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3. AUTHOR(S)

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9. ABSTRACT

The long-term goal of this project is to discover whether genetic control of Aedes aegypti is feasible under East African conditions. This Yellow Fever Mosquito is one of the half-dozen most important insects affecting mankind. It has spread to almost all tropical and subtropical areas of the world, and it transmits many viruses, including yellow fever, dengue, Asian hemorrhagic fever, Chikungunya, and quite possibly elephantiasis. The mosquito is widespread and abundant in East Africa. It is difficult and expensive to control chemically. Thus genetic means are being sought. Chromosome translocations, sex ratio distortion, genes for sterility, and other genetic devices have shown marked promise in the laboratory, but field research and trials are required to allow proper evaluation of these newer methods. Work conducted during the reporting period in East Africa was focused in several small villages in the Rabai area, west of Mombasa. The villages are satisfactory for experiments in genetic control. These villages have 10-25 buildings and a population of about 1,000 adult Aedes aegypti, with a natality rate of 500-100 per day. The selected villages are sufficiently distant from one another that each population can be considered a separate "ecological island." The mixture between the domestic, container-breeding, indoor form and the sylvan, tree-hole-breeding, feral form is probably not a problem, and certainly not during the dry season. Facilities and plans have been developed for mass production and release of mosquitoes bearing chromosome translocations. Genetic control experiments over the next two years of the project will involve translocation homozygotes in an attempt to replace the indigenous mosquitoes with a population of a genetic structure designed in the laboratory. This concept (the Serebrowski hypothesis) has yet to be field demonstrated. If replacement is feasible, years five and six of the project will be used to introduce conditional lethals that act seasonally; otherwise those years will be used for releases of multiple translocation heterozygotes conferring sterility.

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ECOLOGICAL STUDIES ON AEDES AEGYPTI IN EAST AFRICA

Annual Report July 1972 - June 1973

Department of Biology

University of Notre Dame

Notre Dame, Indiana 46556

9. Narrative Summary - Accomplishments:

The first half of year 2 was covered by an earlier report. In the second half, we have largely completed Phase I, background ecological studies. We have determined that the small villages in the Rabai area, west of Mombasa, are satisfactory for genetic control experimentation. These villages generally have 10-25 buildings and a population of about 1000 (± 500) adult *Aedes aegypti*, with a natality rate of 50-100 per day. Distance between selected villages is adequate so that each population can be considered to be on a separate "ecological island." Mixture between the domestic, container-breeding, indoor form and the sylvan, tree-hole-breeding, feral form is probably not a problem; this is certainly true in the dry season. Facilities and plans have been developed for mass production and release of mosquitoes bearing chromosome translocations. Genetic control experiments over the next two years (years 3 & 4 of the project) will involve translocation homozygotes in an attempt to replace the indigenous mosquitoes with a population of a genetic structure designed in the laboratory. This concept (the Serebrowski hypothesis) has attracted great interest but has yet to be demonstrated in the field. If replacement is feasible, years 5 and 6 will be used to introduce conditional lethals that act seasonally; otherwise, these years will be used for releases of multiple translocation heterozygotes conferring sterility.

ANNUAL RESEARCH REPORT

A. General Background

The Yellow Fever Mosquito, Aedes aegypti, is one of the half dozen most important insects affecting mankind. Although the species originated in Africa, it has spread with man (via shipping) to almost all of the tropical and subtropical world; it transmits many viruses, including yellow fever, dengue, Asian hemorrhagic fever, Chikungunya, etc. As demonstrated by the present contract, it may also play a significant role in the epidemiology of filariasis (elephantiasis).

In East Africa, Aedes aegypti is widespread and abundant; at present, even minimal control is not feasible in most areas. To compound the problem, there is evidence of two populations within the species. One occurs in villages, breeding in domestic water containers and throw-away containers such as tin cans and rubber tires. The other is feral, breeding in tree-holes and sylvan situations that are far from human habitation. The relative importance of these two forms to disease transmition and control and the genetic relationships between them have never been established.

Control of Aedes aegypti is expensive and technically difficult. Larvae breed in containers that are inaccessible to conventional mosquito control techniques such as drainage and larviciding. Human activities such as urbanization, water storage and poor sanitation have increased the threat of this mosquito. Vast eradication campaigns directed against this species have failed dismally, in large part because control weapons are inadequate.

In recent years, entomologists have been searching for alternative, non-chemical control methods which might avoid the twin hazards of insecticide resistance and environmental pollution. Among the most promising biological approaches is genetic control, which involves turning a species against itself. While the best-known method is the "sterile-male technique," where released radiation-sterilized males compete for field females, there are many other methods of genetic manipulation which could achieve a similar end. For Aedes aegypti, chromosome translocations, sex ratio distortion, genes for sterility and other genetic devices have shown marked promise in the laboratory. However, field research and trials are required to allow proper evaluation of these newer methods.

B. Project Objectives

The long-term goal of this project is to discover whether genetic control of Aedes aegypti is feasible under East African conditions. As originally proposed to AID in 1970, this goal would require five years of research and a total budget of about $1,000,000. Subsequently, the period was extended and the level of effort per year reduced. The first three years of the contract were allocated to background biological research, a fact reflected in the title, "Ecological Studies...Preliminary to Genetic Control." Specific research objectives for the first three years are as follows:

1. Develop a study area where the population of A. aegypti is thoroughly and intensively known.
   a. Discover the absolute number of mosquitoes in the area and determine population fluctuation on a daily, seasonal and annual basis.
   b. Develop predictive life tables on a specific population, using data on fecundity, mortality, growth rate and ecological parameters.
2. Establish 10-20 replicate villages (ecological islands) which can be used for later releases. Monitor each population for at least one year.
3. Determine the genetic and ecological relationships between indoor-breeding and feral populations of A. aegypti.
4. Develop chromosome translocations and other genetic mechanisms potentially useful for genetic control.
5 Develop facilities for mass-production of mosquitoes.
6. Make preliminary field releases of laboratory-reared mosquitoes to develop technical methods.
7. Conduct subsidiary bionomic research on related mosquito disease vectors.

In addition to scientific objectives as listed above, other objectives of the contract include:

1. Develop a major research component, the Mosquito Biology Unit (MBU), of the International Centre for Insect Physiology and Ecology (ICIPE).
2. Provide training opportunities for African scientists and technicians.
3. Serve as a mechanism for strengthening the East African Community by affiliation with both research institutes and universities.

C. Continued Relevance of Objectives

The objectives of this project, as stated in the Project Statement of March, 1971, remain unchanged. After two years of operation of this Contract, it is evident that the original objectives were reasonable and are attainable in the three-year period. As recognized at the initiation of this Contract, the funding and the 3-year period are not adequate to develop a final answer to the question, "Is genetic control of A. aegypti feasible?" However, we believe we have made marked progress in developing a baseline for a sound approach to this question.

We now request extension of the Contract for an additional 3 years, from July of 1974 through June of 1977. This extension would allow us to carry out a field program to test the genetic control concept. It should be recognized that the background ecological work done to date was preparatory; this ecological portion, designated Phase I, will soon be completed. We are now developing plans (see ANNEX 18) for Phase II, a 2-year program involving the use of released chromosome translocation homozygotes to replace a natural population. Provided the Contract is renewed, this Phase would occupy years 3 and 4 of the Contract. The final period, years 5 and 6, would be devoted to population suppression by released translocations.

D. Accomplishments to Date

Description of Facilities

The laboratory facilities for the Mosquito Biology Unit remain unchanged since the previous report. The laboratory is in a rented 14-room house on the shore at Nyali, just north of Mombasa. This building has insectaries, general laboratory room, library, radiation source room, store and animal rooms, mass rearing room and both living and laboratory space for visiting scientists. The MBU has been designated as the ICIPE Coastal Research Centre; numerous research workers from Nairobi and elsewhere have been provided with temporary facilities.

The field site was fully described, illustrated and mapped in previous reports to AID. It is located in the Rabai district west of Mombasa, a 20-minute drive from the laboratory. It consists of many small villages in the coconut palm belt.

The staff of MBU, listed in the Annual Report for Year 1, remains largely unchanged. It consists of:

3 Research Scientists (1 is Field Director)
1 Bookkeeper-Administrative Aide (part-time)
4 Laboratory Technicians
2 Carpenter-Maintenance Men
4 Field Assistants
Various casual helpers for field work, hired on a daily basis.

Research Findings

1. Nature of the mosquito.

At the Rabai study site, which is representative for a large part of the Kenya Coast, A. aegypti exploits two fundamentally different habitats. The peridomestic and sylvan habitat where the mosquito is dependent on rainfall and the domestic habitat where it is independent of rainfall thanks to man's habits of water storage. The two habitats are occupied by two genetically different forms (Annex 1).

A. aegypti type form occurs regularly in domestic habitats, occasionally in peridomestic and very rarely in sylvan habitats. The sylvan form, subspecies formosus is found in woodland and peridomestic, but never in domestic habitats.

Probably due to a scarcity of large larval habitats like tires, rainwater drums, and other such containers, A. aegypti type form is considerably less successful than ssp. formosus in the peridomestic environment. Most of the larval habitats are steps in coconut trees and treeholes which dry out fast and put the type form with its slower development (Annex 2; Machado-Allison, 1971) at some disadvantage except during the peak rainy season.

Although the two forms mingle during the rainy season in peridomestic habitats the separation is maintained by behavioural differences such as host preference (Annex 3, Trpis; Annex 4, Fanara), preference for oviposition site (Annexes 5 and 6), preference for hunting site (Annex 7) and infraspecific mating preference (Hartberg and Craig, 1968; Nijhout and Craig, 1971). Hybrids do occur, however, and the adults can be recognized by the brownish cuticle of the postnotum and bare prescutellar spot and do thus not fit our definition for A. aegypti ssp. formosus. They seem to occur only during the rains and mostly in peridomestic habitats.

2. Population dynamics

The two forms differ clearly in their population dynamics (Annex 8).

The population density of ssp. formosus is low through most of the year but shows a steep increase of about 10 fold, three to six weeks after the first heavy rains. Thereafter the population decreases again even if the rains continue.

All attempts at trapping adult A. aegypti ssp. formosus in peridomestic and sylvan habitats in sufficient numbers for mark-release-recapture experiments have failed so far. No population estimates, or estimates of life expectancy or dispersal have therefore been made.

The population density of domestic A. aegypti type form is almost stable throughout the year if the Rabai area is considered as a whole. On the village and house level, however, the populations fluctuate with time mainly in response to domestic water consumption and the sporadic cleaning of water containers. Basic differences in population density also exist between villages and seem to be caused by standard of living, proximity and quality of water sources and village size. The only significant increase and decrease in domestic A. aegypti - about 2 fold - coincides with the main rainy season and the occurrence of type form in peridomestic habitats (Annex 8).

In an average Rabai village of 15-25 houses the total number of A. aegypti type form may at any given day range between less than 100 to over 500 individuals of which 20-40% may be males. The daily emergence rate in a village may vary between 10 to 50% of the adult population (Annexes 9 and 10).

Mark-release-recapture experiments with individually marked, field caught mosquitoes established a life expectancy of 4.75 days for marked females and 1.13 days for marked males. If the marked individuals survived the first 24 hours after marking, life expectancy increased to 8.08 days for females and 2.17 days for males. Marking seemed thus to increase mortality at least for the first day after marking. Alternative marking methods were explored but proved to be less efficient (Annex 11). It is assumed that the life expectancy of newly emerged, unmarked females is somewhat higher than 8 days. The longest life span observed for individuals was 43 days for females and 12 days for males (First Annual Report, Annex 7).

3. Dispersal

Domestic A. aegypti type form moves regularly between houses of a vil-

lage and frequently also between villages. Dispersal distances of up to 500 m tangential to, up to 1,000 m within and up to 150 m against the predominant wind direction have been observed. The proportions of immigrants and emigrants vary with the villages and with time between more than 50% and less than 10% of the resident population. Dispersal seems not to be restricted to just one period of adult life (Annex 12). Mating in particular which occurs in the first 48 to 120 hours of adult life, does not affect dispersal (Annex 13).

The dispersal between houses seems to be selective insofar as mosquito density is higher in some houses than in others. The adults seem to spend more time in attactive houses, which usually also show an increased output in pupae (First Annual Report, Annex 7; Annex 7; Sheppard et al. 1969).

