, Craig
   d. Population Biology - Crovello, Saul, Craig
   e. Systematics - Crovello
   f. Biochemical Studies - Fuchs
   g. Behavior Genetics - Craig

5. Field Research Programs

   a. Mombasa, Kenya: Ecological Studies of *Aedes aegypti*. Preliminary to Genetic Control (With AID & ICIPE)
   b. Delhi, India: Genetic Control of *Culex fatigans* and *Aedes aegypti* (with WHO & ICMR)
   c. U.N.D. Environmental Research Center, Gogebic Co., Upper Mich.

Research supported by National Institutes of Health, Atomic Energy Commission, Communicable Disease Center, National Science Foundation, U.S. Agency for International Development, United Nations Development Program and World Health Org.

SYSTEMS DIAGRAM: AEDES AEGYPTI ON THE KENYA COAST



The population dynamics of larval *Aedes aegypti*

Raymond J. Russo
(NIH Trainee in Mosquito Biology and Graduate Student, Univ. of Notre Dame)

A key aspect to the population dynamics of any insect species is larval development rate. For a species like *Aedes aegypti* where most of the mortality occurs in larval stages, a larval life table is of upmost importance. Many investigators have built larval life tables of *A. aegypti* under laboratory conditions (Trager, 1937; DeMeillon, Goldberg and Lavoipierre, 1945; Christophers, 1960). Few investigators have attempted to build life tables under field conditions (Southwood et al., 1972; Trpis, unpublished).

During the summer of 1973 I spent six weeks with MBU to investigate questions of population dynamics. Dr. Hausermann pointed out a series of experiments which would lead help to clarify the complex picture of population dynamics in indoor breeding *Aedes aegypti*. This is a report of that set of experiments.

Materials and Methods

Experiment I - larval development rate

This experiment sought to estimate the mean larval development time of indoor breeding Aedes aegypti under field conditions. Dr. Hausermann noted that over several years of olling at A. aegypti from Rabai Location that he had not seen any *A. aegypti* with the abdominal mutant S^g in homozygous condition. Since the allele was encountered occasionally in heterozygous condition he developed a homozygous strain from selected heterozygous individuals. This strain could be used as a marker strain for field releases without being genetically too different from the local population. Before the field release, both the marker strain and the wild strain should have been compared in the lab controlling the concentration of food and ratio between strains. However, my time in Africa was limited there so we decided to compare the strains in the lab once I returned to Notre Dame. A field release was planned involving the placement of 50 genetically marked first instar larvae into each of 80 water pots in two representative villages in the Rabai area. We would then return daily and remove all pupae. As they emerged they were inspected for the mutant S^g. Since they were all released simultaneously we would get an emergence distribution over time from which we could calculate mean larval development time.

As a byproduct of this experiment, we were able to determine the frequence at which the water jars were cleaned. This cleaning operation would be considered a catastrophe to the mosquito population for all individuals in the cleaned jar were dumped out.

Experiment 2 - Laboratory comparison of marker and wild strains

Once I returned to Notre Dame I proceeded to compare the mean larval development times of the S^g/S^g and the S^+/S^+ strain under varying food concentrations. Each strain was grown by itself and also in a 1 to 1 mixture with the other strain. The density in each pan was held constant and the feeding patterns were as follows:

A 1 - 20 ml of liver powder soln. on the first day
A 2 - 10 ml of liver powder soln. on the first day
A 3 - 10 ml total; 5 ml on first day, 2.5 ml on 10th and 13th days
A 4 - 7.5 ml total; 2.5 ml on 1st, 10th, 13th days

Each combination of food and method grown had two replicates.

Experiment 3 - Determination of common age distribution

An essential aspect of population dynamics is the age structure of the population. Although adult population numbers have been estimated for the Rabai village, the determination of age structure of the larval populations had not been made. Over a three week period beginning June 4 to June 23, we collected larval population by emptying the water jar through a plankton net. The mosquito larvae were separated into stages and counted. Measurements were taken on the water jars so as to classify them by size of water containers.

Results

Experiment 1 - Larval development rate

The releases of first instar S^g/S^g larvae went as planned. A total of 4000 genetically marked individuals were divided among 80 waterpots (50 larvae in each pot). The collection of pupae was continued for 32 days after the release. Unfortunately, the recovery rate was much less than expected. Only 21 marked individuals were recovered. This low recovery can be based on two factors: 1) During the time of the experiment the families whose pots had been selected as experimental containers were to proceed as normal with respect to their water jars. Many families regularly clean their water jars thus eliminating our genetically marked populations. 2) The other possible factor to cause the low number of mosquitoes recovered is larval mortality due to competition for food. Our genetically marked first instar larvae were at a tremendous competitive disadvantage when placed in a container having older and thus larger larvae.

From the 21 individuals recovered we can estimate the mean length of larval development to be 15.04 days $\pm$ 7.36 days. However, by careful examination of figure 1, we see that 57% of the marked individuals were recovered by 11 days after release. The remainder of the marked individuals were spread over the 20 remaining days. If we calculate a development time based upon this first group which came through, we would get 9.5 days $\pm$ 1.38 days.

The second accomplishment of this experiment was the determination of the frequency of pot washings in a village at this time of the year. Figure 2 shows the number of pots remaining unwashed each day. From this we can calculate the average number of pots washed / day and estimate the minimum length of time till a pot is washed. The average number of pots washed / day was .97 ± .92. The minimum average length of time till a pot is washed was 11.91 days ± 6.98 days.

Experiment 2 - Laboratory comparison of marker and wild strains

The results from the comparison of S^g/S^g and S^+/S^+ under varying food conditions in the laboratory. Mean length of time for larval development in days.

	Food concentration			
	A1	A2	A3	A5
S^+/S^+ alone	5.89	6.27	6.50	8.03
S^g/S^g alone	6.25	6.70	7.43	12.69
S^+/S^+ mixed	5.87	6.25	8.90	9.85
S^g/S^g mixed	6.10	6.75	6.82	10.27

If we use multiple comparisons by F ratio as described by Kirk (1968) to compare the mean larval development time of S^+/S^+ alone versus S^g/S^g alone at all food levels, we find a significant difference at food levels A 2, A 3 and A 4. When we use the same test to compare mean larval development time of S^+/S^+ mixed versus S^g/S^g mixed at all food levels, we find a significant difference at A 3.

The indication is that when grown by themselves there are significant differences in the mean larval development time whenever the food concentration is not maximal. When the two strains are grown together there is probably intraspecific competition for the food. By comparing S^+/S^+ alone with S^+/S^+ mixed and S^g/S^g alone with S^g/S^g mixed, as food concentration decreases, S^+/S^+ takes longer to develop when mixed than alone and S^g/S^g takes a shorter amount of time to develop when mixed than alone. This intraspecific competition was not known, when the S^g/S^g strain was used as the marker stock in the field release.

Experiment 3 - Determination of common age distribution

The third experiment was to provide a common larval age distribution. It was thought that the most important factor affecting the age distribution would be size of the container. Therefore the water jars examined were assigned to one of four groups:

1. Small pots: circumference 125 cm, height at girth 20.7 cm
2. Large pots: circumference 140 cm, height at girth 26.6 cm
3. Small drum: circumference 122 cm, height 95 cm
4. Large drum: circumference 180 cm, height 190 cm

The number of containers sampled for each of these groups is 25, 12, 4, 2, respectively. This ratio somewhat represents the frequency of occurrence of each type of container in the villages.

The average number of each larval stage and the percent of total larvae are presented in the following chart.

Mean number and (percent) found in each stage

	L_1	L_2	L_3	L_4	P
Small pots	27.3(17.8)	36.2(23.6)	49.6(32.4)	33.5(21.9)	6.3(4.1)
Large pots	32.2(19.0)	52.6(31.1)	44.9(26.5)	24.7(14.6)	14.6(8.6)
Small pots	3.0(7.0)	31.5(74.1)	7.0(16.4)	1.0(2.3)	0 (0)
Large drum	512.0(52.0)	78.5(7.9)	58.0(5.9)	318.0(32.3)	16.5(1.6)
All containers together	48.9(26.3)	42.3(22.7)	44.7(24.0)	41.3(22.2)	8.5(4.5)

Discussion

There appear to be three ways of obtaining larval life table information.
1. Run the entire experiment in the lab under controlled conditions.
2. Set up several containers in the field so that the investigator controls what goes into and out of the containers but not the abiotic factors like temperature.
3. Use the containers in the field under no control by the investigator and somehow label the individuals which are in the experiment. Each method has advantages and disadvantages, usually one must sacrifice reality for precision or vice versa. I could not find in the literature any work concerning use of a genetically marked strain to determine a larval development time in mosquitoes. This was the most interesting aspect of this experiment. Obviously, a major assumption in the method is that no significant difference exists between the marker strain and the wild strain. This should have been tested before the field experiment was conducted, but it was impossible to do it before the field work. To make the most of my time in Africa, we started the field experiment and put off the laboratory test till I returned to Notre Dame.

Conclusions

1. Since the recovery of genetically marked individuals was spread over the entire experiment, the concentration of food in every water jar

is not the same, but actually varies from household to household.

2. If we consider the 80 pots chosen an adequate and representative sample, then the average larval development time is 15.04 days.

3. If we are concerned with the minimum development time which could exist in the village at this time of year, we should consider the group of individuals which formed the peak in figure 1. Their mean development time was 9.5 days. An interesting note is that of the 12 individuals which pupated by the eleventh day, eight of them were collected from pots which did not produce marked individuals later in time but did continue a high production of unmarked individuals. This indicates that the genetically marked individuals placed in these jars either developed quickly or died. This implies that these jars had a greater food supply and thus competition was less.

4. The rate of pot washing was calculated to be .97 pots/day. Note that this is like an average loss rate from a set number of pots. Once a pot is removed from a population by being washed, it was not considered again.

5. The minimum average length of time until a pot is washed was calculated to be 11.91 days with a standard deviation of 6.98 days. This value can only be calculated if the assumption is made that the rate of washing of each jar as determined from the beginning of the experiment is constant throughout the experiment. We could have achieved a more accurate estimate of average length of time until a pot is washed by having all the people washing their pots on the first day of the experiment and continue to observe the pots for the entire experiment.

6. If we consider the relative effects of competition for food and pot washing on larval mortality, food competition accounts for about 99% of the mortality and pot washing for only about .5%. The laboratory comparison of S^g/S^g and S^+/S^+ strains showed that when grown by themselves their mean larval development times were significant different while when grown together intraspecific competition brought their development times close enough so that they were significantly different at only one food concentration. However, this competition distorts the development time in such a way that it would be difficult if not impossible to use the genetically marked strain to monitor developmental rates in the field. If a marker strain could be found which responded more closely to a wild strain, this method would be usable. An alternative approach would be to sacrifice some reality for more control over the experiment as did Southwood (1972).