4. Défaunation

In view of the low numbers of adults present in a village it was assumed that domestic populations are very vulnerable. It was therefore attempted to eradicate a domestic population from a medium size village by systematic cleaning of water pots together with indoor spraying with pyrethrum against the adults. While the adult population dropped immediately in response to the spraying and the removal of larvae and pupae, it proved to be extremely difficult to get rid of the large store of eggs (Annex 14). After the control measures were stopped the population returned to precontrol level within five weeks.

5. Translocations

At present there are 46 translocations maintained at MBU. Thirty-seven were induced in indoor strains, seven in outdoor strains, and two in a peridomestic strain. Of the 46 translocations, 19 involve linkage groups one and two, 17 involve linkage groups two and three, and 10 involve linkage groups one and three (Annex 15).

Of the 46 translocations, 39 have been tested for homozygosis. Of these only one has given homozygotes in the genetic tests. Further testing will be conducted to verify homozygosity for the translocations, before the strain can be used for releases.

Until recently all mosquitoes irradiated for isolation of translocations have been from strains of wild populations and have given a very low return for homozygotes. Recently a highly-inbred strain has been irradiated and this strain may be more easily capable of furnishing homozygotes, as it is already conditioned to a high degree of isogenicity and presumably the recessive lethal mutants have been selected out. It may be the presence of recessive lethals in translocation heterozygotes that determines the inviability of translocation homozygotes.

In view of the time schedule for releases homozygous translocation strains have been obtained from the University of Notre Dame and the incorporation of these translocations into local strains has started (Annex 16).

Operational significance of findings

Although in the Rabai villages the domestic populations of A. aegypti are neither isolated from each other nor from peridomestic ssp. formosus, experiments at displacement through genetically altered stock seems nevertheless promising for the following reasons:

1. The domestic populations of single villages are in general fairly small, less than 1,000 even in large villages. Natality rates are usually less than 100 per day.

2. Provided a vigorous translocation stock with the right behaviour pattern can be produced, even modest daily releases should thus suffice to affect the indigenous population of a village.

3. A large number of similar villages is available. Various release strategies and translocations can be tested simultaneously and in replicates.

4. Since the village populations respond rapidly to house spraying with pyrethrum or the cleaning of the household water containers, releases can as an alternative be integrated with conventional control methods to assure the introduction of translocations. Translocation populations thus established can be monitored over time and the effects of interbreeding with wild type immigrants assessed.

In summary, the scarcity of large peridomestic larval habitats and a short rainy season prevent A. aegypti type form to establish a strong continuous peridomestic population. However, the water storage habits of the Rabai people offer suitable larval habitats inside houses and this leads to small, vulnerable, more or less separate village populations of this species. These populations constitute ideal experimental units to test the idea of genetic control propagated first by Serebrowsky (1940) and later by Curtis (1968).

Considering the time schedule the first releases will have to be made with translocation homozygotes obtained from the Vector Biology Laboratory of the University of Notre Dame. These homozygotes will be outcrossed to local A. aegypti type form in July and August. Behavioural field tests will be carried out in September. The first large scale field release is planned for late December (Annex 16).

E. Dissemination of Research Results

1. Several manuscripts have been completed and submitted for publication. There should be at least 6 papers in print from this project in the next year.

2. Excellent relationships continue with the Division of Vector-Borne Diseases, Kenya Medical Department. See Annex 14 for an account of six months of training at MBU by Mr. Philip Bebora, Senior Entomological Field Assistant of DVBD.

3. Cooperative research on A. aegypti as a vector of filariasis continues with Dr. Wenceslaus Kilama, Senior Lecturer, University of Dar-es-Salaam, Tanzania. Evidence continues to accumulate that certain East African populations of this mosquito are highly susceptible to Wuchereria bancrofti and may play a role in its epidemiology.

4. Dr. Hausermann is serving as thesis advisor to Mr. S. K. Sempala of the East African Virus Research Institute, Entebbe, Uganda. Mr. Sempala is doing a Ph.D. thesis on bionomics of Aedes (Stegomyia) africanus.

5. MBU continues to serve as a major component of ICIPE, with Research Fellows from Nairobi making extensive use of our laboratory and field facilities for diverse entomological research. The following ICIPE-related visitors were recorded during January to June, 1973:

   Prof. Thomas Odhiambo, Nairobi U., ICIPE Scientific Director
   Prof. Jan DeWilde, Waginenen U., Netherlands
   Prof. Koji Nakanishi, Columbia U., U.S.A.
   Prof. Detrich Schneider, Max Planck Inst., Munich, Germany
   Mr. Joel Ojal, ICIPE Business Manager
   Dr. G. Wagner, ICIPE Immunochemist
   Drs. D. Elder, T. Gebrejesus, P. Scheltes, ICIPE Research Fellows

   MBU visitors from Notre Dame during this period include Professors G. Craig, T. Crovello and M. Trpis. In addition, a Trainee in Mosquito Biology at Notre Dame, graduate student Raymond Russo, spent six weeks at MBU in June-July. His report on population dynamics of larvae will be included in the next report.

6. Other visitors to MBU in Jan.-June 1973 include:

   Professor Ruben Olembo, Chairman, Botany Dept., Univ. Nairobi (interested in placing a graduate student in genetics at MBU)
   Prof. Paul Weinstein, Chairman, Biology Dept., U. Notre Dame
   Dr. John Roberts, Head, Div. Vector-Borne Disease, Kenya Med. Dept.
   Dr. John Raybold, East African Inst. Vector-Borne Disease, Amani, Tanzania
   Mr. A. H. F. Afifi, Trainee Health Inspector, Kenya Medical Services
   Mr. Jaffet Kisiuji, Health Inspector, Municipality of Mombasa
   Mr. J. K. Nyaswade, Entomological Field Officer, D.V.B.D.

   (The last three were being trained in mosquito biology).

7. Finally, we had a site visit from an AID team consisting of Drs. David Adams, Michael Rechcigal and Rajindar Pal (WHO).

F. STATEMENT OF EXPENDITURES

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

Expenditure	Budgeted 3 year total	Year 2 (July 72 - June 73) Proposed	Actual	Remainder
AT NOTRE DAME				
1. Salaries & Benefits (3 scientists plus accountant-aide)	$ 98,960	$ 35,520	$ 34,694	+ 826
2. Supplies	9,405	1,000	1,831	(- 831)
3. Bank Charges	1,500	500	0	+ 500
4. Travel	20,600	6,867	11,095	(- 4,228)
5. Capital*	-	8,201	5,140	+ 3,061
6. Indirect - to U.N.D.**	12,935	4,667	4,167	+ 500
	143,400	56,755	56,927	(- 172)
IN KENYA				
7. Salaries & Benefits (lab & field personnel)	21,000	7,000	7,330	(- 330)
8. Housing Allowance	10,800	5,544	5,614***	(- 70)
9. Rental - Space	12,000	4,000	5,443***	(- 1,443)
10. Supplies	7,695	3,500	3,780****	(- 280)
11. Travel	12,000	4,000	5,216	(- 1,216)
12. Capital	28,905	9,000	6,698	+ 2,302
13. Indirect - to ICIPE**	23,100	8,025	8,274	(- 259)
	115,500	41,069	42,355	(- 1,296)
TOTAL:	258,900	97,824	99,282	(- 1,468)

* Purchase and shipment to Mombasa of a portable irradiation source, Cesium-137.

** Notre Dame receives 14% of Salaries & Wages paid at Notre Dame. This is standard University rate for overhead for off-campus research. ICIPE receives 25% of direct costs in Kenya. Total for overhead = 14% of contract.

*** Building rental and Hausermann housing allowance paid in advance for the period July-December, 1973 ($1,887 + 857 = 2,744).

**** Includes bank charges of $58.

Budget comments:

1. When the advanced rental payment of $2,744 is discounted against the deficit of $1,468, the final total for the year is a <u>surplus</u> of $1,276. This was accomplished by stringent fiscal policies.

2. The project has been hit hard by devaluation of the U.S. dollar. When the contract was initiated, a rate of 7.4 Kenya Shillings to one dollar prevailed. The current bank rate in Nairobi is 6.65 K.S. to the dollar. This rate plus rising rental rates on the coast have seriously eroded salaries of the Research Scientists.

3. The overseas travel budget, as proposed in the initial contract, is grossly inadequate. Note the deficit for this year of over $4000 and the fact that almost nothing is left for travel in year 3. The initial budget allowed for 18 trips (or 6 trips per year) at $1200. Rising airline costs have brought air ticket alone, South Bend-Mombasa and return, to over $1300, without any allowance for maintenance. Moreover, this budget item must cover travel by three Principal Investigators, several Consultants, University Accountant, plus initial duty travel and travel to meetings and outside collaboration by the three Research Scientists in Mombasa. As an example of the problems being encountered, Research Geneticist Paul McDonald, after 2 years of duty at Mombasa, is now on a month-long trip involving: 1. Coordination with WHO Genetic Control Project in Delhi, India, 2. Attending and giving a paper on this contract at the International Congress of Genetics, San Francisco, 3. Visiting Notre Dame to coordinate field research plans. His air ticket alone costs $2,200; additional per diem costs must be added, making this trip equivalent to nearly three trips on the original budget (half the total for the year).

4. The amount budgeted for Salaries in Kenya is inadequate. We have a fine staff of laboratory and field workers; they have shown loyalty, diligence and enthusiasm for the project. However, we do not pay them adequate salaries. New regulations (insurance, medical benefits, retirement, workman's compensation, housing allowance) and a new salary scale has been established by ICIPE. We must be able to pay our employees on that basis (which follows salary scales and benefits for the Kenya Ministry of Education).

5. We have encountered difficulties in the budget item for travel in Kenya. We budgeted $4000 and were forced to pay out $5,216. This item covers both petrol and per diem costs during travel and maintenance on the three MBU vehicles. The latter item has been especially expensive. Vehicle repair facilities in Mombasa are poor and we have been unable to find competent mechanics. We spend large sums on repair and get very little in return. ICIPE-related travel has also been more expensive than expected. The Field Director of MBU is an ex-officio member of the ICIPE Resident Research Scientist Council, which meets every month in Nairobi. We try to see that all MBU scientists visit ICIPE-Nairobi at least every two months and, preferably, monthly. This travel and per diem is expensive.

6. We foresee problems in the future for rental of laboratory space. The space now occupied is ideal for this project. The landlord wants to raise the rent by 20% or more and he would be fully justified in doing so, based on current rental prices on the coast. We will continue at our current rental for another year, to June of 1974, but must then expect a significant rise.

G. Work Plan and Budget Forecast

1. The work plan for the coming year 3 of the contract is given in ANNEX 18. The work plan is based on the tentative assumption that the project will be continued for three additional years (years 4-6). The project then breaks down into three time components:

Years 1,2: Ecological background, translocation development
Years 3,4: Initial releases, release technology, genetic engineering
Years 5,6: Sustained releases in control trials

In the event that the project is not renewed, it will terminate on 30 June 1974. Early notice of AID action on renewal is essential. If the project is terminated, Year 3 should be spent in rounding out and completing the ecological work, not in making preparations for release programs.

2. There will be some changes in personnel during Year 3. Drs. Hausermann (Team Leader) and McDonald (Geneticist) will remain through June of 1974, but Dr. Fanara (Ecologist) will be leaving on 1 December to take up a position with WHO Vector Biology and Control. Because of the difficulty in moving overseas, we do not intend to replace Dr. Fanara until we learn whether the project has been renewed. Note that another Ph.D.-level scientist will join the team in December, 1973. Dr. Fidelis Ogah, a Nigerian and an ICIPE Research Associate, has completed the Ph.D. at Cornell University in Population Genetics. He will be working on electrophoretic analysis of enzymes as a means for measurement of genetic differences in domestic and sylvan populations of Aedes aegypti. Although Dr. Ogah will be paid by ICIPE (via funds from UNDP), his work will contribute to objectives of this AID project.

3. The greatest difficulty encountered by this project is financial. The project was underfunded for the nature of its activities. Devaluation of the American dollar plus rapid inflation in Kenya have seriously eroded our capacity to operate. However, we will end Year 3 with a balanced budget.

5. Anticipated budget - Year 3 (1 July 1973-30 June 1974)

Expenditure	Budgeted 3 year total	Year 3 Initially proposed	Year 3 Currently proposed
AT NOTRE DAME			
1. Salaries & Benefits	98,960	34,595	34,000
2. Supplies	9,405	3,405	145
3. Bank Charges	1,500	500	-
4. Travel	20,600	6,866	6,400
5. Indirect costs - UND (14% of S&W)	12,935	4,522	4,760
	143,400	49,888	45,305
IN KENYA			
6. Salaries and Benefits	21,000	7,000	12,000
7. Housing Allowance	10,800	3,600	4,500
8. Rental - Space	12,000	4,000	4,000
9. Supplies	7,695	2,695	3,000
10. Travel	12,000	4,000	5,200
11. Capital	28,905	5,905	1,000
12. Indirect costs-ICIPE	23,100	6,800	7,425
	115,500	34,000	37,125
TOTAL:	258,900	83,888	82,430

Budget Comments:

1. Salaries at Notre Dame are for the three Research Scientists plus half-time bookkeeper-managerial aide.
   Salaries at Kenya are for local laboratory technicians and field collectors. The increase in this category, from $7,330 spent in year 2 to $12,000 proposed for year 3, reflects two factors:
   a. Increased scales and benefits at ICIPE.
   b. Need for additional collectors and field hands, as project moves into field releases and evaluations requiring many hands.
2. Bank charges are now paid by the Kenya budget (Supplies) instead of the Notre Dame budget.
3. Increased Housing Allowance (Kenya) reflects action taken in year 2 to insure that Research Scientists live near MBU laboratory.
4. Increased budget for Travel (Kenya) reflects high cost of vehicle maintenance plus increased vehicle usage during releases and evaluations.
5. Budget for Travel (Notre Dame) is nowhere near adequate. However, this category had to be pared because of lack of funds. The proposed figure will reduce or eliminate visits by consultants, reduce visits by Principal Investigators and reduce travel by Research Scientists to international meetings and for coordination with others (i.e., WHO).
6. Total expenditure, 3 years = $258,900
   Year 1 = 77,188; Year 2 = 99,282; Year 3 = 82,430

H. Annexes

1 Research Report - Relationship of domestic and sylvan A. aegypti - Hausermann

2. Research Report - Larval development time of treehole Stegomyia - Chesang and Hausermann

3. Research Report - Host preference - Trpis

4. Research Report - Domestic and sylvan A. aegypti - Fanara

5. Research Report - Oviposition site choice - Hausermann

6. Research Report - Habitat definition - Fanara

7. Research Report - Domesticity - Trpis, Hausermann

8. Research Report - Seasonal abundance - Hausermann

9. Research Report - Population fluctuations - Fanara

10. Research Report - Population fluctuations - Hausermann

11. Research Report - Marking methods - McDonald

12. Research Report - Age: Influence on dispersal - McDonald

13. Research Report - Time of insemination - Hausermann

14. Activities Report - Philip Bebora, DIBD, Kenya

15. List of available chromosome translocations - McDonald

16. Plans for translocation research - Rai

17. References used in this report

18. MBU Project Outline, Years 3 and 4

Genetic relationship between domestic A. aegypti type form and wild A. aegypti ssp. formosus. Interim Report.

Walter Hausermann

According to Mattingly (1957) A. aegypti ssp. formosus ranges from fully wild to nearly domestic and A. aegypti type form from semiwild to fully domestic behaviour, while A. aegypti var. queenslandensis behaves always fully domestic.

On the Kenya Coast A. aegypti covers with representatives of all three categories the whole adaptive range of the species from fully wild to fully domestic. In the past year A. aegypti type form and ssp. formosus were occasionally found to exploit the same outdoor habitats like treeholes, coconut steps, seedpods, tires and discarded domestic containers. A clear distinction between specimens of the two categories when collected from the same habitats was often difficult. Specimens with white scales on the first abdominal tergite which were otherwise ssp. formosus and specimens with a totally black first tergite which were otherwise type form were quite common. Genetic exchange between type form and ssp. formosus populations seemed therefore very likely. The simultaneous presence of dark type form A. aegypti and var. queenslandensis as adults and as larvae inside houses indicated at the same time the possibility of genetic exchange on the domestic front between type form and var. queenslandensis.

To recognize quantitative shifts in gene frequencies of peridomestic and domestic populations the following colour definitions for the infraspecific categories of the species were adopted:

Table 1: Definition of infraspecific categories of *A. aegypti* on the basis of habitat preference and associated colour characters.

Infraspecific category	Habitat	Colour characteristics	Remarks
A. aegypti ssp. *formosus*	Exclusively peridomestic and sylvan. Ovipositing in treeholes, seedpods, tires and discarded domestic containers	1. Cuticle *always* black (bare prescutellar spot, post-notum, pleurites). 2. Abdominal "black" scales *always* black and densely set, giving a black lustre. 3. "White" scales on abdomen and back of head *always* pure white.	The mutant Black tergite Bt, described by Machado-Allison (1971) and used as criterion for ssp. *formosus* by Mattingly (1957) could not be used as a single criterion. However, if white scales on first tergite are present, they are always pure white.
A. aegypti type form a) "dark"	Domestic and during rains peridomestic. Ovipositing in all above mentioned habitats plus domestic water containers	1. Cuticle dark brown or dark grey. 2. Abdominal "black" scales black, densely set and producing a black lustre. 3. "White" scales all pure white. Extension of "white" scales minor.	At least one of the three characters is not as in ssp. *formosus* above. First abdominal tergite occasionally all black, but usually with a few too many pure white scales. This dark form corresponds to lab produced hybrids between type form and ssp. *formosus*.
b) "brown"	Domestic and during rains peridomestic. Ovipositing as "dark" type form	1. Cuticle dark to light brown, light grey or pale. 2. Abdominal "black" scales dark and usually loosely set, not producing a lustre. 3. "White" scales on abdomen and back of head usually pale cream. Extension of "white" scale patterns remarkable.	A highly domestic *A. aegypti*, pale in total appearance, but not carrying the mutants Gold mesonotum *G*, spot abdomen *s* or pale abdomen *pa*. White scales of first abdominal tergite always pale cream or yellowish, never pure white.
c) "pale"	Domestic, exceptionally peridomestic during rains	Colour characters as in brown *A. aegypti* type form, but combined with one of the mutants *G*, *s* or *pa*.	Corresponds to Mattingly's definition of var. *queenslandensis*. All alleles of spot abdomen are observed (Craig-Hickey, 1967; McClelland 1971, 1967)

Table 2: Frequencies of selected genes affecting body colouration in domestic, peridomestic and sylvan *A. aegypti* in Rabai. Samples taken during rainy season 1973 from larval habitats and oviposition traps.

Gene:		Gene Frequencies			
		A. aegypti type form domestic	*A. aegypti* type form peridomestic	*A. aegypti* ssp. *formosus* peridomestic	*A. aegypti* ssp. *formosus* sylvan
Sample size:		232 ♀♀	130 ♀♀	241 ♀♀	112 ♀♀
1st abdominal tergite all black	Bt	0.06	0.11	0.83	0.87
Mesonotal scales					
black	B	0.35	0.19	1	1
brown	+	0.58	0.17	0	0
gold	G	0.07	0.10	0	0
All alleles of spot abdomen affecting lateral spots and white scaling of tergites	s	0.03	0.04	0	0
Palps with white speck on segment III	Sp	0.31	0.34	0.52	0.67
Halteres covered with black scales	h	0.28	0.30	0.67	0.56

Time of larval development in *Aedes aegypti* and other *Stegomyia* collected from treehole habitats in Rabai.

Rosebella Chesang, Walter Hausermann

In the course of rearing eggs collected with oviposition traps in the Rabai area the following *Stegomyia* species were collected in sequence of frequency: *A. aegypti* ssp. *formosus*, *A. simpsoni*, *A. metallicus*, *A. aegypti* type form, *A. heischi*, *A. soleatus*, *A. calceatus* and *A. vittatus*. Among these species *A. aegypti* ssp. *formosus* seems to be the most successful, for it outnumbered all other species together in egg collections with oviposition traps as well as in larval collections from peridomestic habitats. The main reason for its success seems to be the higher speed in larval development which gives it an advantage in the fast drying larval habitats. Laboratory colonies have been established for several of these *Stegomyia* and a listing of the time of larval and pupal development is given below.

Table: Time of larval development for various Stegomyia during cool - wet season and hot - dry season

Species	Temperature range	% ♀♀	Time of pupation in days after hatch		Time of adult emergence in days after hatch	
			Start	90%	Start	90%
A. aegypti ssp. formosus	23 - 27° C (cool - wet season)	50	5	6	7	7
A. aegypti type form	"	48	6	7	7	8
A. metallicus	"	36	6	7	7	8
A. heischi	"	31	6	7	8	8
A. vittatus	"	57	5	6	6	9
A. aegypti ssp. formosus	25 - 30°C (hot - dry season)	43	4	5	6	6
A. aegypti type form	"	48	5	5	6	7
A. metallicus	"	50	5	7	7	9
A. heischi	"	36	5	7	7	9
A. vittatus	"	37	4	6	5	8

Title: Host preference in feral, peridomestic and domestic populations of Aedes aegypti from the Rabai area.

Milan Trpis

Aedes aegypti has been the subject of a very large number of papers. It is surprising how little is known on host preference of this species. The direct field studies using the method of precipitin test appeared to have been those of Davis and Philip (1931), Teesdale (1955), Lumsden (1955), McClelland and Weitz (1963). Teesdale's results refer to the Mombasa area, the closest to our studied area. In his results from 51 specimens tested, only eight (16%), were proved to be positive to man. The rest of the specimens (43) were negative against all tested antisera (monkey, ox, goat, cat, dog, rat, fowl).

Haddow (1945) and Garnham et al (1946) found that wild forest forms of ssp. formosus rarely or never bite man. As against this, var. queenslandensis because its strongly domestic habit is expected to bite man readily. Both of these cases, in fact, appear in the Rabai area, where wild populations of ssp. formosus as well as peridomestic and domestic populations of type form and var. queenslandensis occur in the same area, but live in different habitats. Endophilic behaviour of var. queenslandensis and exophilic behaviour of ssp. formosus was demonstrated in a field experiment in the Rabai area by Trpis and Hausermann (See Annex 7). The results of their experiment show that the intermediate, peridomestic type form was partly endophilic, and partly exophilic.

The aim of the present report is to demonstrate variation in host preference in domestic, peridomestic and wild populations of Aedes aegypti from the Rabai area by means of a plastic olfactometer in the laboratory. Gouck and Schreck (1965) proposed an olfactometer for use in study of mosquito attractant which, with certain modifications, Gouck (1973) used for testing of mosquitoes for host preference. Using the same basic idea we developed at the University of Notre Dame an olfactometer which allows us to use rearing colony cages for testing mosquitoes for host preference in large series.

Description of the olfactometer.

It consists of three basic parts: a) host chambers; b) rearing-testing cages; c) fan. Cylindrical host chambers are connected with mosquito traps to prevent feeding of mosquitoes on the tested hosts. The fan draws air through the host chambers, takes odour from the tested hosts to the testing cages. The tested females get activated with the odour and having two choices fly to the chosen host. After ten minute exposure of the hosts to the mosquitoes the entrances into the olfactometer are closed with vertically moving slides. The mosquitoes trapped are removed and counted. The tests were done in a walk-in insectary at 26°C and 75-86% RH. Fluorescent light was placed in the center above the olfactometer. The tested mosquitoes were from 3 to 7 days old.

Mosquito populations tested.

Six populations (4 domestic, 1 peridomestic and 1 feral) from the Rabai area were tested (Table 1).

Hosts used in the tests.

Five different hosts (man, rat, chicken, frog, turtle) as representative of five groups: man, small mammals, birds, amphibians and reptiles, were tested.

Results of host preference.

In the first series Man was tested against animals. This test yielded results on anthropophily and zoophily of the three basic populations. The X^2 test shows a significant difference in host preference between domestic and wild populations (Table 2).