The common age distribution when considering all pots was 1st instar = 48, 2nd instar - 42, 3rd instar = 44, 4th instar = 41, pupae = 8. Usually the information one is looking for when concerned with age distributions is the age specific survivor rate or what percent of each day makes it to the next day. However, these data cannot give this information. First, since the data are expressed in stage of individual and an individual does not spend the same amount of time in each stage, there is no transformation to convert stage of individual to age of individual. We might be able to do this if we knew from the literature the lengths of time spent in each stage

at various concentrations of and the percent of pots having each concentration of food. The second problem is that since the water jars are filled and cleaned at varying times depending on many ephemeral factors, the maturity of the age distributions in a village's water jars will have a tremendous variance.

Summary

A summer was spent to collect data on the larval dynamics of an *Aedes aegypti* indoor breeding strain in East Africa. A genetically marked strain was used to monitor larval development time and an estimate of 15.04 days were calculated. The rate of catastrophe (pot washing) was determined for the village for this season. A common age distribution was determined but its use is questioned unless a good deal more information is used.

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Figure 1

Frequency of Recovery of Marked Mosquitoes

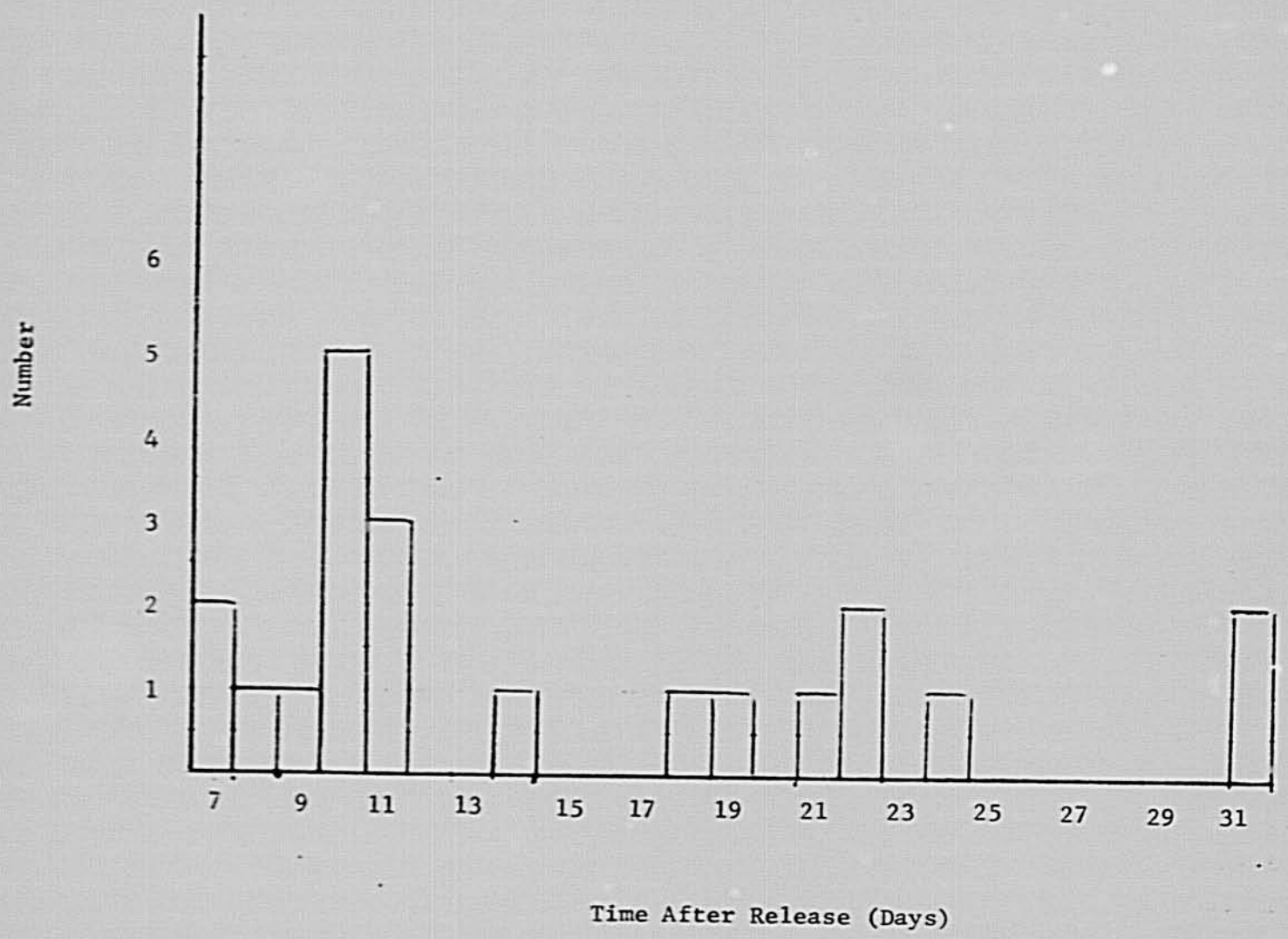
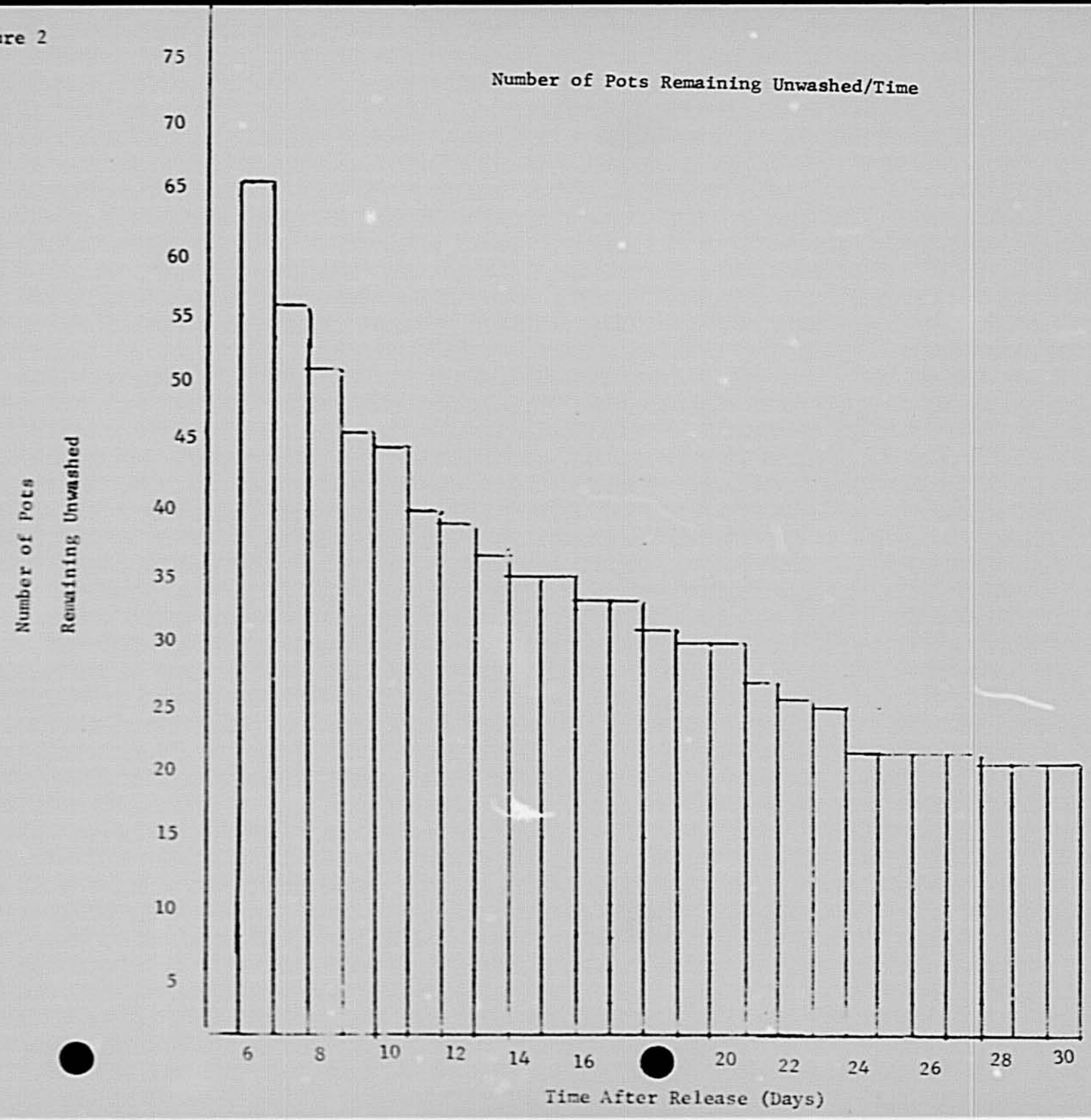


Figure 2


Number of Pots Remaining Unwashed/Time
Number of Pots Remaining Unwashed
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
6
8
10
12
14
16
20
22
24
26
28
30
Time After Release (Days)

Fecundity of domestic *A. aegypti* populations

W. Hausermann

The following two methods were adopted to estimate the daily egg production of domestic *A. aegypti* populations:

Method 1: Number eggs per female and day

Assumptions:

1. The daily egg production of field collected females when kept in the laboratory without additional bloodfeeding is the same as if these females had not been caught and had not taken another bloodmeal for the rest of their life.

2. A sample of females caught in one day's landing-biting catch does not necessarily represent the real proportions of the different physiological stages in the population. However, if the landing-biting catch is carried out daily for at least the average time interval between host visits and the oviposition rate of the sample is measured for the same period following the catch, the daily oviposition rate of the sample reflects essentially the daily oviposition rate of the field population for the same period.

Procedure:

The average time interval between subsequent host visits was found to be four days (Annex 10). Samples of females were collected daily for several 4-day periods with the landing-biting catch method and then kept in gallon cages for oviposition. The eggs produced by these samples were counted daily. An average oviposition rate per female and day was calculated for the first four days period after the catch.

Results:

A summary table for the results is given below:

Season	No. ♀♀ caught	No. eggs produced / ♀ / day — Days after capture 1	2	3	4	5	6	7	8	Total days 1 - 4	Average per ♀ and day
Cool	678	4.4	3.0	2.4	2.1	2.0	1.5	1.4	1.4	11.9	3.0
Hot	291	0.8	2.4	5.0	5.8	5.2	3.5	2.5	1.9	14.0	3.5

In the populations sampled the average oviposition rate was thus estimated as 3.0 and 3.5 eggs per female and day. This amounts to a fecundity of approximately 24 and 28 eggs per adult female, when her life expectancy is 8 days as estimated earlier.

The validity of this method will be discussed with a statistician in the near future.

Method 2: Number egg oviposited per house and day

Assumptions:

1. By placing sufficient uncovered oviposition jars into a house close to 100% of the oviposition is diverted from the customarily covered containers for domestic water into these oviposition traps.