In the next series Chicken was tested against other animals. A significant difference was found between chicken and rat in domestic and feral A. aegypti (Table 3). The results between chicken and turtle, and chicken and frog are not significant (Table 3). Both populations are attracted to amphibians and reptiles which is showed in the test-combination rat-turtle, rat-frog and turtle-frog, but difference in host preference in these cases is not significant. Similar results are anthropophily and zoophily were achieved in other populations tested; however, the character of this report does not allow me to discuss the results fully. Instead, on the bases of these tests I present a table which shows orders of preference in all six populations for the five hosts (Table 4). The feral population is strictly zoophilic and the domestic one strictly anthropophilic. In the peridomestic populations, in some of them, preference for man in others for animals were found.

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Table 1: Various populations of *Aedes aegypti* from the Rabai area tested for host preference.

Strain	Taxonomic category	Color	Habitat
BEJUMA (1)	*A. a. formosus*	Black	Sylvan
MOYO (28K)	*A. a. aegypti*	Brown	Peridomestic
MBAA (TIRE)	*A. a. aegypti*	Brown	Peridomestic
MOMSABU (2)	*A. a. aegypti* some *queenslandensis*	Pale	Domestic
MOYO (IN)	*A. a. a.*, some *queenslandensis*	Pale	Domestic
MBAA (IN)	*A. a. a.*, some *queenslandensis*	Pale	Domestic

Table 2: Test for Anthropophily and Zoophily in *Aedes aegypti* of Bejuma (1) and Momsabu (2) Populations

HOST COMBINATION	POPULATIONS *		X^2
	BEJUMA (1)	MOMSABU (2)	
MAN CHICKEN	6 74	81 19	$P < 0.001$
MAN RAT	2 81	50 39	$P < 0.001$
MAN TURTLE	8 17	77 13	$P < 0.001$
MAN FROG	16 18	70 16	$P < 0.001$

* = Number of mosquitoes out of 100 entering trap for the host indicated.

Table 3: Test for host preference among diverse vertebrate hosts

Vertebrate tested	Host combination	Mosquito response (N = 100)*		X^2
		BEJUMA (1)	MOMSABU (2)	
Bird	Chicken	55	25	$P < 0.001$
	Rat	33	59	
	Chicken	78	51	N.S.
	Turtle	11	14	
	Chicken	62	3[illegible]	N.S.
	Frog	29	19	
Rat	Rat	91	88	N.S.
	Turtle	1	4	
	Rat	38	40	N.S.
	Frog	28	28	
Cold blooded	Turtle	8	11	N.S.
	Frog	32	30	

* = Number of mosquitoes out of 100 entering trap for host indicated.

Table 4: Order of host preference in six populations of *A. aegypti* from the Rabai area.

	BEJUMA (1) Sylvan	MOMSABU (2) Domestic	MOYO (28K) Peridomestic	MOYO (IN) Domestic	MBAA (TIRE) Peridomestic	MBAA (IN) Domestic
1.	CHICKEN	MAN	MAN	MAN	CHICKEN	MAN
2.	RAT	RAT	CHICKEN	CHICKEN	RAT	CHICKEN
3.	FROG	CHICKEN	RAT	FROG	MAN	RAT
4.	TURTLE	FROG	FROG	RAT	FROG	FROG
5.	MAN	TURTLE	TURTLE	TURTLE	TURTLE	TURTLE

Relationships of domiciliary and extradomiciliary demes of *A. aegypti*

Dean M. Fanara

The field cage in Bejumwa forest has been utilized for two experiments involving host preference by the males and females of the pale, domiciliary Mwamsabu Dry strain, the intermediate, extra-domiciliary Bejumwa Wet strain and the dark, extra-domiciliary Bejumwa Dry strain. The results shown in table 2 clearly demonstrate that the dark, extra-domiciliary form does not prefer to bite man in the forest. The pale, domiciliary form appears to be somewhat inhibited. The intermediate, extra-domiciliary strain is the most aggressive feeder being four times as active in feeding upon man whether in the semi-hungry state (less than 48 hours after emergence) or in the hungry state (over 72 hours after emergence). These results greatly support the conclusions from the previously reported mark-release-recapture experiments.

An experiment is being conducted to compare the strains in a village hut situation for comparison with the field cage results.

Intensive laboratory and field preparation is under way for a genetic marker study of the three afore mentioned strains. A different recessive marker is being bred into each of the strains which are now approaching 92.75% "Africanization." The genetically marked mosquitoes will be released into their respective habitats. Then larval collections will be made in the forest and in the village. The laboratory reared adults will be backcrossed to a homozygous laboratory strain which contains all three markers. The resultant progeny should yield information as to oviposition-site selection and amount of genetic contact between the three demes.

Table 2: Field cage results of mark, release and recapture of three strains of *Aedes aegypti* from Chang'ombe Sublocation

	Males landing				Females biting			
	48 hours old		72 hours old		48 hours old		72 hours old	
Ecotype	% Total	Ratio	% Total	Ratio	% Total	Ratio	% Total	Ratio
Domestic	9.3	28	11.1	16	14.3	26	40.0	30
Sylvan	5.0	15	26.5	37	7.0	13	21.4	16
Interm.	18.6	57	32.8	47	33.7	61	70.7	53
		100		100		100		100

Releases were 50 ♂♂ 100 ♀♀ of each strain for the 48 hours old (since molting from pupal stage), and 100 ♂♂ 100 ♀♀ of each strain for the 72 hours old.

Strains used were:

Domestic - pale, domiciliary Mwamsabu Dry marked with Helecon Red
Sylvan - dark, extradomiciliary Bejumwa Dry marked with Helecon Green
Inter. - intermediate appearing strain from forest marked with Helecon Yellow

Preference for artificial oviposition sites in *A. aegypti*

Walter Hausermann

Egg collections made with black metal tins and claypots indicated that the claypots used for water storage inside houses are the preferred oviposition site also in the peridomestic environment for *A. aegypti* type form. *A. aegypti* ssp. *formosus* on the other hand showed some preference for the narrow oviposition tins.

Table: Summary of observations made in April and May, during the rainy season, 1973.

Kind of trap	No. trap days	Domestic habitat No. eggs		Peridomestic habitat No. eggs	
		A. aegypti type form	*A. aegypti* ssp. *formosus*	*A. aegypti* type form	*A. aegypti* ssp. *formosus*
OPJ *	300	8,416	0	233	279
OPT **	1,200	0	0	54	1,060
Peridomestic *** larval habitats		-	-	509	1,029

* OPJ = Oviposition jars = fired earthenware pots of about 5 l volume, spherical shape and a narrow opening of 12 cm diameter. Oviposition on total inner surface above waterline. Jar is 3/4 filled with water.

** OPT = Black painted tins of 12 cm height and 6 cm diameter. Oviposition on inserted strip of leather cloth, 12 cm long and 1.5 cm wide. The tin is ½ filled with water.

*** 80 peridomestic larval habitats sampled five times in two week intervals for water and larvae.

Environmental definition of indoor and outdoor habitats

Dean M. Fanara

In rearing through larva collected from Bejumwa forest treeholes it was noted that there were no pale adults similar to the Mwamsabu Dry and Mwamsabu Wet strains. However, landing-biting catches in the same forest have indicated that the domiciliary forms do enter the forest. This was also confirmed by marked releases and recaptures. In cooperation with Dr. William Wood of I.C.I.P.E. at Nairobi we are investigating the ovipositional attractancy of Mwembe tree extracts (mango). To date it has been demonstrated that there is a definite difference in response between the strains. However, the results are confused by what appears to be a difference in strain response to age at time of oviposition, light intensity and age of extract preparation.

A new location, Bombwe village and Bombwe forest, is being investigated for comparison with Mwamsabu village and Bejumwa forest.

The search continues for locating the night time resting site of the dark, extradomiciliary A. aegypti. A mass release of blood-fed, laboratory reared, dark, extradomiciliary A. aegypti into the forest is being prepared. The mosquitoes will be marked with fluorescent dyes and a nighttime search will be conducted. Blood fed females collected in quantity from known resting sites would be valuable for precipitin tests to determine host preference.

Domesticity in *Aedes aegypti*

Milan Trpis, Walter Hausermann

Definition: Domesticity = Tendency to hunt for man inside houses and to oviposit inside houses.

A test for domesticity was made by releasing marked *A. aegypti* adults of different origin in a peridomestic habitat and recapturing simultaneously inside houses and in the release environment. In each release the two sexes were released in equal numbers. Recovery of marked individuals was made with landing-biting catches.

Two releases were made, the first one comparing laboratory strains from different origins, the second one comparing these two strains in a later generation with their reciprocal crosses. The releases were made in April and May 1973.

Results:

Strain	Origin	Type	Generation of lab.	No. released	No. recovered in	
					Domestic habitat ♀/total	Peridomestic habitat ♀/total
MNAZI	peri-domestic	ssp. *formosus*	F_{10}	ca. 2000	41/72	no catches
MWAMSABU	domestic	type form	F_5	ca. 2000	146/335	no catches
MNAZI			F_{12}	900	1/3	29/44
MWAMSABU			F_7	900	99/215	4/20
Cross MNAZI F_{11} x MWAMSABU F_6				900	26/35	13/24
Cross MWAMSABU F_6 x MNAZI F_{11}				900	25/27	20/26

Conclusions:

1. The strains tested were not 100% domestic resp. 100% peridomestic in their behaviour, but nevertheless basically constant in their habitat preference, i.e. domesticity is an inherited trait.

2. The crosses failed to produce information on the mode of inheritance because the sampling was biased in two ways:

   A. Sampling by landing-biting catch means that only the anthropophilic components of the test strains and the crosses are sampled.

   B. Sampling outdoors is less efficient per unit effort due to greater dilution.

Seasonal abundance of A. aegypti in domestic and peridomestic habitats.

Walter Hausermann

Seasonal abundance of A. aegypti was monitored in peridomestic and domestic habitats in weekly intervals for a whole year (First Annual Report, Annex 6). The methods used were oviposition traps, larval surveys, landing-biting catches and handnet catches. The most rewarding methods were oviposition traps in peridomestic habitats and landing-biting catches in domestic habitats.

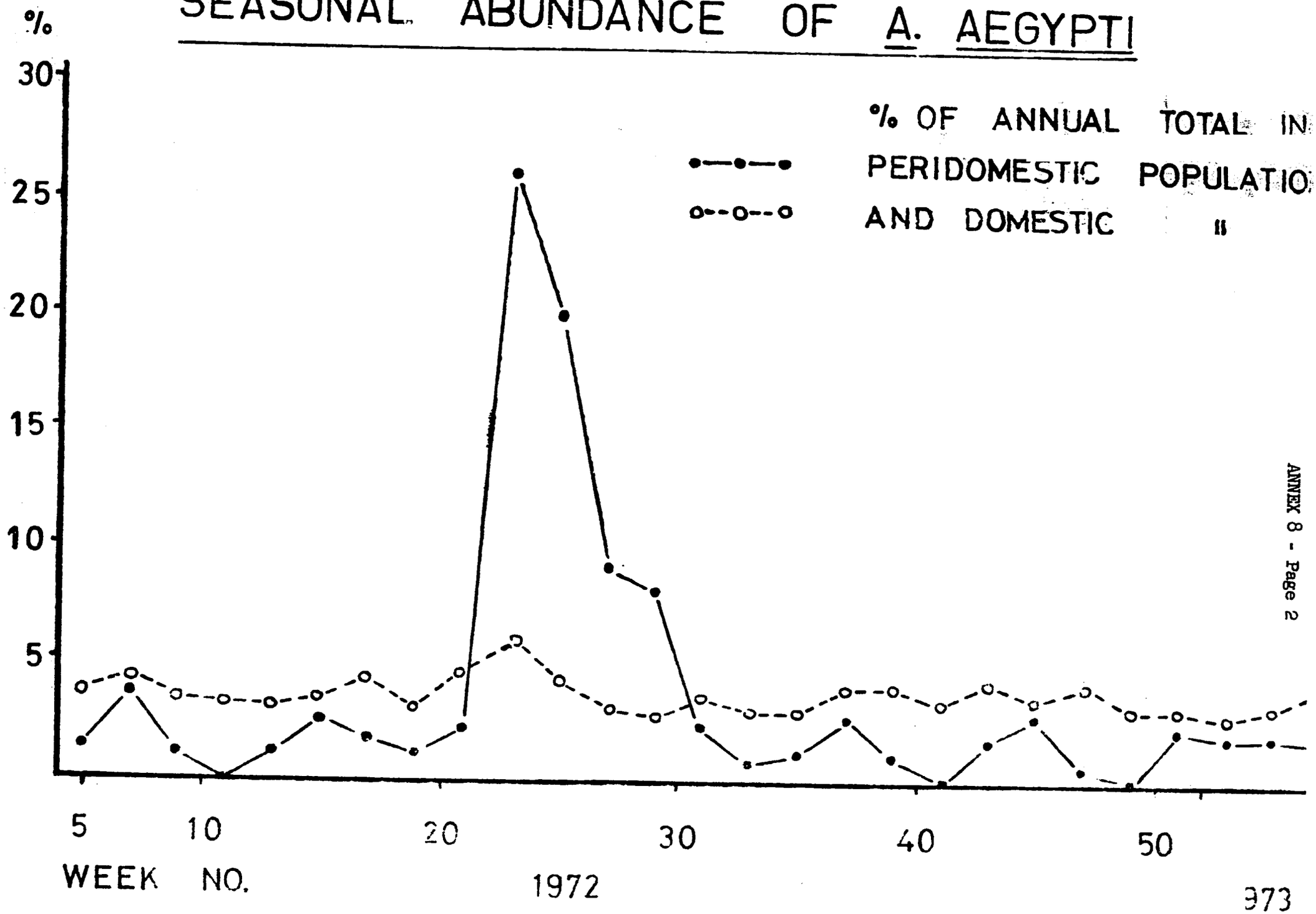
In a preliminary evaluation the results of egg collections by oviposition traps and adult collections by landing-biting catch are plotted as biweekly percentages of the total annual collections (figure 1). The total annual collections were 7,433eggs in peridomestic habitats and 5,945 adults in L-B catches in domestic habitats. The dependence respective independence of peridomestic and domestic populations is evident when the population fluctuations are related to the seasonal changes in rainfall and temperature (figure 2).