2. The placing of oviposition jars in a house does not increase the oviposition rate for the village.

Procedure:

Two and three oviposition jars were placed into 18 houses of 3 villages and filled half way with water. After 3 days the jars were removed, emptied and slowly dried. Six days later the jars were filled to the rim with water and reflooded daily until no more larvae would hatch. The hatching larvae were counted daily and the total taken as an estimate of the number of eggs oviposited in the jars.

Results:

A summary table of the results is given below.

Village	Dates traps set	Total No. traps set	No. larvae recovered (= eggs)	No. eggs per house and day	Estimated number of ♀♀ per house + day
Rabai	2-5 /11	12	1646	91.4	28.1
	16-19/11	18	1944	108.0	30.8
Shika Adabu	2-5 /11	12	1119	62.2	17.8
	16-19/11	18	2315	128.6	36.7
Simakeni	2-5 /11	12	1049	58.3	16.7
	16-19/11	18	1919	106.6	32.8

A remarkable increase in number eggs collected was noted when the number of oviposition jars per house was increased from 2 to 3. Experiments with 4 jars are now in progress. The number of oviposition jars per house may have to be increased even to five before 100% of the eggs can be assumed to be harvested in the traps.

Combining method 1 and method 2 preliminary estimates of the numbers of *A. aegypti* females ovipositing in these Rabai houses can be made. For

November 1973 these estimates vary between 30 and 36 females per house. These per house estimates sum up to estimated village populations of 550 females for the 15 houses of Shika Adabu, 525 females for the 16 houses of Simakeni and well over 1500 females for the large village of Rabai. These estimates are consistent with estimates made earlier with mark-release-recapture methods in other villages.

The method of estimating female numbers by oviposition will be used in conjunction with mark-release-recapture methods in preparation of the releases.

Intervillage movement of mosquitoes

Paul T. McDonald

A series of experiments has been undertaken in order to determine the relative isolation of village mosquito populations from each other. The basic workplan calls for a series of experiments to determine the influence of several factors on mosquito immigration into a village. These factors are 1) climatic 2) distance 3) availability of alternative sources of food, shelter and bloodmeals 4) age of mosquito and 5) genetic factors.

For the first series of experiments the village of Kwa Dena was selected. It has 14 houses and lies in the western edge of the field study area. From a central point in the village, sites were selected in four directions, NE, SE, NW, SW, at 200 meters and at 400 meters. The 200 meter sites were all in open fields. Three of the 400 meter sites were in other nearby villages, the SW site being in an open field.

The first two experiments were conducted during the period of the SE monsoon, intermittent rain and somewhat shifting breezes. Mosquitoes of the RABAI DOMESTIC strain were released at dusk at distances of 200 meters in 4 directions and 400 meters in 4 directions, each direction receiving equal number of release mosquitoes. In addition mosquitoes were released in the village itself. The mosquitoes were captured in landing-biting (L-B) catches for seven days subsequent to the releases, at 14 man hours per day. Mosquitoes released at 200 meters were all dusted Day Glow Green and those released at 400 meters were dusted Day Glow Red. Those released within the village were dusted Day Glow Yellow. All mosquitoes were newly emerged (0-48 hours old) when released. The recoveries of marked mosquitoes during the L-B catch periods is given in table 1, along with the details of the numbers released in each of the first two experiments.

A more detailed investigation of the direction of immigration was undertaken with releases of newly emerged RABAI DOMESTIC mosquitoes made at 200 meters from the center of the village in 4 directions. With mosquitoes from each direction marked with a different fluorescent dye it was possible to determine the origin of each marked mosquito as before the mosquitoes released with Kwa Dene were also dusted. Table 2 gives the details of the numbers released and recovered in each of these experiments. Experiments III and IV were conducted during a period of variable wind direction as the predominant wind direction shifted from south to northeast, becoming established during the course of experiment IV. At the time of release (dusk) of Experiment III it was quite still while during the time of release (dusk) of Experiment IV the wind was light from the southern direction.

The recovery of marked mosquitoes which were released in the village gives an approximation of the effectiveness of the capture method. If the assumption is made that the same ratio of captured: not captured holds for marked mosquitoes of each different color, then an estimate of the number

of mosquitoes of each sex entering from both distances can be made. The percentage of released mosquitoes that enter the village from each distance outside can then be determined. The results of the calculations are presented in Table 3.

The results from Experiment I and II indicate a considerable ability to enter a village across a distance of 200 meters with 28 percent for males and 42 percent for females. At 400 meters the entry rate is much reduced with one percent for males and eight percent for females. The results from Experiments III and IV also indicate a large influx of mosquitoes from 200 meters, however, with a much higher estimate of male immigration, being 57 percent. The influx of females is, as before, slightly greater than 40 percent.

The direction from which mosquitoes enter a village has been determined for Kwa Dena during the period specified above. Estimates of percentages of released mosquitoes entering from the different directions of release is presented in Table 4. The direction probably reflects on climatic conditions prevailing at time of flight and would be expected to vary especially with wind direction. While it is necessary to conduct further tests with various changes in marking color choice, it appears that the influence of wind direction at time of release is quite important as all directions were represented when the releases were made during a lull and predominantly southern releases were represented when southerly breezes occured during the release period.

The significance of immigration into a village as determined in these experiments for mosquitoes released outside can only be evaluated for the relevant situation when tests with alternatives for survival available at the release points are undertaken.

Table 1: Capture of marked mosquitoes entering Kwa Dena

Experiment	Location	Sex	No. released	Dust	Captures	% Captured
Expt. I	within	♂	98	DGY	48	49
		♀	97	DGY	20	21
	200 m	♂	395	DGG	62	16
		♀	393	DGG	37	9
	400 m	♂	395	DGR	2	1
		♀	392	DGR	5	1
Expt. II	within	♂	65	DGY	34	52
		♀	93	DGY	30	32
	200 m	♂	395	DGG	49	12
		♀	371	DGG	46	12
	400 m	♂	389	DGR	2	1
		♀	370	DGR	12	3

Table 2 : Captures of marked mosquitoes entering Kwa Dena from 200 meters

Experiment	Location	Sex	No. released	Dust	Captures	% captured
Expt. III	within	♂	77	DGY	8	10
		♀	79	DGY	19	24
	NE	♂	79	DGR	6	8
		♀	77	DGR	8	10
	SE	♂	79	HR	7	9
		♀	75	HR	3	4
	NW	♂	80	DGG	2	3
		♀	80	DGG	10	13
	SW	♂	76	HG	9	12
		♀	80	HG	8	10
Expt. IV	within	♂	157	DGY	65	41
		♀	158	DGY	51	32
	NE	♂	159	DGR	6	4
		♀	160	DGR	7	4
	SE	♂	159	HR	44	28
		♀	156	HR	25	16
	NW	♂	160	DGG	2	1
		♀	160	DGG	9	6
	SW	♂	157	HG	71	45
		♀	159	HG	45	28

Table 3 : Estimates of mosquitoes entering Kwa Dena

Experiment	Location	Sex	No. entering	% released entering
Expt. I	200 m	♂	126	32
		♀	176	45
	400 m	♂	4	1
		♀	24	6
Expt. II	200 m	♂	94	24
		♀	144	39
	400 m	♂	4	1
		♀	38	10
Expt. III	200 m	♂	240	76
		♀	121	39
Expt. IV	200 m	♂	300	47
		♀	269	42

Table 4 : Estimates of mosquitoes entering Kwa Dena from 200 meters

Experiment	Location	Sex	No. entering	% released entering
Expt. III	NE	♂	60	76
		♀	33	43
	SE	♂	70	89
		♀	12	16
	NW	♂	20	25
		♀	42	53
	SW	♂	90	118
		♀	33	41
Expt. IV	NE	♂	15	9
		♀	22	14
	SE	♂	107	67
		♀	78	50
	NW	♂	5	3
		♀	28	18
	SW	♂	173	110
		♀	131	82

Progress Report

Dean M. Fanara

During the period since the last report the major emphasis has been on the "Africanization" of a recessive marker strain for each of the 3 main strains. The Bejumwa Dry strain (A. aegypti formosus taken from tree holes during the dry season) was crossed with a BLACK TARSI homozygous recessive. The Bejumwa Wet strain ("type form" taken from a treehole during the heavy rains) was crossed with a BLACK PEDICEL homozygous recessive. The Mwamsaby Dry strain (A. aegypti queenslandensis taken from water pots indoors during the dry season) was crossed with a BLACK PALPI homozygous recessive. Outcrosses and sibling crosses with selection for marker were made for 10 generations to produce 96.875% Africanized stocks. This consumed 7 months of labor (21 days per generation).

The experimental design called for the mass release of these marker stocks in their appropriate habitat. Larvae were to be collected from labeled water pots (1 per house) and 100 bamboo "section" hung throughout Bejumwa forest. By crossing the reared virgin females with a multiple marker male laboratory stock (developed by combining and selecting marker stocks obtained from the Vector Biology Laboratory of the University of Notre Dame) it would have been possible to determine:

1. Direction of gene flow (domestic to forest or vice versa or none).
2. Amount of sibling mating.
3. A concept of numbers needed for a release to produce a determined amount of interaction with the domestic Aedes aegypti.

Unfortunately my contract with this project expired before this experiment could be fully executed. A delay in the experiment was caused because the laboratory mixed marker strain was more difficult to produce than was anticipated. The parent recessive marker stocks appeared to contain more genes affecting the discrimination of desired markers than was suitable for the proposed experiment. It is also unfortunate that no one has been hired to fill the position I am vacating so that this experiment could be completed.

During this last period a visit was paid to the U.S. Naval Medical Research Unit No. 3, Research Detachment Ethiopia. This unit is responsible for conducting basic and applied research on tropical and infectious diseases prevalent in Ethiopia. On the staff is Lt. Cdr. Lance Sholdt, an entomologist who is conducting some mosquito trapping tests. Also at NAMRU 3 is Mr. P. Neri who has been surveying the mosquitoes of Ethiopia for many years. From him I obtained the strain Langano of A. aegypti that is formosus

in appearance and comes from a forested area where no man biting has been observed. It is useful as a comparative tool with the Bejumwa Dry strain.

Table 1 depicts the results of field cage, domestic, peridomestic, and forest releases of the strains that were being developed for release in the gene flow experiments. It is seen that the Bejumwa Dry strain has a host other than man. Dr. Milan Trpis of the University of Notre Dame, after laboratory tests, considers it to have birds as its preferred host (personal communication). Mr. P. Neri considers that the Langano strain prefers reptiles (based upon field observations). In testing it against the proven anthropophilic gold mesonotum strain from Mwamsabu village, it was 6 fold less attracted to man under sylvan conditions. The "type form" Bejumwa Wet periodomestic mosquito is more adapt at seeking man than the Mwamsaby Dry domestic mosquito under conditions of indoor release, peridomestic release and field cage release. This mosquito may pose a real threat to any project attempting control of A. aegypti by consideration of only the domestic form.