Peridomestic A. aegypti mostly ssp. formosus shows a drastic 10 fold increase with the onset of the rains, but then decreases steeply to its prerain level despite ongoing rains. Domestic A. aegypti type form is almost stable throughout the year and shows only a minor increase during the rainy season which is due to some additional breeding in outdoor habitats.

A detailed analysis of the monitoring data is made by Prof. T. Crovello at Notre Dame and is expected to be available by June 1974. In the mean time the monitoring of domestic and peridomestic populations continues by means of oviposition traps alone. As far as can be judged by now the seasonal pattern is repeated rather precisely.

Studies to correlate oviposition rates and landing-biting rates with absolute population size are in progress and should eventually permit to estimate absolute population size on the basis of relative measurements, at least for domestic populations.

SEASONAL ABUNDANCE OF A. AEGYPTI



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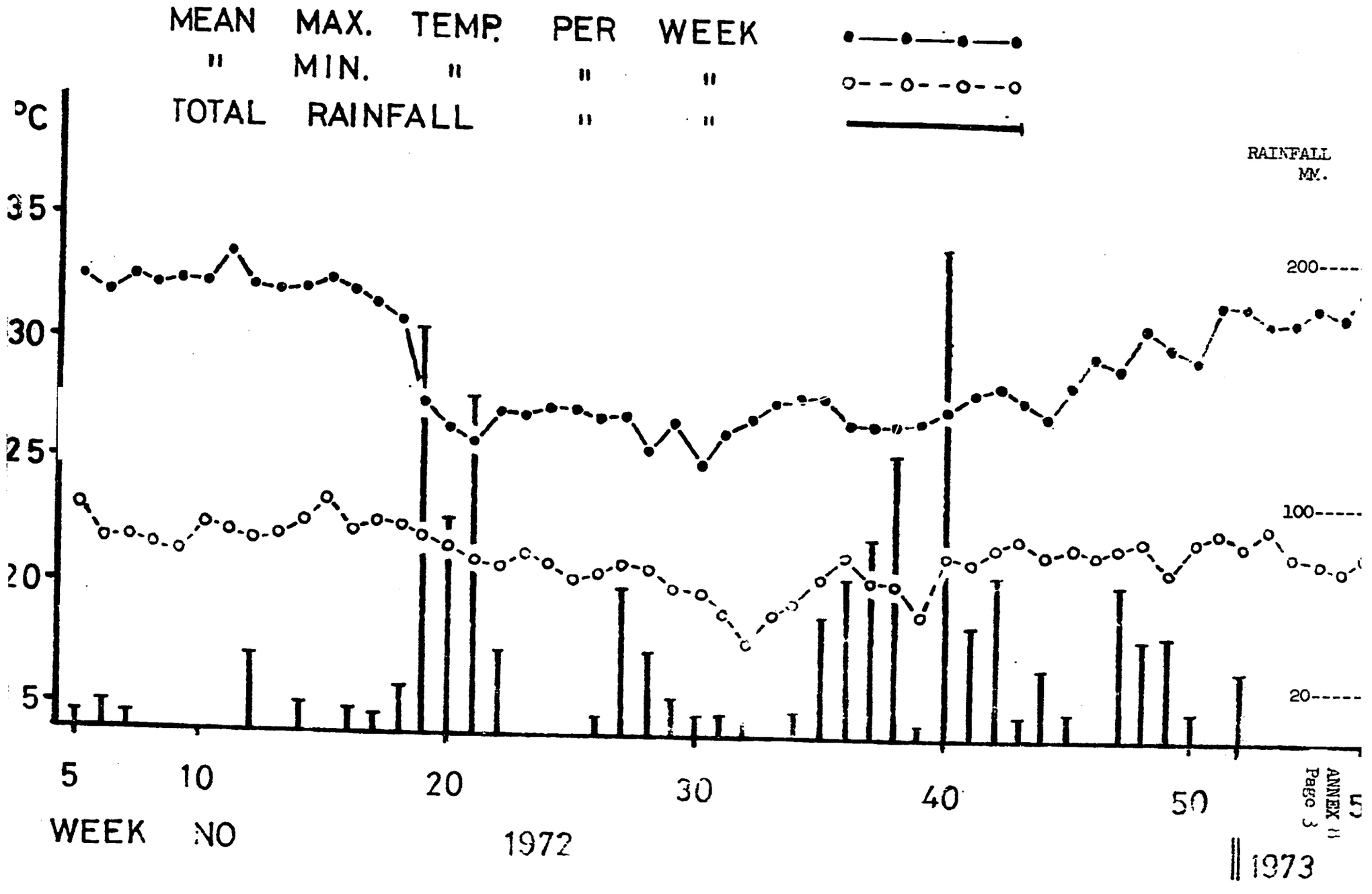

Description of population fluctuations

Dean M. Fanara

A study of dry season landing-biting by A. aegypti in relation to the number of its pupae per water container was conducted at Mwamsabu village. The observations were made during the 3 months of the dry season with one hour catches and counts made at each house at one time (on a different day) during the period 0800 to 1800 hours local time (see table). The mean number of pupae per house was 4.3 (2.4 per pot with a mode of 2 pots). The mean number of biting females was 3.7 and the mean number of landing males was 1.9. Females outnumber males 2 to 1. In 15 of the 22 houses the mean number of males and females landing-biting correlated closely with the mean number of pupae. Houses were either below the mean or above the mean in all categories (pupae per pot, pupae per house, males landing and females biting).

Night landing-biting activity by A. aegypti during the wet season was investigated. Indoors at Mwamsabu a low level of landing-biting was recorded all during the night. Meanwhile, with the exception of 2 females, no A. aegypti were taken during the night in Bejumwa forest. The daytime biting rate was low at 2.4 females per hour during the wet months of April and May.

Table 1 : Indoor landing biting catches compared with density of pupae in houses at Mwamsabu village during dry season (January - March, 1973).

House No.	A. aegypti				All counts correlated	
	Pupae		Adults			
	Mean No. per house	Mean No. per pot	Mean No. males	Mean No. females	Above	Below
1	0.6	0.4	0.2	1.3		x
2	0.9	0.5	0.4	1.1		x
3	4.3	2.0	1.2	2.2		x
4	5.4	2.4	1.2	2.2		
5	6.1	2.3	0.8	1.2		
7	0.0	0.0	0.9	2.1		x
8	1.2	1.1	2.8	4.8		
9	0.8	0.8	1.2	2.8		x
10	4.4	1.8	1.4	3.4		
11	6.5	5.4	1.7	3.4		
12	3.2	0.8	1.4	3.5		x
13	2.7	1.4	1.4	3.9		
14	9.7	5.1	3.9	7.1	x	
15	0.0	0.0	1.0	1.5		x
16	1.3	0.5	2.2	5.4		
17	17.6	6.8	4.0	9.4	x	
18	3.1	1.8	1.1	2.4		x
19	6.2	6.2	4.0	5.8	x	
21	10.6	8.8	5.7	9.0	x	
22	0.0	0.0	0.4	0.8		x
23	9.6	4.8	4.1	7.8	x	
24	0.0	0.0	0.2	0.6		x
Means	4.3	2.4	1.9	3.7	5 +	12 = 15 out of 22

Fluctuations in adult *A. aegypti* populations in Rabai villages

Walter Hausermann

The number *A. aegypti* caught in landing-biting catches in different villages fluctuate widely between villages (table 1) and with time. The reasons for these fluctuations are manyfold and only partially understood.

One of the main reasons for the fluctuations is doubtless that the domestic larval habitats constitute a high risk environment. Usually 10 - 20 % of the domestic water containers of a village contribute 60 - 80 % to the pupal production. Cleaning of such pots results in a drastic drop of emergence of adults, followed by increased hatch caused by refilling the pots and after 7 - 10 days by an increase in emergence of adults. If occasionally the cleaning efforts of housewives coincide (often stimulated by our investigations), these fluctuations are even more accentuated. Thus a village population of *A. aegypti* can never be considered stable (tables 2 + 3).

If for several days all individuals caught in a L-B catch or all emerging adults are marked, it could be expected that the proportions of marked adults caught in L-B catches increase steadily from day to day until the total population is marked. While an increase in the proportions of marked individuals was indeed observed, the populations were never totally marked (tables 2 + 3). This was very likely due to survivors from previous marking days and to immigrants. Especially intermittent drops in the proportions of marked individuals of an L-B catch seem to indicate sporadic immigration.

Table 1: Incidence of *A. aegypti* in different villages during one visit in dry season.

Name of village	No. houses	No. people	No. containers with water	No. (%) containers with larvae and/or pupae	No. pupae on sampling day	L-B catch on sampling day ♀ ♂
SHAURI MOYO	16	58	21	16 (76.2)	135	4.12/1.68*
KIPEVU	22	85	34	26 (76.5)	192	2.08/1.22
GOVA	17	65	29	23 (79.3)	19	0.59/1.18
SWAFI	18	38	23	16 (69.6)	40	1.48/0.67
MGANDINI	34		56	31 (55.4)	117	1.81/0.56
Selected houses of 4 villages	40	151	48	23 (47.9)	69	1.35/0.90

* per man hour

Table 2: Absolute population density of *A. aegypti* during a 15 day interval. Shauri Moyo: 16 houses, 56 people, 21 water pots.

Date	Pupal output	L-B catch * (16 man hours)		Proportion of marked mosquitoes in L-B catch	Estimates of adult numbers	
		Total	♀♀		Total Lincoln index	Total Jolly method
April 17	54	93	66	----	741	----
18	45	133	108	0.11	465	----
19	67	91	77	0.29	272	----
20	60	103	82	0.23	189	164
21	31	81	63	0.46	290	199
22	42	90	61	0.23	144	259
23	67	46	39	0.54	129	184
24	83	69	40	0.33	274	190
25	55	93	66	0.22	495	281
26	59	99	52	0.17	299	291
27	69	87	54	0.29	207	220
28	78	73	35	0.27	187	266
29	82	103	82	0.60	113	241
30	89	125	90	0.67	164	256
May 1	108	92	75	0.73	----	198
		1,378	990			
		♀ ratio	0.7184			

* All individuals caught in L-B catch released with individual marking. Recaptured mosquitoes released again.