Table 1 : Tabled results of man biting activity by various strains of *Aedes aegypti*

Strain		Release			Recapture		
Designation	Description	Site	Total	Age(hrs)	Time(min)	%	Ratio
Mwansabu Dry	queenslandensis from water pots during dry season	Field	100	48	60	14.0	26
		Cage	100	72	45	40.0	30
Bejumwa Dry	formosus from tree holes during dry season	Field	100	48	60	7.0	13
		Cage	100	72	45	21.0	16
Bejumwa Wet	"typeform" from tree holes during rains	Field	100	48	60	33.0	61
		Cage	100	72	45	71.0	53
Mwansabu Dry		Indoors	100	48	75	54.0	30
Bejumwa Dry		Indoors	100	48	75	41.0	23
Bejumwa Wet		Indoors	100	48	75	85.0	48
Mwansabu Dry		Peri-domestic	250	72	60	10.4	31
Bejumwa Dry		Peri-domestic	250	72	60	3.2	10
Bejumwa Wet		Peri-domestic	250	72	60	20.4	61
Bejumwa forest	formosus from bamboo sections in forest	Forest	400	48	15	11.8	26
Langano	formosus from Ethiopian forest	Forest	120	48	15	5.0	11
Gold mesonotum	Adults from village - homozygous recessive	Forest	300	48	15	23.3	62

Studies on habitat selection

W. Hausermann

One of the major reasons for the limitation of geneflow between domestic A. aegypti type form and A. aegypti ssp. formosus is the genetically determined difference in habitat preference and the absence of a distinctly peridomestic habitat with an abundance of man made containers, where the two could meet. It is only during the rainy season from late April to June, when frequent rains permit continuous breeding outdoors, that the two subspecies meet and hybridization takes place. These hybrids look like dark A. aegypti type form and disappear again when the rains end.

Outdoor releases of domestic type form, ssp. formosus and reciprocal hybrids demonstrated the immediate separation of the two parental species into domestic and feral habitats. The hybrid dispersed more evenly. The recapture figures did not permit a genetic analysis because of different dilution factors.

In an attempt to study the genetics of this behavioural trait a chamber was developed in which the two subspecies separate to a similar degree as in the field. The environmental cues to which the mosquitoes respond are not yet fully understood. Investigations to this end are in progress. The design of the chamber is improved at the same time.

Preliminary experiments with the chamber demonstrated that habitat preference is an inherited trait and consistently present in strains collected from many different areas. The results are shown in the table below.

Strain + origin	Laboratory generation	Method and place of collection	% recovery in domestic chamber
RABAI DOMESTIC RD	P	Oviposition jars from inside houses in Rabai	65
	F_1		58
	F_2		40
SIMAKENI DOMESTIC SD	P	Oviposition jars from inside houses in Simakeni	58
	F_1		51
	F_2		38
SHAURI MOYO	F_n	Larvae from domestic water container	43
MWAMSABU	F_n	Larvae from domestic water container	46
RABAI FERAL RF	P	Oviposition tins set among trees in Rabai	16
	F_1		10
	F_2		13
SIMAKENI FERAL SF	P	Oviposition tins set among trees in Rabai	11
	F_2		13
	F_4		8
MNAZI feral	F_{15}	Larvae from steps in coconut trees	5
TREEHOLE feral	F_{12}	Larvae from treeholes	9
LANGANO feral	F_n	Larvae collected by I.Nori in Langano forest, Ethiopia	10

Translocations

Paul T. McDonald

In July a decision was reached that the major emphasis with translocations would be, in order of priority, the following: 1) Africanization of translocation homozygote strain of T (2:3) c from Notre Dame stocks in preparation for releases in the field. 2) Maintainence of selected strains of African translocation heterozygotes as back-ups for later releases. 3) Completion of tests for homozygosity of strains not already tested before.

During consultation with the ICMR/WHO Genetic Control Unit in New Delhi two important factors became clear. First, the best suitable homozygous line T (2:3) c had consistently showed low fitness compared with heterozygotes for the same translocation and second, such type of homozygote stock could not be expected, on theoretical grounds, to replace a resident isolated population of non-translocation individuals. Quite importantly, genetic monitoring required for the homozygote release strategy would be very extensive and a considerable lag between releases and assay of effectiveness of releases would occur.

When alternative plans were considered (Table 1), a decision was made to release a double heterozygote derived from the ROCK laboratory strain homozygote line and the African MAZERAS DOMESTIC strain sex-linked heterozygote (III of Table 1). Such a strategy was thought to represent optimization of mass production efforts with production of a behaviourally appropriate type of release mosquito. In addition such release mosquitoes would possess heterotic qualities as they arise from the synthesis of two genetic backgrounds. The decision to make genetic releases of males would be expected to reduce the possibilities of resistance of villagers to releases of large numbers of mosquitoes.

Translocation homozygote

Samples from Notre Dame were contaminated with heterozygotes with and without pseudolinkage of $\underline{s}^+$ and $\underline{blt}^+$ and non translocation individuals as well. A sample of 20 individuals taken from the F_2 of this stock was analyzed for fertility and pseudolinkage and while 14 were heterozygotes none were homozygotes (Table 2).

A program of Africanization, purification and reconstitution of homozygotes was undertaken with the scheme of Table 3. The stock has been Africanized in the multiple marker (ru / ru; Si s / Si s ; blt / blt) Africanized DOMESTIC strain for three generations. Heterozygotes have been crossed together and resultant progeny phenotypically appropriate for homozygotes have been crossed with each other in single-pair crosses. If

both parents of a single pair are homozygous then all F_1 and F_2 should be phenotypically homozygous as well. If only one parent is homozygous (the other resulting from a crossover, homozygous for $\underline{Si}^+$ / $\underline{Si}^+$ but not for the translocation) then the F_2 will reveal this while the F_1 could reveal if both parents are $\underline{Si}^+$ / $\underline{Si}^+$ and neither homozygous for the translocation. Thirty-eight potential homozygote x homozygote crosses were made. Only eleven gave sufficient progeny for analysis and all were heterozygote x heterozygote. Another fifty-eight crosses have been set and 37 have left progeny which are soon to be analyzed. Once lines of homozygotes derived from a single-pair have been established, they will be multiplied and pooled for mass production. At the same time several separate lines of homozygotes will be maintained in the event that one becomes contaminated.

The fertility associated with the T (2:3) c translocation heterozygotes and controls have been estimated by hatchability of egg papers from single-pair cages with multiple marker females crossed with heterozygote or normal males. The results take the form of the distribution table of table 4 and indicate a general fertility for this translocation heterozygote of 57 percent compared with 94 percent for control (non translocated) males.

The T (2:3) c translocation strain has been tested during the process of Africanization and the results are in Table 5. The strain has a rather mediocre performance in a test cage designed for testing endophily in the laboratory and appears to be refractory to selection of endophily with the use of the laboratory cage.

Translocation heterozygotes

In the last report it was noted that 46 translocation lines of African origin were being maintained and tested for homozygosis whenever possible. In July there were no definite homozygote lines available and the decision was made to discard several lines which had not given homozygotes after several trials and had relatively more problems in maintaining because of loose pseudolinkage of markers used. For maintainence as back-ups, twelve heterozygote lines have been kept (Table 6). The sex-linked strains of domestic origin are being tested to select male-linked crossover translocation heterozygotes, and to date two lines (1:3 p and 1:2 n) have given the crossover phenotypes required. These individuals are being tested for the M-linked translocations. The most promising will be produced for field releases.

Search for homozygotes

For purposes of testing for homozygosis the strains which had not been successfully tested for five times were kept until they had been tested at least five times. If the tests were negative and the translocation strain was not needed for other purposes, it was discarded. The current listing of translocation strains that were tested after the last report, with results,

are included as Table 7 (A). The translocation stock AT 2:3 n has on two occasions produced progeny which have tested as homozygotes. The isolation of the homozygous line is extremely troublesome because 1) the homozygotes have fertility similar to heterozygotes and 2) the normal heterozygote produces 25 percent crossovers between pseudolinked markers. Such crossovers may produce individuals phenotypically the same as heterozygotes but not heterozygotes for the translocation.

An experiment to determine if irradiated inbreed lines would yield translocation homozygotes more readily is still in progress and the results to date are included in Table 7 (B).

Table 1 : Translocation release alternatives

	I Homozygote	II Double heterozygote from homozygote	III Double heterozygote from homozygote x heterozygote	IV Double heterozygote from heterozygotes
Source:	ROCK	ROCK	ROCK / MAZERAS	MAZERAS
Fitness of stock:	Hatch 40 % Larval survival 77 %	----	"Heterotic vigor"	----
Rearing for production of release mosquitoes:	No marker selection required	No marker selection required	Marker selection for males of hetero-zygotes only	Marker selection required for both strains
Release:	Both sexes	Both sexes optional	Males only	Males only
Expected sterility from release:	50 %, declining slowly after termination of releases	Increasing to 75 % until slow post releases decline	Increasing to 75 % with rapid post releases decline	Increasing to 75 % with rapid post releases decline
Does translocation become fixed in population ?	Only if village swamped with releases and until immigration of normals	No	No	No
Monitoring release effectiveness:	By genetics and sterility	By genetics and sterility	Sterility measurements only	Sterility measurements only

Table 2 : Genetic testing of T (2:3) c stock

Presumed type	No. tests	Results			
		Δ	♦	Normal	Indeterminate
Homozygote	20	0	14	0	6
Heterozygote	20	0	17	0	3
Control	20	0	0	20	0

Δ Homozygote

♦ Heterozygote

Table 3 : Africanization and purification of homozygous T (2:3) c stock

G_1 (M)	Δ	x	MMA	⟶	♦MMA$_1$	
G_2 (M)	♦MMA$_1$	x	MMA	⟶	♦MMA$_2$	(selected)
G_3 (M)	♦MMA$_2$	x	MMA	⟶	♦MMA$_3$	(selected)
G_4 (M)	♦MMA$_3$	x	♦MMA$_3$	⟶	ΔMMA$_3$	(selected)
G_5 (S-P)	ΔMMA$_3$	x	ΔMMA$_3$	⟶	ΔMMA$_3$	(selection)
G_6 (B-S)	ΔMMA$_3$	x	ΔMMA$_3$	⟶	ΔMMA$_3$	(selection)

G = Generation

M = mass mated

S-P = single-pair mated

B-S = brother-sister mated

Δ = Homozygote

MMA = multiple marker Africanized stock

♦ = Heterozygote

Table 4 : Distribution of hatchabilities of egg papers from marker females with heterozygote or control males

Hatchability	Number of egg papers	
	Heterozygote	Control
1 - 10	0	0
11 - 20	0	0
21 - 30	1	0
31 - 40	1	0
41 - 50	9	0
51 - 60	17	0
61 - 70	13	1
71 - 80	8	3
81 - 90	1	4
91 - 100	0	22
Total	50	30

Average hatch of heterozygote : 57 %
Average hatch of control : 94 %

Table 5 : Endophily response of T (2:3) c stock in lab cage

T(2:3)c line	no.tested	captured		lost	% in - going
		in	out		
ΦMA_3	100 ♂♂	7	34	59	7/41 = 17
	100 ♀♀	30	52	18	30/82 = 37
F_1 (ΦMA_3)	118 ♂♂	23	82	13	23/105 = 22
	131 ♀♀	29	102	0	29/131 = 22
F_2 (ΦMA_3)	39 ♂♂	1	31	7	1/32 = 3
selected "in"	29 ♀♀	6	21	2	6/27 = 22
F_2 (ΦMA_3)	38 ♂♂	5	30	3	5/35 = 14
selected "out"	29 ♀♀	9	15	5	9/24 = 38

Table 6 : Selected translocations maintained at IBU

Translocation code	Background	Pseudolinkage	Fertility
1 : 2 c	indoor	.93 (280/302)	50 %
1 : 2 n	"	1.00 (117/117)	50 %
1 : 2 t	"	.99 (137/139)	30 %
1 : 2 r	outdoor	.93 (143/153)	50 %
1 : 2 u	"	.94 (294/314)	35 %
1 : 3 f	indoor	.97 (287/297)	50 %
1 : 3 p	"	1,00 (234/235)	70 %
2 : 3 c	indoor	.95 (288/302)	50 %
2 : 3 j	"	.95 (363/382)	50 %
2 : 3 n	"	.76 (645/849)	70 %
2 : 3 r	outdoor	.99 (321/323)	50 %
2 : 3 z	"	.88 (473/533)	50 %

Table 7 : Translocations tested for homozygosity

	Homozygosis tests (+/-)		Current status
	June 73	December 73	
A. African strains			
1 : 2 a	0/0	0/0	T
1 : 2 b	3/0	3/0	L
1 : 2 j	3/0	7/0	D
1 : 2 l	4/0	4/0	L
1 : 2 r	1/0	1/0	T
1 : 2 t	4/0	8/0	T
1 : 2 x	0/0	5/0	D
1 : 2 ad	0/0	0/0	D *
1 : 3 b	2/0	8/0	D
1 : 3 i	3/0	9/0	D
1 : 3 n	3/0	6/0	D
1 : 3 p	2/0	8/0	D
1 : 3 q	0/0	0/0	L
2 : 3 f	3/0	3/0	T
2 : 3 n	3/0	11/2	T
2 : 3 u	0/0	0/0	D *
2 : 3 aa	0/0	0/0	L
2 : 3 ad	0/0	0/0	L
B. Inbreed strains			
1 : 2 ae		0/0	L
1 : 2 af		3/0	T
1 : 2 ag		0/0	L
1 : 3 r		3/0	T
1 : 3 s		0/0	T
2 : 3 ae		7/0	D

D = Discontinued testing

L = Lost

T = Testing continued

* = produced no homozygote phenotypes

Mass Production I

Paul T. McDonald

The mass production facility has made considerable advances since the last report. The physical facility has been improved with the replacement of the large rearing trays with four shelves for holding the pans for larval rearing as well as the emergence containers and gallon cages for adults. The shelves are arranged to make three aisles much like a library. The necessary plasticware have been purchased and are being prepared for use.

In the past a troublesome aspect of the mass production has been the acquisition of a pupal separation facility. We now have the Sharma type pupal separator from WHO/ICMR in New Delhi with additional ones of the same design made at the ICIPE workshop in Nairobi. This type of separator is quite effective in the first trials. The stirring devise for providing uniform alliquots of first instar larvae had already been tested and found satisfactory.

The release experiments planned for the near future call for the availability of newly emerged mosquitoes on a daily basis for periods up to six weeks. A decision was made that this could be most effectively accomplished if production were regularized so that the daily releases were not dependent upon a store of eggs but rather on the daily production of eggs sufficient to yield the necessary number of release mosquitoes after the period of embryonation and development. The general procedure would be the daily setting of crosses of new cohorts and collecting of eggs from the older cohort such that each cohort is responsible for egg production on two occasions, each being at an interval after a bloodmeal.

For each cohort the maximization of production is dependent upon the particular regime as applied to all cohorts in the overall production scheme. Preliminary to establishing a particular regime, the especially important parameters of fecundity and larval development have been the subject of experiments (see Annex). Improvements in production are being introduced in accordance with findings from these experiments.

For these investigations, the temporal sequencing of hatching, pupae separation, bloodfeeding and collecting of eggs have been scheduled so as to simulate the actual continuous production scheme, with a new cohort starting through the regime each day. This has had the benefit of familiarizing the staff with the overall mass production scheme involving the daily advancement of each cohort through the regime.

The overall outcome of the mass production efforts has been:

1. Physical facility ready
2. Staff trained
3. Regime improved for fecundity and larval development
4. Pupal separation operative.

Continuing investigation on egg collection and storage procedures have begun and the best methods will be incorporated into the regime as soon as the results of these experiments are known.

Based on experience to date requirements for mass production have been determined for two types of releases: 1) release of mixture of single and double heterozygote males derived from two different heterozygotes and 2) release of double heterozygote males derived from homozygotes and heterozygotes (Table 1). The initial production has begun for the first of the two alternatives and the current schedule calls for releases in the middle of March.

Table 1 : Mass production requirements for releases of double heterozygotes

A. Mixture of single and double heterozygotes from m-linked heterozygote x M-linked heterozygote at 2000 males per day

Eggs daily	Cross
12	MMA x MMA
20	♠(m) x MMA
800	MMA x ♠(m)
400	MMA x ♠(M)
18,000	♠(m) x ♠(M)

B. Double heterozygotes from autosomal homozygote x M-linked heterozygote at 2000 males per day

Eggs daily	Cross
14	MMA x MMA
1,310	▲ x ▲
510	MMA x ♠(M)
22,222	▲ x ♠(M)

MMA - Multiple Marker Africanized
▲ - Homozygote
♠ - Heterozygote
(m) - m - linked
(M) - M - linked

Mass production II

Paul T. McDonald

Investigations have been made to determine the effect of various changes in operational procedures to cohorts of mosquitoes in order to determine the necessary procedures for maximizing productivity. The major emphasis has been with testing the variables influencing fecundity. In addition trials have been run with alterations of feeding level for larval development, and a first trial run from hatch to adults has been undertaken with pupal separation by the Sharma type separator.

The basic regime for each cohort is a varient of the one in standard use at MBU. Table 1 depicts the initial and improved regimes. For the initial series of trials the multiple marker Africanized stock was selected. This strain is continuously needed for genetic studies and will be a mass produced strain in the field release program.

Cohort characteristics

The number of females per gallon cage has been varied and the effect on fecundity investigated. Results indicate that at least up to 40 females can be kept in a gallon for production (Table 2).

The sex ratio has been tested at two females per male and four females per male in cages with 32 females. Slight advantage of only two females per male was suggested.

Blood feeding

The age of female when first bloodfed has been varied from day 11 to day 14 after hatching, and it has been found that day 11 females are much more productive under the blood feeding regime tested (Table 4).

The frequency of feeding has been varied from two to three bloodmeals over five days, and it has been found that more frequent offering of bloodmeal results in substantial gains in production (Table 5).

Larval development

Eleven groups of 200 larvae were put through the feeding regime selected as the best from earlier trials. The overall percentage of pupation on day 6 at 140 hours after hatching was 93 percent before the first male emerged. Survivorship through development was 88 percent. Of the initial group of first instars, 82 percent were pupae at 140 hours with 42 percent being male and 40 percent female.

Pupal separation

The best Sharma type separator was the grid with spaces of 1.11 mm between strings for passage of males with retention of females. This separator achieved

separation which left a smaller size group containing 89% of the males placed in the separator and of 99% male composition (Table 6).

The total result of the larval development - pupal separator trial was that 38 percent of first instar larvae were passed through the larval development regime and became separated out as males, and this is at 140 hours post hatching.

Table 1 : Regimes of maintaining cohorts for mass production

Time	Starting regime	Improved regime
Day 0	hatch	hatch
1	---	---
2	---	---
3	---	---
4	---	---
5	pick	---
6	pick	pick
7	pick	---
8	mate (20 ♀♀ x 10 ♂♂ /gal.)	mate (40 ♀♀ x 10 ♂♂ / gal.)
9	---	---
10	---	---
11	first blood meal	first bloodmeal
12	---	---
13	---	second bloodmeal
14	---	---
15	second bloodmeal	third bloodmeal
16	---	---
17	first egg collection (C-I)	first egg collection
18	---	---
19	---	---
20	hatch C-I possible	hatch C-I possible
21	second egg collection (C-II)	second egg collection
22	---	---
23	---	---
24	hatch C-II possible	hatch C-II possible

Table 2 : Fecundity of females caged at various densities and subject to same regime of feeding

Females / cage	Eggs / female
10	102
10	58
20	57
20	39
30	60
30	65
40	90
40	89

Table 3 : Sex ratio effect on fertility and fecundity

Cohort composition (female : male)		Eggs / female	Fertility
32 ♀ / 16 ♂	I	60	90 %
	II	90	92
	III	46	60
	Average	65	84
32 ♀ / 8 ♂	I	64	68
	II	43	88
	III	48	84
	Average	52	78

Table 4 : Fecundity of females with varying times of onset of bloodfeeding

Age at first of two bloodmeals	Eggs per female		
	Group I	Group II	Group III
11 days (post hatch)	91	94	103
12 "	35	71	63
13 "	26	87	59
14 "	21	60	59

Table 5 : Fecundity with varying frequencies of bloodfeeding within five days

Bloodfeeding available on days (post hatch)	Eggs per female
12, 16	40
12, 16	21
13, 17	27
13, 17	39
12, 14, 16	58
12, 14, 16	64
13, 15, 17	47
13, 15, 17	44

Table 6 : Pupal separation with grid of 1.11 mm spaces

	No. males	No. females	misfits
Passing through	827	9	1 %
Retained	103	861	11 %

Visit as temporary advisor to WHO-ICMR Unit, New Delhi

Paul T. McDonald

I visited the World Health Organization/Indian Council of Medical Research's Unit on Genetic Control of Mosquitoes from 13-17 August as a temporary advisor and at the invitation of Dr. R. Pal of Vector Biology and Control.

The purpose of the visit was to become acquainted with the research goals, priorities and methodology of this unit, and the exchange of information about MBU projects and similar ones at New Delhi. Another benefit of the visit was to consult with Dr. K. S. Rai who is a principal investigator of the MBU contract and also a consultant with the WHO/ICMR unit, making for a further coordination of genetic control activities with translocations for *Aedes aegypti*.

An initial visit with Dr. G. D. Brooks, the project leader, provided a general orientation toward the scientific endeavors of the Unit, and the appointments for consulting with both Dr. Curtis, geneticist, and Dr. Yasuno, ecologist, were arranged.