Table 3: Mgandini

Date	Pupal output	Emergence of adults		L-B catch (25.5 man hours)		
		Total	♀♀	Total	♀♀	Proportion marked
Jan. 18	117	-----		--	--	---
19	79	33	10	--	--	---
20	75	76	29	122	91	---
21	62 *	57	27	104	69	---
22	41	50	26	89	45	0.30
23	61	42	16	104	71	0.10
24	63	58	31	51	33	0.10
25	82	39	13	81	46	0.11
26	89	84	27	74	35	0.27
27	25	76	26	104	56	0.27
28	45	40	17	69	31	0.19
29	20	30	15	58	42	0.16
30	59	26	13	63	36	0.19
31	59	27	13	75	33	0.21
Feb. 1	73	41	19	45	24	0.22
2	81	69	29	53	25	0.15
3	68	68	31	60	18	0.45
4	124	43	20	53	42	0.24
5	--	89	38	39	22	0.18
6	--	--	--	45	27	0.40
	1,223	948	400	1,309	746	
		♀ ratio 0.422		♀ ratio 0.570		

* From January 21 onwards all emerging adults were marked with fluorescent dusts. All adults captured, marked or unmarked, were removed.

Marking methods in study of survival and dispersal, migration

Paul T. McDonald

A single field experiment was designed to give information on methods of marking A. aegypti as well as dispersal and migration. Three neighbouring villages were selected and the study was conducted from 9-14 March during the hot dry season.

1. Self dusting. In an attempt to improve upon a marking technique for newly emerged mosquitoes, several self dusting chambers were constructed and tested. The most successful has a cover of parallel 0.5 cm strips of paper towelling covered with dust and separated by 0.1 cm spaces through which the mosquitoes escape the emergence cup. With such a device all mosquitoes emerging were dusted and left within two days under laboratory conditions.

In the field the self-marking of females was compared with dusting with an atomizer into a single pair cage containing 50 newly-emerged adults. The results are given in table 1, and indicate that both methods are comparable in terms of recovery and dispersal of treated individuals.

2. Transfer of dust on mating. A method of marking the ventral surface of the abdomen of males with fluorescent dust was developed and males so marked were placed with virgin females to see if transfer of dust occurred on mating. In the laboratory cages the method was initially successful.

In the field trial, 900 males were marked and 176 virgin females released with these males. In L-B catches for five subsequent days only 9 of 311 females collected in the villages of release (B, C) and 2 females of 309 captured in a neighbouring village (A) were carrying dust transferred from these males. Three females dusted at emergence in the neighbouring village (A) were mated with these release males: one had migrated to B village, one to C village while the third was captured in the village of its emergence. Since 53 percent of males captured in the village where the marked males were released, the return of only 3 percent marked females indicates that a more effective marking method needs to be found.

3. Migration and Dispersal. The dispersal and migration of newly emerged mosquitoes from a colonized strain of indoor A. aegypti was determined in the field under conditions different for males and females. Males were released 225 per house in two houses well separated in a village, for two villages. Females were released 100 per house in a five house cluster in another village. The intra-village displacement (dispersal) and the inter-village displacement (migration) were ascertained from data in the L-B catches made for five days after the releases. The results are in Table 1.

The dispersal of females released from a cluster of houses was 7 percent, much less than that found in other experiments, in which the exact house of release was known. This indicates that most dispersal within a village occurs within a cluster of neighbouring houses within the village

and not over the whole village.

The male dispersal was 37 percent from release houses well distributed among the collection houses in the villages. This is in general agreement with results from other release experiments where the exact house of release was known.

Migration of both sexes is extremely low with only two of 500 release females found in neighbouring villages and three of 900 males found in a neighbouring village.

Table 1

Release Group	Site (No. Houses)	Total Recovery+	Dispersal	Migration	Transfers of Marking	Release houses % marked L-B catch	Non-release houses % marked L-B catch
250 ♀♀ yellow self dusted	A* (12)	60 (24%)	4 (7%)	1	--	48%	7%
250 ♀♀ red atomizer dusted	A* (12)	60 (24%)	5 (8%)	1	--		
900 ♂♂ green	B* (6) C* (6)	159 (18%)	57 (37%)	3	11	80%	33%

+ L-B catches daily for 40 minutes per house
* Village A is to the NW of village B and village C to the NW of village A. Predominating winds are from the NE, village A is 150 meters from B and from C.

Age dependence of mosquito survival and dispersal

Paul T. McDonald

Introduction

Previous research has shown that only 36 percent of mosquitoes with marks were recaptured in Mgandini even after marking over 75 percent of newly emerged mosquitoes of the village for six weeks. It has been postulated that this situation is due to immigration into the village by either mosquitoes emerging outdoors or indoors in other villages. Experiments conducted by W. Hausermann at Mgandini have demonstrated that migration of indoor mosquitoes from nearby villages into Mgandini does indeed occur.

When survivorship curves were constructed for the Mgandini population of marked mosquitoes it was found that the survivorship on the first two days after marking was drastically lower (males 21%, females 25%) compared with two day survivorship afterwards (males 60%, females 80%). While the low recovery of marked mosquitoes was undoubtedly partially due to marking treatment and sampling method, it was thought that it could also have been attributed to migration out of the village by the marked mosquitoes soon (within two days) after emergence. An experiment was designed to test the hypothesis that newly-emerged mosquitoes of both sexes migrate to a greater extent than older mosquitoes. If this is true then (1.) newly-emerged mosquitoes would have lower recoveries than older ones in L-B catches after release and (2.) the newly-emerged mosquitoes would be found in greater numbers leaving release houses and in other houses in the village to which they have dispersed.

Materials and methods

The village of Mgandini was selected for this experiment. A strain from Mgandini, colonized for two generations in the laboratory was raised and, when newly emerged, individuals were painted with a dot of Humbrol on the mesonotum. Release of mosquitoes of three different age groups together was made on three different days. Mosquitoes had been kept in gallon cages in the laboratory until attaining the appropriate age for release. Table 1 gives the scheme of releases. All houses of release were outfitted with window traps. The number of mosquitoes released in any one house was 120, in keeping with the numbers that ordinarily be emerging in; a house with heavy breeding. Releases were made in the early evening and collections in L-B catch were begun the day after the first release continuing for twelve days. All 32 houses of the village were sampled with two twenty-minute LB catches, and each afternoon the twenty window traps were examined and all mosquitoes recovered. No captured mosquito was returned to the village.

The experiment was conducted from 20 May to 1 June. The long rains season continued and rains fell daily especially during the mornings. The sky was grey and overcast until late afternoon or for the whole day. The temperatures were near the lowest of the year. Two exceptional days

occurred. May 2 had intermittent sunny periods and high winds and May 29 was warm and sunny.

Results

Survivorship

The survivorship to day one after release was determined by summing all captures after release during the twelve days of collections. Results are entered in table 2. A total of 229 marked mosquitoes were caught in L-B catches and 41 were captured in the exit window traps. Male recaptures were 58/120 = 39 percent for 2 days old and 43/120 = 36 percent for 4 days old. This indicates that younger females have no different survivorship potential than older females in this experiment.

Dispersal

Dispersal in the village over the twelve days of this experiment has been determined by summing the number of mosquitoes caught leaving the house of release plus those found in another house. Table 2 shows that dispersal is not influenced by age at time of release, as determined at the termination of the experiment or when measured by L-B catches within two days after release. The window traps caught too few mosquitoes to reveal any pattern.

The number of dispersed vs. non-dispersed males and females has been determined for up to 3 days after release for all release groups, and is given in table 3. This shows that dispersal reaches its maximum by two days after release for both sexes, and thereafter does not increase appreciably. This suggests that functionally there may be sedentary and moving components in the population.

Dispersal as measured by captures in window traps varies greatly while the L-B catch remains relatively constant, decreasing gradually over the period of the experiment (table 4). The sudden increase in window trap captures may be due to increased movement of mosquitoes following warm sunny periods during otherwise overcast cool days.

Conclusions

The movement of mosquitoes away from the release house is not dependent upon the length of time before release that the mosquito has been held in a gallon cage. This indicates that there is no internal gate for dispersal (or migratory) movement at 0 - 2 days after emergence which closes before 2 days after emergence. The results are consistant with a view that the mosquito is moving as opportunity permits at any age (newly emerged to 4 days old) and strongly suggests that the lower survival from day 0 to day 2 found in an earlier study is not due to movements out of the field area, but to the marking treatment and sampling procedures employed. The improved sampling technique and laboratory marking has given a recovery of marked newly-emerged mosquitoes (male 48%, female 35%) considerably greater than earlier experiments (male 21%, female 25%).

Table 1

Release Date	Age at Release	House of Release	Number ♂♂	Number ♀♀	Marking
20 May	0	14	20	20	VI cream - Left
		19	20	20	VI cream - Right
	2	14	20	20	II black - L
		19	20	20	II black - R
	4	14	20	20	I blue - L
		19	20	20	I blue - R
22 May	0	7	20	20	VII green - L
		33	20	20	VII green - R
	2	7	20	20	V navy - L
		33	20	20	V navy - R
	4	7	20	20	III white - L
		33	20	20	III white - R
24 May	0	29	20	20	IX olive - L
		4	20	20	IX olive - R
	2	29	20	20	VIII grey - L
		4	20	20	VIII grey - R
	4	29	20	20	IV yellow - L
		4	20	20	IV yellow - R

Table 2

Age at Release	Sex	Total Released	Total Captures	Not Dispersed	Dispersed	Percent Dispersal	L-B catch Captures within 2 days of Release	Percent Dispersal	No. caught in first 2 days in window traps of house of release
0	♂	120	58	35	23	23/58=40%	35	13/35=37%	4
	♀	120	42	26	16	16/42-38%	18	7/18-39%	3
2	♂	120	46	26	20	20/46=43%	26	11/26=42%	1
	♀	120	47	18	29	29/47=62%	13	6/13-46%	2
4	♂	120	30	15	15	15/30=50%	20	11/20=55%	0
	♀	120	43	20	23	23/43=54%	16	9/16-56%	2

Table 3

Day after Release	Captures ♂	Captures ♀	Percent Dispersal ♂	Percent Dispersal ♀
1	44	28	17/44 = 39	10/28 = 36
2	37	19	18/37 = 49	12/19 = 63
3	29	16	11/29 = 38	7/16 = 44
4	8	14	3/8 = 38	7/14 = 50
5	8	10	3/8 = 38	6/10 = 60
6	2	15	1/2 = 50	9/15 = 60
7	4	10	3/4 = 75	4/10 = 40
8	1	11	1/1 =100	10/11 = 91

Table 4

Date	L-B catch	Window traps
20 May *	---	---
21 May	165	1
22 May *	116	3
23 May	170	4
24 May *	143	2
25 May	157	37
26 May	105	25
27 May	106	9
28 May	130	14
29 May	96	6
30 May	118	32
31 May	117	27
1 June	110	19

* Days of releases

Onset of mating and dispersal in domestic *A. aegypti* in Rabai

Walter Hausermann

Procedure

Male and female pupae were collected daily from domestic water containers and kept separately until emergence.

The emerging adults were collected each day, marked with fluorescent dust and released in selected houses.

Adults were captured every day singly in test tubes and marked females dissected for insemination. The catch data are shown in annex table 2. In 16 days 336 females and males were marked and released. Recaptures were continued up to day 21.

Results

See table 1.

Conclusions

1. The recovery of marked females and males of different age classes are low due to the low numbers of newly emerged adults entering into the village population daily. It is thought that these numbers depict the actual conditions better than a large scale release which would guarantee large numbers of recoveries for statistical comparison.

2. The insemination of domestic *A. aegypti* females in the field takes place at a slightly lower speed than in the laboratory. Successful matings take place as early after emergence as in the laboratory cage but the last matings take place one to two days later (GWADZ, 1968).

3. Dispersal started at the same time as mating, i.e. within 24 hours after the release or 48 hours after emergence. There was no indication that dispersal would enhance or reduce the chances of successful mating, however with increased age females were increasingly dispersed.