Visit to genetics section

Discussions were made with the following scientists:

Dr. C. Crutis, Geneticist
Reverend E. Hallinan, Consultant
Ms. N. Lorrimer, Consultant
Dr. Suguna, Senior Scientist
Dr. Uppal, Scientist
Dr. Kazmy, Scientist

The emphasis of genetic work with *Aedes aegypti* is in the following two areas:

1. Double heterozygote translocations

The preliminary experiments have indicated the best two of four available homozygote translocations for releases. Because the fitness of even the best translocation homozygote in the Delhi background is less than that of the heterozygote of the same translocation, releases of double heterozygote males had been decided upon as the best strategy, and releases began on 12 August in the *Aedes* field site, Mohavir Colony. An extensive program of monitoring for sterility differences, and incorporation of translocations in the population, and adult population estimates, had been outlined and was ready to go into effect. Unfortunately problems of translocation homozygote strain purity and low fitness had developed and mass production had as yet not become operative.

A visit to the field site for releases was made in the company of Fr. Hallinan. The site includes some 165 houses and at the time was characterized by an extremely low density of A. aegypti. Breeding occurred in water storage containers which were kept in courtyards inside the houses. Release points had been determined on the basis of data on dispersal and varying breeding densities.

A social worker, a valuable liaison person, is included with the release team. She explained features of the release program to the residents and acted as interpreter for the scientific staff with casual employees.

2. Meiotic drive coupled with translocations

At the present time a meiotic drive factor to which 80% of the Delhi strain m-linked chromosomes are sensitive has been isolated and steps are underway to link this meiotic drive factor to a multiple translocation.

Discussions with Dr. Curtis touched also on the genetics program with Culex fatigans where experiments had just begun with male releases of cytoplasmically incompatible strains carrying M-linked compound translocations. The advantage of cytoplasmic incompatibility in preventing mixing of genetic material of introduced with resident populations was pointed out. The production of viable fertile offspring between resident and introduced population does present a problem for control by translocation homozygotes.

Visit to ecology section

Discussions were made with the following scientists:

Dr. Yasuno, Ecologist
Dr. Rojagopalan, Senior Scientist

The emphasis in ecological work with Aedes aegypti has been with population dynamics and survivorship of larvae in natural containers. Considerable experience with ecology of Culex fatigans has been gained at the unit and discussions of methodology and results were entered into.

The Culex experiments have stressed use of mark-release-recapture methods for estimating daily loss rate of released males as well as population size estimates. The method of releasing only males and then estimating the change in the sex ratio of the population provides estimate of population size that has corresponded well with the Lincoln Index method.

Studies have indicated the importance of estimating seasonal changes in such factors as longevity and fecundity. The unit has also placed emphasis on studies of migration and the procedures and results were discussed.

A trip to the field sites for the Culex releases of cytoplasmically incompatible strains was made in the company of Drs. Yasuno and Rojagopalan and the ecological monitoring of the experiment in progress was seen at first hand. One feature of releases made by the Delhi unit was the marking (fluorescent dusting) of all release mosquitoes and determination of ratios

of marked mosquitoes in the field subsequent to release.

Visit to mass production section

Discussions were held with Dr. K. R. P. Singh.

Until recently facilities were available for the mass production of Culex fatigans and the first production with Aedes aegypti, at a reduced scale, had just begun; the routine had not yet become established although several procedures had been selected for production. An extensive tour of the Culex mass production facility provided opportunity to see and discuss several techniques which the Delhi unit has developed. The quality control of all released mosquitoes is made to ensure that mosquitoes of known and uniform standard are released.

Seminar

I delivered an illustrated seminar on the Progress of Ecological Studies Preliminary to Genetic Control of Aedes aegypti on 17th August at the WHO/ICMR Unit with the project leader, consultants, and scientists in attendance.

Other business

1. Dr. V. P. Sharma has volunteered to provide MBU with three pupal separators of his design for use in our facility at Mombasa.

2. Arrangements to receive monthly reports from the WHO/ICMR Unit have been made.

3. Invitations have been extended to Dr. C. F. Curtis and Dr. Yasuno to visit MBU. Rev. Hallinana and Ms. Lorrimer will likely visit the unit with Rev. Hallinan's visit in either October or November.

Summary

The visit to the WHO/ICMR Unit on the Genetic Control of Mosquitoes provided a stimulating and revitalizing experience. Everywhere I was met with an enthusiastic and cooperative reception. The visit provided opportunities for valuable exchange of information and experiences. As a result of the visit tighter linkages between MBU and the Delhi unit were established and some much appreciated assistance with experimental design and methodology was given at a critical time in MBU's development.

Population dynamics of *Aedes aegypti* in East Africa in view of genetic control

Paper given by W. Hausermann at the 9th International Congress on Tropical Medicine and Malaria in Athens, on October 15, 1973

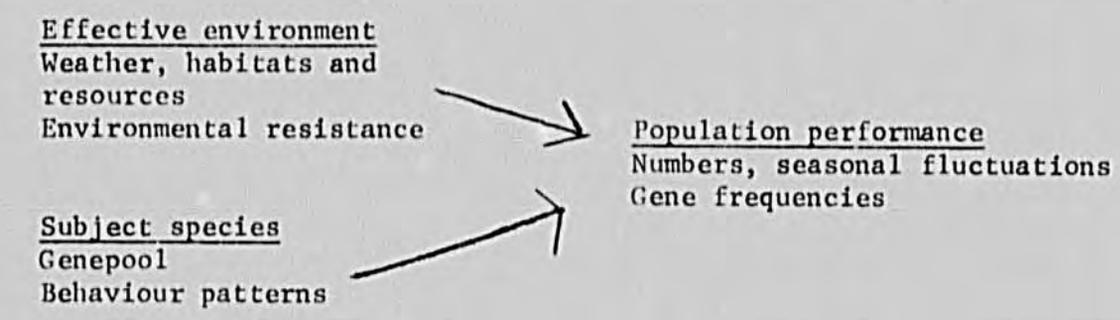
1. Introduction

Genetic control techniques must be developed specifically for the target populations in their particular environments. Detailed information on ecology, behaviour and genetics of the population is a must.

In preparation of control of *A. aegypti* by means of chromosome translocations such studies were carried out by the Mosquito Biology Unit of ICIPE in Nairobi, Kenya. After preliminary surveys in East Africa the Rabai Location near Mombasa was selected as study area and work started in late 1971.

2. Life system of A. aegypti

The population studies were based on the following concept of the *A. aegypti* life system:



Note: Conventional control and most other control methods increase environmental resistance. Genetic control renders subject species less fit.

3. Effective environment

3.1. Weather

The climate of the Kenya Coast is characterized by three seasons (Fig. 1):

A. The hot dry season with mean max. temperatures around 33° C.
B. The wet season with frequent heavy rains and temperatures dropping by about 5°-6° C.
C. The intermediate season with intermittent rains and temperatures rising again.

3.2. The habitats

They can be grouped into feral and peridomestic habitats in which breeding is determined by rainfall and domestic habitats in which the water is supplied by man (Table 1). In the Rabai area the feral habitat consists of coconut plantations set in a climax vegetation of wooded grassland. Breeding occurs mostly in treeholes and steps of coconut trees. In these coconut plantations are numerous more or less isolated villages of 5 to 50 houses. There is virtually no peridomestic habitat as man made containers like tires, drums etc. are scarce. The domestic habitat permitting independence of rainfall is limited almost totally to the inside of houses. Breeding occurs in domestic water containers (5 colour slides).

4. Subject species

Based on colouration and behaviour A. aegypti is after Mattingly divided into three forms:

a. black wild to semidomestic ssp. formosus
b. brown to black semiwild to domestic type form
c. pale, fully domestic var. queenslandensis

In our study area the whole range of colour variations is observed, however, on the basis of their distribution and behaviour only two types can be readily distinguished: ssp. formosus and type form. Our distinction of the two forms rests on two colour characters. With ssp. formosus black scales are totally black and white scales are pure white. Any A. aegypti in which the scales are not totally black or entirely pure white is considered type form. Our definition of ssp. formosus includes also specimens with white scales on the first abdominal tergite, which by convention would be type form A. aegypti. In addition ssp. formosus has generally a black cuticle, while in A. aegypti type form the cuticle is often brown, yellowish brown or yellowish grey. Contrary to ssp. formosus A. aegypti type form exhibits great variability in colouration. E.G. the mesonotal scales may vary from black over brown to pale gold or the abdomen from total black without white marks or silver spots to almost white or cream with no black or brown scales (3 colour slides).

The frequencies for some of these most conspicious colour variations indicate that in Rabai, there is very little genetic exchange between populations of these two forms (Table 2).

The separation between the two forms made arbitrarily on a colour-basis is correlated with behavioural traits which keep their populations largely apart.

a. ssp. formosus is strictly exophilic and type form mostly endophilic. This behaviour can be tested in field releases and in a field-hut simulator. The results are repeatable with subsequent generations of laboratory colonies of ssp. formosus and type form and with strains from different locations on the coast (Table 3).

b. The same difference is demonstrated in site preference for oviposition. ssp. formosus prefers treeholes and small oviposition traps made of tins, type form prefers larger containers like earthenware jars and rarely uses small oviposition traps or treeholes (Table 4).

5. Population performance

During 1972 populations of the two forms were monitored weekly with landing-biting catches, oviposition traps and larval surveys. The rainfall pattern followed not too closely the general pattern of the area. The wet season ended rather abruptly with no rain in June (Fig.2).

The feral and peridomestic ssp. formosus population showed a dramatic increase in oviposition rate and breeding level, following the onset of the rains. It decreased, however, rapidly again in July after a dry June. The population did not recover when the rains returned (Fig. 3).

The domestic type form populations were stable through most of the year. However, modest increase in the landing-biting rate occurred during the rains. This increase coincided with a slight decrease in the Breteau index. It was only during this period that outdoor breeding of type form was observed in natural habitats (Fig. 4).

This was also the only period when both forms could be caught on man outside houses.

In 1973 both populations showed so far the same fluctuation pattern again although the rains continued strongly through June and July. In both years in the month following the rain an increase in frequency of white scales on the first abdominal tergite was observed in our ssp. formosus population. Also it was in this period that the few ssp. formosus individuals with alleles for spot and gold mesonotum were found. We therefore suspect that geneflow between these two populations is established only during a few rainy months.

From mark-release-recapture experiments we know that the domestic type form populations in the Rabai villages are fairly small and in many cases fairly isolated (Table 5). The population studies have therefore concentrated mostly on these village populations. They offer a singular opportunity to test chromosome translocations, for population replacement and suppression.

Our hopes for successful releases, however, encountered a major obstacle. We have so far not obtained a homozygous translocation, which is sufficiently fit to replace a native population. Although we still hope for such a translocation homozygote our efforts concentrate now towards production of a double translocation heterozygote for population suppression.