4. Males and females seemed not to differ principally from each other in their dispersal after eclosion.

Table 1: Age at insemination

Age in hours after emergence	Total No. ♀♀ re-captured	Total No. ♀♀ insemi-nated	% insemi-nated	No. ♀♀ emigrants	No. ♀♀ emigrants insemi-nated	% emigrant ♀♀ inseminated	Total ♂♂ recaptured	♂♂ emigrants recaptured
24 - 48	12	1	8.3	7	1	14.3	18	10
48 - 72	22	5	22.7	7	0	0.0	43	9
72 - 96	14	9	64.3	6	5	83.3	28	9
96 -120	12	11	91.7	10	9	90.0	8	8
120 -144	7	6	85.7	7	6	85.7	13	8
144 -504	53	53	100.0	38	38	100.0	44	28
Total	120	85		75	59		154	72

Seven months with the Mosquito Biology Unit of ICIPE

Philip Bebora, Senior Entomological Field Assistant,
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Ministry of Health, Kenya

What MBU wants to do:

The Mosquito Biology Unit wants to describe and understand the population fluctuations of *A. aegypti* in domestic, peridomestic and sylvan habitats. To achieve this goal, information on dispersal, longevity, fertility and rate of larval development is collected continuously in field experiments and observations. In addition, MBU tries to produce genetically altered strains of *A. aegypti* which are sterile or semisterile. These "new" sterile mosquitoes will eventually be released in selected areas and it is hoped that they will sterilize and gradually eradicate native *A. aegypti* populations.

What I did during my stay:

During my stay I learned various techniques of collecting *Aedes* mosquitoes. I have become familiar with how to rear *A. aegypti*. The techniques are different from those of *Culex* and *Anopheles* species because the eggs of the latter can not be dried and stored. I was also involved in making surveys for *A. aegypti*, spray catches, dissection for insemination, landing-biting catches, larval sampling indoors and outdoors, tree hole scratching and marking of mosquitoes by dusting with fluorescent dust. I also carried out insecticide resistance tests on various strains of *A. aegypti* from different habitats. Finally, under the supervision of Dr. W. Hausermann, I controlled and almost eradicated the *A. aegypti* population of a village and then monitored the reappearance of the mosquito. For my future use and further reference I have written short reports on all my activities.

How to make a survey of *A. aegypti*:

1. Material needed:

Before commencing a survey in rural areas the following equipment must be in hand:

1. Torch with cells
2. Plastic cups
3. Ladle
4. Small handnet for larvae
5. Spoons
6. Pencil
7. Scissors
8. Aspirator
9. Test tubes
10. Sticky labels
11. Long and short pipettes
12. Small plastic bags

13. Handnet for catching mosquito adults
14. Plastic pans
15. Note book
16. Cotton wool
17. Specimen box
18. Forceps
19. Marking pen
20. Single pair cages
21. Netting and chimneys
22. Rubber teats
23. 2.5 x 10 cm test tubes

2. Procedure

Search for water containers of all sorts indoors and outdoors. Check for larvae or pupae with a pipette, flashlight and a handnet. Collect samples of larvae in plastic tubes for later identification in the laboratory. Check for resting mosquitoes in houses, on walls and objects hanging from the ceiling, under beds and on loose roofing materials. Also check for landing-biting mosquitoes and capture in test tubes. Label and identify on the spot or later in the laboratory.

If no rain has fallen for a long period and everything is dry, check for eggs. Scratch treeholes, broken pots, rock pools, coconut shells and waste bins around a village. Put the dirt in plastic bags and label. Reaching the laboratory flood the dirt in plastic pans, then examine.

When making a village survey, the following data should be noted:

- Name of village
- Location and Sublocation
- Date of survey
- Number of houses
- Number of inhabitants
- Description of village: Topography, vegetation, agriculture, sources of water, date of last rainfall, if recent.
- Breeding indices: No. of water receptacles indoors and outdoors
  No. of receptacles with larvae
  No. of receptacles with pupae
  Total number of pupae in village
- No. adults caught in landing-biting catch.

Note also the time spent in different activities like:

How many man-hours are spent on the following:
1. Searching for larvae outdoors
2. Searching for larvae indoors
3. Landing-biting catch outdoors
4. Landing-biting catch indoors.

Collection of different teams at different times can then be compared on a yield per unit - effort basis.

The indices used are:

1.	Landing-biting catch	= No. mosquitoes landing or biting man per man hour
2.	Breteau index	= No. of containers with larvae per hundred houses or premises
3.	Container index	= % of containers positive with larvae of all containers with water
4.	House index	= % houses with either larvae in domestic water containers or adults landing-biting.

How to rear *A. aegypti*

Adults:

Adults collected in the field or obtained from field collected or laboratory reared larvae are kept in cages of various sizes.

A. The colony cage is a wooden cage of 56 x 30 x 30 cm. The back is completely covered with hard board, the sides with netting and the front side partly with netting and partly with hardboard and a sleeve for access. This cage can keep up to 500 adult mosquitoes.

B. The gallon cage is a plastic container of 25 cm diameter and 10 cm height. The container is covered with fine netting. Access to the container is again through a sleeve. The cage can keep up to 150 adult mosquitoes.

C. The single pair cage is an ordinary one pint plastic cup of 10 cm diameter and 9 cm height. This cage also is covered with fine netting on top. Access is through a circular hole in the bottom covered by a rubber membrane with a slit.

Single pair cages (= one pint ice cream cups) and gallon cages (= plastic crispers) are obtained from EMCO PLASTICS in Mombasa. The colony cages are made in a carpenter's workshop from wood and ordinary plastic mosquito screening.

The adult mosquitoes are given blood meals two or three times a week from mice or guinea pigs which have been anaestesized with Nembutal solution. The solution is prepared from commercially available Nembutal in the following manner:

Nembutal	3 ml
Absolute alcohol	12 ml
Distilled water	45 ml

The dosage depends on the weight of the mouse. For each 10 gr body weight 1 ml of the solution is injected intraperitoneally.

Eggs:

Eggs are collected on moist paper towelling. A paper cup lined with

a piece of paper towelling is put into a colony or gallon cage which contains mosquitoes. For single pair cages paperlined vials of 2 cm diameter are used. The paper cup or vial is left for three to seven days. Then the remaining water is drained off and the container put into an egg box to air dry slowly for three days. At the same time the paper cup is replaced by a new one. When the egg paper is dry it is put into a small plastic bag, labelled and stored in a plastic container and used when needed. Eggs of Aedes can be sotred for up to 12 months.

Larvae:

When eggs hatch the larvae are distributed in plastic pans of 30 x 30 x 10 cm, about 200 per pan, paying attention that there is no overcrowding. Feeding depends on the number of larvae in a container. Liver powder is recommended as food for larvae. Ground dog food or ground mouse or guinea pig food can also be used.

Pupae:

Depending on temperature the larvae will grow to pupae in 5-10 days. At about 28°C the first pupae will appear 5 days after hatching. Then the pupae are separated from the remaining larvae in emergence cups. These are plastic cups covered with a chimney the top of which is closed by netting. If need arises the pupae are sexed. The female pupae are bigger than the males. Pupae do not feed. When the adults emerge, they are put in a cage and treated as explained for adults.

Emergence cups are one pint plastic cups obtained from OLYMPIC PLASTICS, Mombasa. Emergence chimneys are made from the same cups by cutting off the bottom and covering the top with netting.

How to make a spray catch:

Material needed:

1. Test tubes
2. Forceps
3. Sticky labels
4. Torch
5. Pencil
6. Pyrethrum insecticide
7. SOLO sprayer
8. ULVA ultra low volume hand sprayer (battery driven)
9. White sheets (americani)

Procedure:

Before spraying a house white sheets are spread out evenly over floors and beds. For spraying a pyrethrum insecticide (FARUTOX) is used following the manufacturer's instructions. Two people do the spraying. One, with the SOLO sprayer on his back, sprays outside around and through the eaves to avoid

the escape of mosquitoes. The other, with the ULVA sprayer, sprays inside the house, under beds, on loose hanging materials on the roof and on loose hanging clothes. After spraying the insecticde is allowed to work for 10 minutes. Then, using a torch, the dead mosquitoes are collected with forceps or battery driven aspirators and put into a tube. The mosquitoes are identified, the name of the village, house number and date noted and everything recorded in a note book.

The marking of mosquitoes with fluorescent dusts

I was involved in an experiment which was designed to find out at what age A. aegypti females mate in the field. Pupae were collected from domestic water containers with a pipette, sexed and then kept separately in plastic cups on moist cotton wool which is covered with paper towelling. The cups were covered with netting to prevent escape. The cups were labelled and left until the pupae emerged.

Within 24 hours of emergence the adults were transferred into a small cage for marking. The fluorescent dust was blown into the cage with a blower made from a rubber bulb and a pipette. Further blowing and shaking ensured that the dust settled on all mosquitoes. After marking, the mosquitoes were released in selected houses. Each day, i.e. every 24 hours, a different colour of dust was used such that the age of recaptured mosquitoes could be recognized as multiples of 24 hours.

Recaptures were made by landing biting catches, whereby each mosquito was kept singly in a glass tube. The mosquitoes caught each day were checked for fluorescence under an UV light in a dark room. Since the releases were made each day in another house of the village, the recaptured marked mosquitoes indicated also the intensity of movement from house to house. The marked females were dissected for insemination and thus it could be observed at what time after release they were inseminated.

Dissection for insemination:

Material needed:

1. Microscope slides
2. Coverslips
3. Forceps
4. Entomological pins mounted in wooden applicator sticks (for use as dissecting needles)
5. Petri dish
6. Saline solution

Procedure:

Female mosquitoes are selected either from the field or from laboratory reared ones. A female is anaesthesized with ether, then put in a drop of saline on a slide for observation through a dissecting microscope. The mosquito is held at the base of the abdomen on the ventral side with an entomological pin. The abdomen is pressed with a second pin and the tip torn off

slowly. The three spermathecae will remain fixed to the eighth segment and can easily be pulled out of the abdomen. They are crushed by placing a coverslip on top and pressing slightly with a forceps. If the specimen is fresh and inseminated, threadlike structures, the sperms, will be seen moving actively in the saline. The use of a phase contrast microscope facilitates the observation of sperm (magnification 100-200 x). Alternatively the crushed spermathecae can be dried and then stained with acetocarmine. The sperms will show up as red threads under the same magnification.

Control of A. aegypti in a selected village

This experiment was conducted by myself and with the assistance of two field helpers. General guidance was provided by Dr. W. Hausermann. The purpose of this experiment was threefold. First to find out, whether control of domestic A. aegypti by source reduction would be feasible. Second to see how long it takes to get rid of all A. aegypti eggs which are stored inside domestic containers. Third to see how long it takes until the population reaches precontrol level after control measures are stopped.

Design of experiment:

Kipevu was the experimental village. It had 22 houses which were given white numbers. Eighty five people were living in the village. The village was situated in Rabai Location, at Mbumo wa Patsa sublocation. During the period of observation, there were in the average 34 water containers in the 22 houses. Forty neighbouring houses in all four wind directions were designated. The houses closest to the experimental village were about 200 meters away. These 40 houses were given blue numbers. They harboured an average of 47 water containers. This experiment was arranged in three phases. Precontrol, control and recovery. What was done during these three phases is summarized in table 1.

Results:

The results of the experiment are shown on Table 2. Before control started the container index (= percentage of containers with larvae or pupae) in the experimental village was 76,5% and the landing biting catch was 3.30 A. aegypti per man hour (2.08 ♀♀). In the neighbouring houses the container index was 49% and the landing biting rate was 2.25 A. aegypti (1.35 ♀♀) per man hour.

In phase two, after cleaning all pots in the village five times in weekly intervals, the container index dropped gradully to 11.8%. The landing biting catch on the other hand dropped immediately after spraying to 0.03 A. aegypti per man hour. Despite the effort, however, it never reached zero for even a period of a week. In the neighbouring houses the container index was during the same period 39.2% and the landing biting catch 1.76 mosquitoes per man hour, which was lower than in the precontrol phase.

In the first week of recovery the container index climbed up again to 18.8% and the landing biting catch to 0.30 mosquitoes per man hour. In the neighbouring houses no breeding index was made but the landing biting catch

was 1.26 mosquitoes per man hour. Within the next four weeks the container index climbed steadily to 97.3% and the landing biting catch to 2.79 A. aegypti per man hour. An increase in landing biting and breeding rates was also observed in the neighbouring villages.