Table 1 : *Aedes aegypti* habitats on the Kenya Coast

Habitats	Resources		Key influences
	Hosts	Larval habitats	
Feral Woodland Plantations	Animals rarely man	Natural	Rain Evaporation
Peridomestic Gardens Dumps	Man and Animals	Natural and man-made	Rain Evaporation
Domestic Yards Houses	Man rarely animals	Domestic water	Water storage and consumption

Table 2 : Frequencies of major colour traits in *A. aegypti* populations of the Kenya Coast

Trait	*A. aegypti* type form		*A. aegypti* ssp. formosus	
	Domestic	Peridomestic	Peridomestic	Sylvan
Cuticle black	0	0	0.914	1.000
Abdominal scales :				
- deep black	0.423	0.438	1.000*	1.000*
- pure white	0.245	0.256	1.000*	1.000*
Mesonotal scales :				
- deep black	0.581	0.406	0.998	1.000
- all golden	0.026	0.053	0.002	0
Abdominal patterns :				
- Tergite I all black	0.008	0.034	0.804	0.671
- Tergites II - VII no extension or reduction of white scales or silver spots	0.710	0.633	0.003	0
No. ♀♀ examined	439	207	620	112

* per definitionem

Table 3 : Endophily in *A. aegypti* populations from the Kenya Coast

	Type	Origin	% Recaptures indoors	
			Total	♀♀
A.	ssp. *formosus*	peridomestic	0.3	0.1
	type form	domestic	23.9	22.0
	hybrid		3.4	5.8
B.	ssp. *formosus*	peridomestic	9.0	10.0
	type form	domestic	50.0	77.0
	type form	peridomestic	57.0	68.0

Table 4 : Site preference for oviposition in *A. aegypti* populations from the Kenya Coast

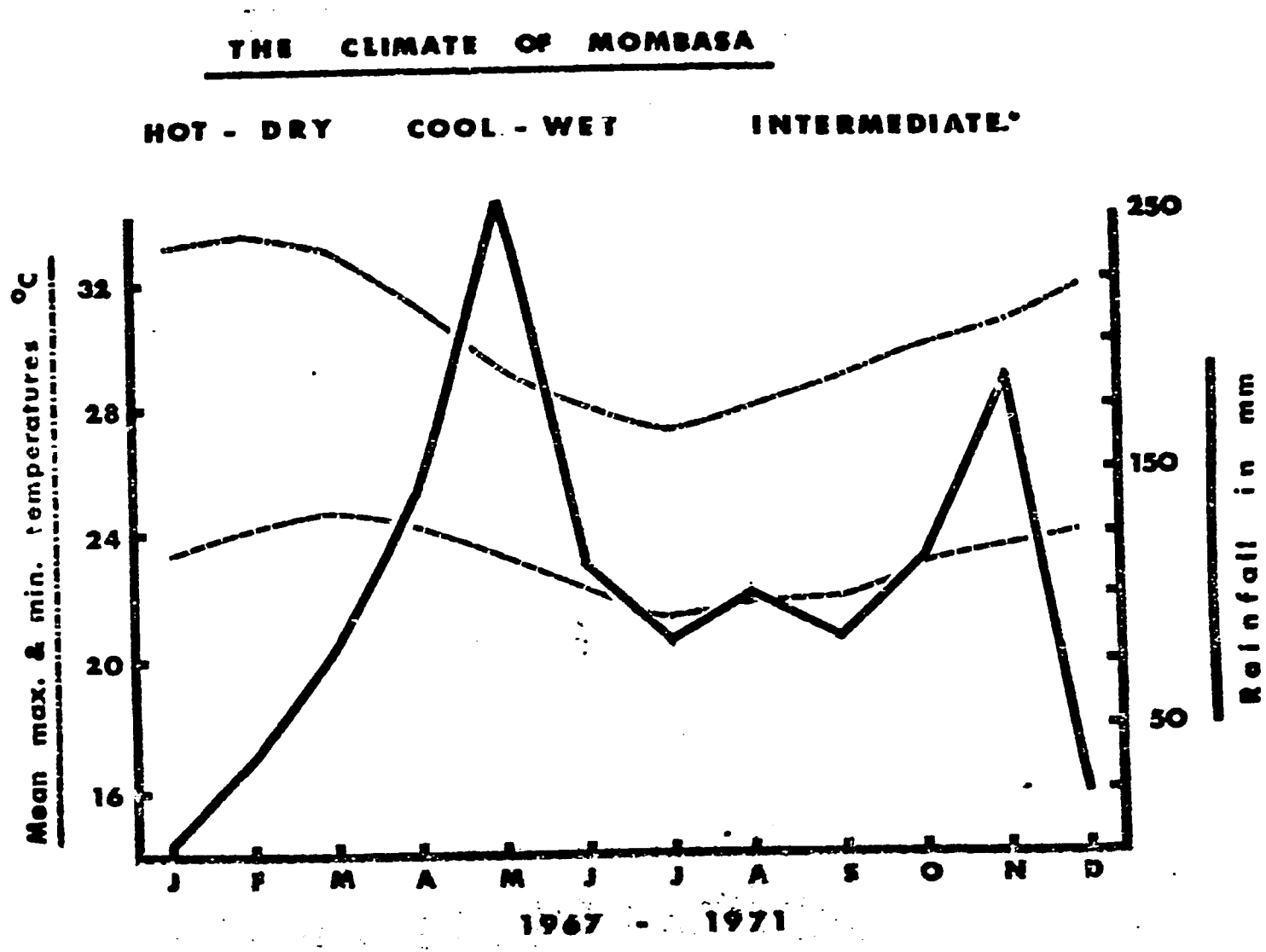
Site	*A. aegypti*	
	% type form	% ssp. *formosus*
Treeholes	22.7	77.3
Oviposition tins (0.3 l)	2.6	97.4
Oviposition jars (5 l)	67.5	32.5

Table 5 : Description of a domestic *A. aegypti* population in a Rabai village (16 houses, 21 water containers, 56 inhabitants)

A. Population size	$\bar{x}$	range
- Daily pupal output (16 houses, 21 water containers)	65.5	31 - 89
- Daily landing biting rate	86.6	46 - 125
- Absolute numbers of adults (Estimates after Jolly)	238.7	184 - 291

B. Behavioural aspects	$\bar{x}$	range
Contact with man (♀) :		
- No. contacts observed	4.30	1 - 11
- No. days between contacts	3.99	1 - 23
Movement within village (♀) :		
- No. house changes between subsequent captures	0.458	
Dispersal :		
- Max. distances observed : downwind		1,000 m
tangential to wind		500 m
against wind		150 m

C. Life table (dry season)	$\bar{x}$
- Developement time from egg batch to pupa	15.5 days (range 6-32 ")
- Survival from 1st instar to pupa	0.1897
- Survival pupa - adult	0.91
- Female ratio at emergence	0.42
- Prereproductive period of ♀ = days to 50 % insemination	2.85
- 24 h survivorship of reproductive ♀	0.89
- Life expectancy of reproductive ♀	8.07
- No. eggs produced per reproductive ♀	25.72


THE CLIMATE OF MOMBASA
HOT - DRY
COOL - WET
INTERMEDIATE
Mean max. & min. temperatures °C
32
28
24
20
16
250
150
50
Rainfall in mm
J
F
M
A
M
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S
O
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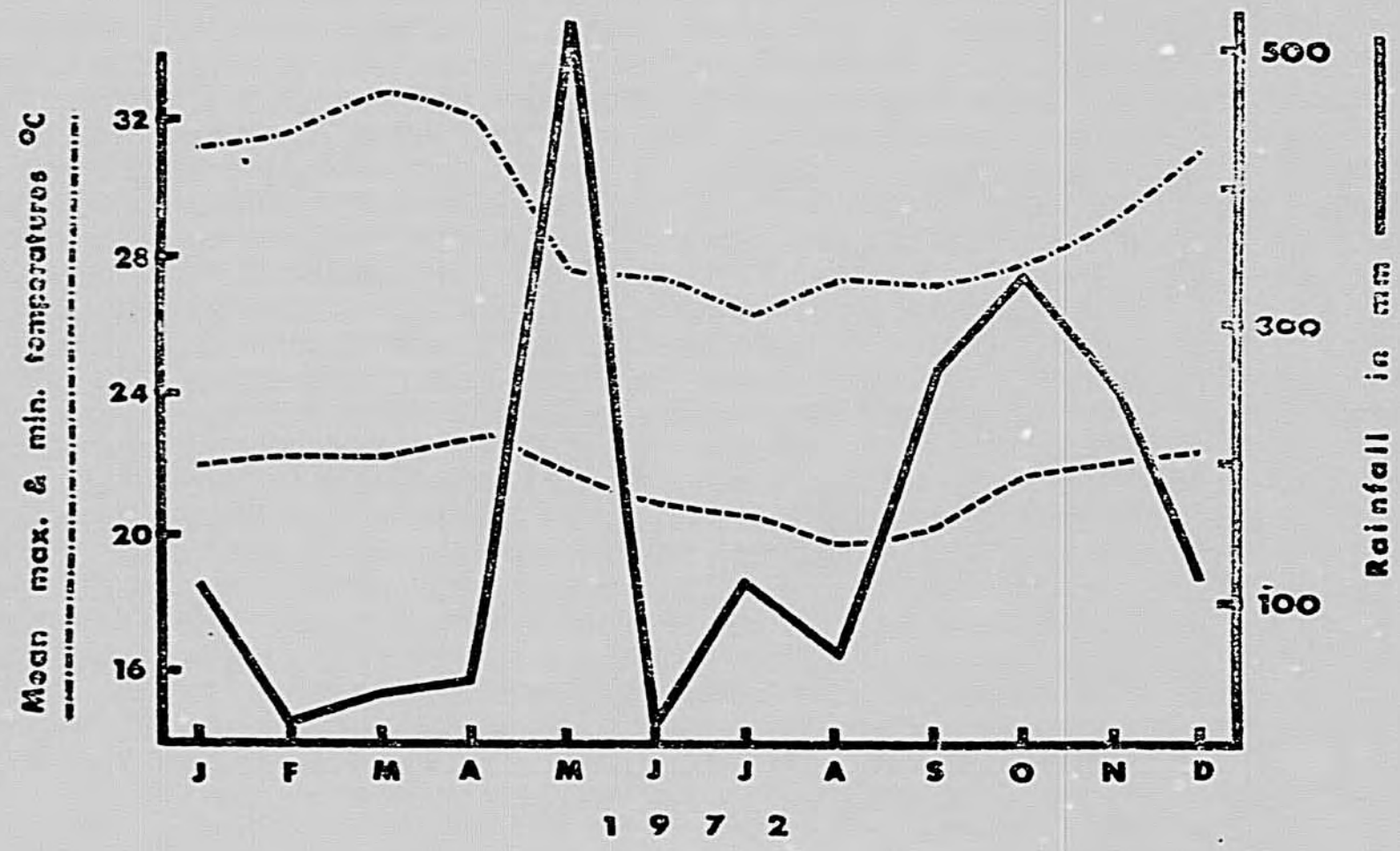
1967 - 1971

Figure 2

RAINFALL AND TEMPERATURES IN RABAI



SEASONAL FLUCTUATIONS IN FERAL HABITATS

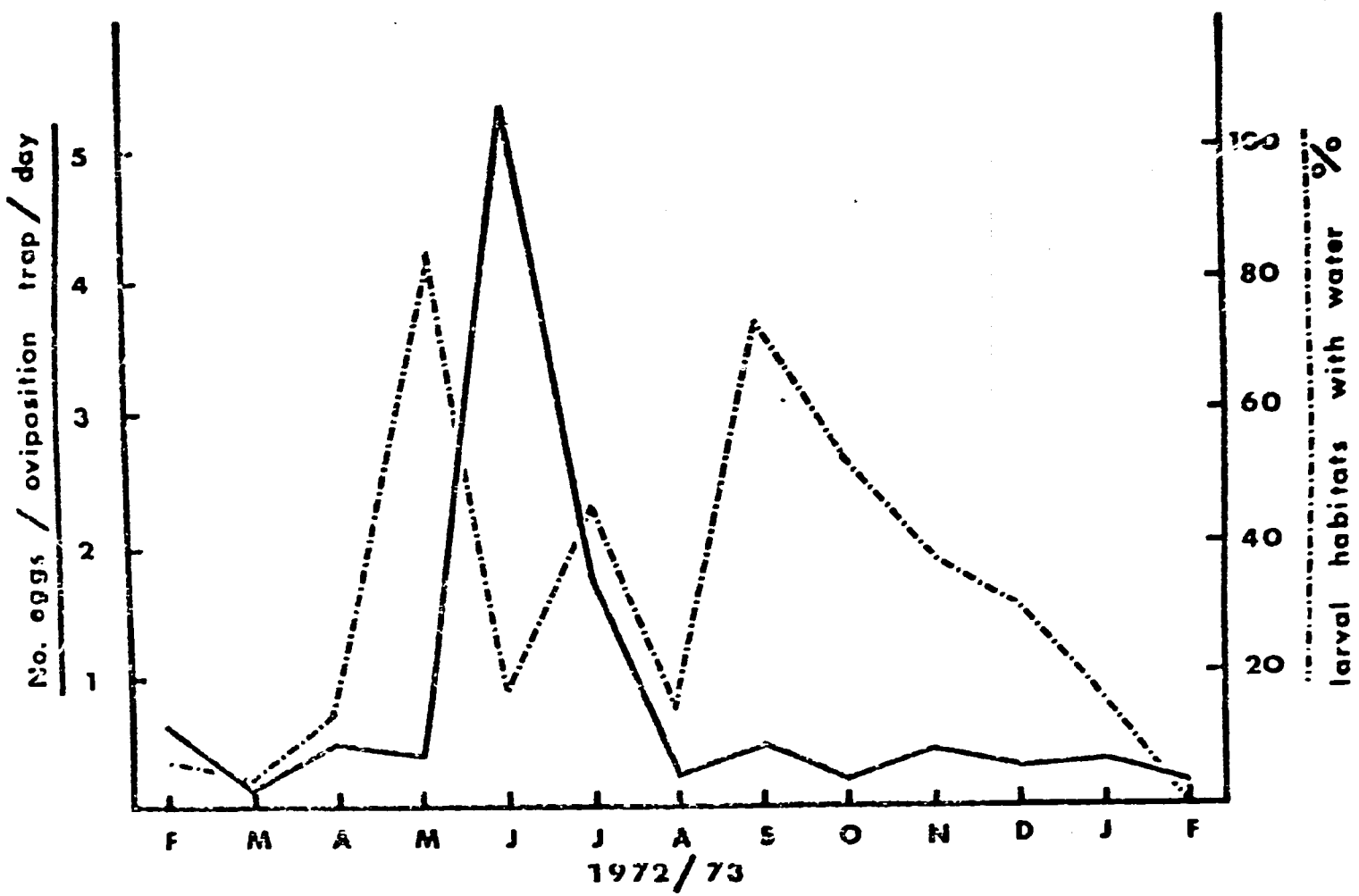
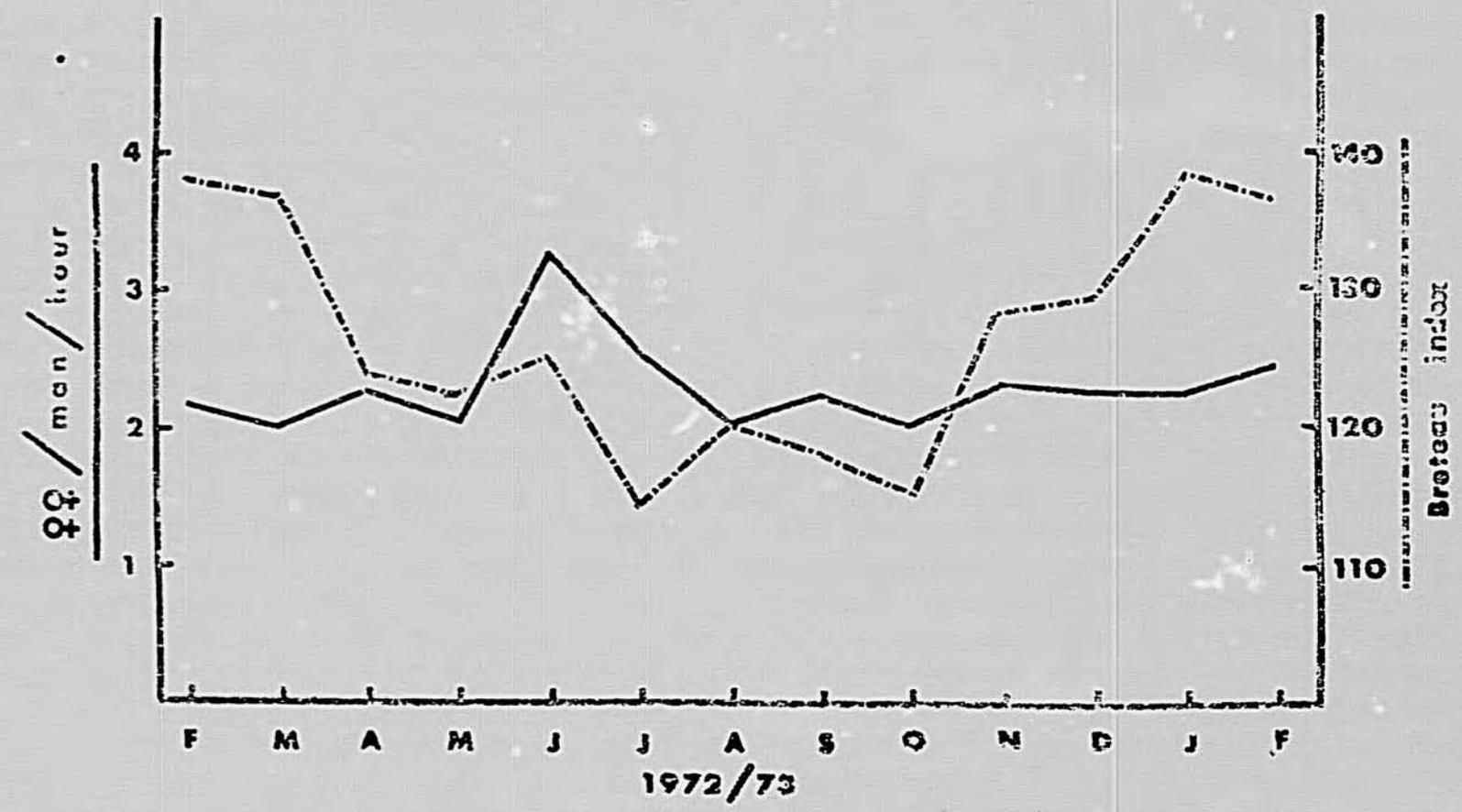


Figure 4

SEASONAL FLUCTUATIONS IN DOMESTIC HABITATS



Comments to WHO computer survey of Stegomyia mosquitoes

Submitted to Dr. Arata, Ecologist of VBC / WHO by W. Hausermann

1. House index, container index and Breteau index are a simple way to express A. aegypti densities at the time of the survey. They do, however, not consider sufficiently the biology and ecology of the surveyed populations and may thus misrepresent the true situation.

   McClelland (WHO/VBC/71.271) has pointed out the need for a better understanding of the ecology of the species. He recommended that domestic and feral populations be distinguished by their independence respectively their dependence on the natural rainfall cycle. This recommendation deserves full attention.

   In East Africa A. aegypti populations from different areas differ considerably in ecological and behavioural aspects. They can be grouped in four distinct categories (Table 1).

   It is easy to see that these populations differ in their vector potential. However, the computer printouts based on Breteau index and related indices cannot adequately consider these aspects.

2. Going through the computer printouts one encounters many maps with P indicating the presence of the species. No density index is given because of insufficient survey data. This must not always be the fault of the surveyor. In East Africa it is fairly easy at any time of the year to establish the presence or absence of A. aegypti. Often it is, however, impossible to obtain a meaningful density index. If some additional ecological information could be entered into the computer program and expressed in the printouts, the P's could become much more meaningful.

3. The computer printouts for the distribution and density of A. aegypti reflect of necessity first the distribution and levels of survey activity. Again more consideration for some selected ecological aspects in the surveyed areas and correlation with climatic, ethnological aspects in the surveyed areas and correlation with climatic, ethnological, agricultural, industrial or sanitary conditions known from hitherto unsurveyed areas could improve the distribution maps by means of extrapolation. Likewise correlations of such background informations over time and from different areas could permit to predict future trends of A. aegypti populations with urban or rural development.

   Informations which seem to me of particular importance when you try to understand the distribution and vector potential of A. aegypti populations are: Rainfall pattern, vegetation cover, range of potential hosts, density of human population, availability of man-made larval habitats, man's habits of water storage, behavioural characteristics of the A. aegypti population such as host preference and the choice of hunting grounds, and lastly, a listing of other potential vectors of

A. aegypti transmitted viruses. If information of this kind could be worked into the computer program, the worldwide survey would come closer to its goal, which I presume is to recognize the danger spots and future developments.

The information could be obtained in different ways. For many areas some information is available from published national surveys. Additional information could be obtained by means of precise questionnaires which are simple to answer. Such questionnaires could be sent out to the field staff of cooperating ministries of health, agriculture or eduction.

Table 1: Major characteristics of *A. aegypti* populations observed in East Africa

Habitat selection	Host preference	Larval habitat	Key factors	Population fluctuations	Areas of occurrence (examples)
feral	zoophilic	1.natural 2.man-made	rain evaporation	seasonal peak(s) and lows	West Nile Bwamba
feral	anthropo-philic	1.man-made 2.natural	rain evaporation	seasonal peak(s) and lows	Mombasa Dar es Salaam Ulanga District Tana River
ambivalent feral and domestic	anthropo-philic	1.domestic water containers 2.man-made 3.natural	domestic water consuption and storage, rain and evaporation	almost stable through year with minor peaks	Kenya Coast parts of Tanzania Coast Southern Tanzania
domestic	anthropo-philic	domestic water containers	domestic water consuption and storage	stable through year	Dry belt parallel to Kenya Coast