Discussion and conclusion:

While regular cleaning of water pots might prevent domestic A. aegypti from establishing itself in a village it seemed not to be a very efficient way to get rid of an established population. The method of removing all mosquito eggs in domestic water containers with brushes was not 100% effective. The reappearance of first instar larvae within 24 hours after cleaning indicates that not all eggs were removed in the cleaning process. The rough inner surface of these pots provides ample hiding places for eggs to survive the most vigorous brushing. On the other hand the use of chemicals is not advisable because the containers are used to keep drinking water. The best would be to use hot water or steam in order to kill all eggs of A. aegypti. This procedure was contemplated, but then thought too cumbersome and uneconomic, since the hot water would have to be prepared on the spot and over fires in large quantities.

During the observation period immigration seemed to be negligibly small as exhibited by the low landing-biting rate during the control phase. From other experiences it was expected that immigration would be substantial and its lack must be judged exceptional and attributed to the unusually low mosquito density in the neighbouring villages.

Insecticide testing

Material needed:

WHO insecticide test-kit for adult mosquitoes containing:

1. Insecticde impregnated papers (DDT and Dieldrin)
2. 20 plastic tubes with 10 slides, colour coded; red for insecticide, green for holding mosquitoes.
3. Brass spring clips for insecticide paper, silvery spring for control paper and for ordinary paper in the holding tube
4. Aspirators
5. Recording forms
6. Ordinary white papers

Procedure:

The preferred mosquitoes for the test are females which have had a blood meal or newly emerged females.

The preliminary test is done with all concentrations of the available insecticide: 0.25, 0.5, 1, 2, and 4% in the case of DDT. The concentrations increase by doubling. The insecticide paper is rolled into a cylinder to line the wall of the test tube and fastened with the brass spring clip.

Then an ordinary paper is rolled into the tube marked with green dots and fastened with a silvery spring clip. The tubes containing the insecticide papers are marked with red dots. The tubes marked green are for holding the mosquitoes before exposure and after exposure. One tube is lined with a control paper impregnated with oil alone, without insecticide. This tube is also used for the one hour exposure, but is marked green.

With an aspirator 15-26 mosquitoes are put into each of the holding tubes through the filling hole. The mosquitoes are then transferred into the testing tubes, five with the different insecticide concentrations and one with oil paper alone. For one hour they are left exposed to the insecticide under conditions of moderate, diffuse illumination. After 1 hour the mosquitoes are gently blown back into the holding tubes. They are left there for 24 hours at a shady place, where the temperature does not go beyond 30°C. If practicable, the maximum and minimum temperatures of the room should be noted during the 24 hours. The tubes are protected from ants by placing them on a table which stands in plastic pans of water or oil. After 24 hours record the mortality. The dead mosquitoes are removed from the holding tube after unscrewing it from the separating slide. Mosquitoes that are unable to walk are counted as dead. The living individuals are removed with the aspirator. Anaesthetics should not be allowed to come into contact with the plastic tubes. Each impregnated paper is used for three weeks or not more than twenty times, after which it is discarded. When the impregnated papers are removed from the package it should be resealed with plastic tape. The insecticide kit should be stored in a cool place, but not in a refrigerator.

Results of the first test

The first test was made with domestic *A. aegypti* mosquitoes from Rabai Location, where DDT was sprayed against Anophelines in 1950. The dose response curve indicated no DDT resistance. LC 50 was 1.65% for young adult females.

Plans for the future

Insecticide testing will continue with mosquitoes collected from different areas along the Kenya Coast under the auspices of the Division of Vector Borne Diseases of the Kenya Ministry of Health. The value of this test lies in its possibility to recognize existing insecticide resistance. It also permits to monitor the selection for insecticide resistance in mosquito populations which are under control. Ideally such insecticide tests should be carried out before control actions with insecticides are started.

Table 1: Activities in the Kipevu control experiment

Phase	Activity	Amount of effort
Week 1	Kipevu:	
	1. Landing biting catch	22 houses, 2 men, 2 day, = 32 man hours
Precontrol	2. Larval index	22 houses, 34 containers, 1 man once = 8 man hours
	Neighbouring houses:	
	1. Landing biting catch	40 houses, 2 men, 4 days = 64 man hours
	2. Larval indices	40 houses, 48 containers, 1 man, 2 days = 16 man hours
Weeks 2-7	Kipevu:	
	1. Spray catches	22 houses, 3 men, 6 days in weeks 2, 3, 4 = 144 man hours
Control	2. Cleaning water pots	22 houses, 34 containers, 3 men, 7 days = 168 man hours
	Neighbouring houses:	
	1. Landing biting catches	40 houses, 2 men, 3 days = 48 man hours
	2. Larval indices	40 houses, 48 containers, 1 man, 3 days = 24 man hours
Weeks 8 - 14	Kipevu:	
	1. Landing biting catches	22 houses, 2 men, 5 days = 80 man hours
	2. Larval indices	22 houses, 34 containers, 1 man, 5 days = 40 man hours
	Neighbouring houses:	
	1. Landing biting catches	40 houses, 2 men, 5 days, = 40 man hours
	2. Larval index	40 houses, 48 containers, 1 man, 1 day = 8 man hours

Table II

Phase		I:Precontr.	II: Control						III: Recovery				
Week		1	2	3	4	5	6	7	8	9	10	11	14
Experimental village	L-B catch:												
	No.mosquitoes per man hour	3.30	No L-B catch made	0.41	0.10	0.10	0.05	0.03	0.30	0.10	0.84	1.84	2.79
22 houses, average of 34 water pots	No. females per man hour	2.08		0.14	0.10	0.07	0.05	0.03	0.14	0.10	0.34	0.92	2.18
	% containers with larvae	76.5	No larval survey	88.2	36.6	37.2	25.8	11.8	18.8	47.8	66.2	75.0	97.3
85 people	% containers with pupae	38.2	No pupal survey	58.9	3.3	0	0	0	1.4	19.4	22.6	32.4	32.5
	Spray catch	--	30 fem. 17 mal.	10 fem. 6 mal.	2 fem. 1 mal.	--	--	--	--	--	--	--	--
	Cleaning of water pots	***	no cleaning	all ** pots	all * pots	all * pots	all * pots	all * pots	--	--	--	--	--
Control village	L-B catch:												
Neighbouring houses in all directions	No. mosquitoes per man hour	2.25	--	--	1.74	1.82	--	1.76	1.26	1.57	1.20	2.32	3.27
	No. females per man hour	1.35	--	--	1.18	1.18	--	1.53	0.83	1.20	0.56	1.16	1.84
40 houses, average of 48 water pots	% containers with larvae	49.0	--	--	61.8	--	39.2	--	--	--	--	--	82.2
151 people	% containers with pupae	17.0	--	--	23.8	--	17.4	--	--	--	--	--	40.0

*** 192 pupae removed ** 1707 larvae, 72 pupae removed * all larvae removed

Listing of translocations presently maintained at the Mosquito Biology Unit of ICIPE

Paul T. McDonald

Translocation		Origin	Marker Stock	Hatch	Pseudolinkage	Homozygosis −	Homozygosis +
1 : 2	a	Mkwaja	Red	50%	523/611 = .86	0	0
	b	Mkwaja	Red	50%	1201/1240= .97	3	0
	c	Mazeras	Rust	50%	137/148 = .93	6	0
	e	Mazeras	Rust	50%	97/114 = .85	9	0
	f	Mazeras	Rust	40%	24/24 = 1.00	8	0
	j	Mazeras	Rust	50%	33/37 = .89	3	0j
	kk	Mazeras	Rust	50%	33/37 = .89	8	0
	l	Mazeras	Rust	50%	72/87 = .83	.4	0
	m	Mazeras	Rust	40%	43/46 = .93	10	0
	n	Mazeras	Rust	50%	13/13 =1.00	16	0
	o	MNAZI	Rust	40%	53/62 = .85	14	0
	r	MNAZI	Rust	50%	31/38 = .82	1	0
	s	Ganga	Red	50%	108/143 = .76	13	0
	t	Ganga	Red	30%	59/59 =1.00	4	0
	u	MNAZI	Rust	35%	259/277 = .94	13	0
	v/w	Mazeras	Rust	50%	182/222 = .82	26	0
	x	Kipevu	Rust	50%	50/58 = .86	0	0
	z	Kipevu	Rust	60%	97/108 = .90	13	0
	ad	Kikomani	Rust	50%	8/10 = .80	0	0
1 : 3	b	Shauri Moyo	Red	50%	1313/1324= .99	3	0
	e	Mazeras	Rust	50%	124/127 = .98	5	0
	f	Mazeras	Rust	50%	185/190 = .97	14	0
	g	Mazeras	Rust	50%	193/203 = .95	6	0
	i	Mazeras	Rust	50%	23/26 = .88	2	0
	m	Ganga	Rust	50%	65/93 = .70	6	0
	n	Kipevu	Rust	50%	73/83 = .88	3	0
	p	Kikomani	Rust	60%	122/123 = .99	22	0
	q	Kikomani	Rust	50%	18/21 = .86	0	0

Translocation	Origin	Marker Stock	Hatch	Pseudolinkage	Homozygosis −	Homozygosis +
2 : 3 b	Mazeras	Rust	50%	252/319 = .79	16	0
c	Mazeras	Rust	50%	207/259 = .80	13	0
d	Mazeras	Rust	50%	102/107 = .95	10	0
e	Mazeras	Rust	50%	111/115 = .97	14	0
f	Mazeras	Rust	35%	195/234 = .83	3	0
g	Mazeras	Rust	60%	229/240 = .95	11	0
j	Mazeras	Rust	50%	245/260 = .94	14	0
l	Mazeras	Rust	50%	124/140 = .89	14	0
n	Mazeras	Rust	60%	161/208 = .77	3	0
p	Mazeras	Rust	50%	173/197 = .88	10	0
r	MNAZI	Rust	50%	170/171 = .99	7	0
u	MNAZI	Rust	30%	112/117 = .96	0	0
x	MNAZI	Rust	50%	189/219 = .86	18	0
z	MNAZI	Rust	50%	333/351 = .95	9	0
aa	Kikomani	Rust	50%	19/23 = .83	0	0
ac	Kikomani	Rust	50%	53/59 = .90	7	0
ad	Kikomani	Rust	50%	27/32 = .84	0	0
mb (1:3 only)	Mazeras	Rust	35%	(1:3 only) 259/283 = .92	0	0

Notes for Mombasa Project (AID) Renewal
(Project Appraisal Report from Bill Long of May 31, 1973)

K. S. Rai July 6, 1973

1. (Point E, para. 1, Page 3 of PAR) During the next approximately six months (July-Dec. 1973), "particular stress will be placed ----- to obtain requisite genetic translocations" in village populations of A. aegypti near Mombasa. Approximately 40 translocations have been already recovered from these villages. Some of these may yet produce viable homozygotes. This has been one of the major thrusts of our work in Mombasa during the last two years. However, during the next year, particularly during the next six months, this area will receive ever greater emphasis. It is hoped that this work will yield needed homozygotes which will then be released in one or more target populations sometime during January-July, 1974.

Backup Strategy: In the unlikely event that no translocation homozygotes from the African populations of A. aegypti are available by December, 1973, one or both of the following steps may be taken:

A. As a result of the work done at the University of Notre Dame during the last two years, the following translocation homozygotes have been recovered:

Homozygotes in ROCK Stock: Out of approximately 30 radiation-induced translocations isolated from the wild type laboratory stock, ROCK, two yielded viable homozygotes - one for a sex-linked T (1:3)b (designated T_1T_1) and one for an autosomal T(2:3)c translocation ($=T_2T_2$) (Lorimer et al 1972). Their mean fertility was 19% and 55% respectively when sib-mated and 45% and 40% respectively when outcrossed to Delhi females. After true-breeding stocks of these homozygotes were established at the University of Notre Dame, they were colonized at the World Health Organization Research Unit on Genetic Control of Mosquitoes in Delhi.

Preliminary data were obtained on the survival rate, insemination capacity and mating competitiveness of the two homozygotes in the original ROCK genetic background and the double heterozygotes, T_1T_2, T_2T_1, obtained by reciprocally crossing the two. Field cage tests of their survival from first instar larvae to four-week-old adults showed that the performance of the two homozygotes was inferior and that of the double heterozygotes superior as compared with the Delhi control. There was not much difference in the insemination capacity of the various stocks tested except that T_1T_2 double heterozygote performed a little better than the Delhi control. The mating competitiveness of T_2T_2 and the two types of double heterozygotes was similar to that of Delhi males whereas T_1T_1 did poorly. Thus by all the criteria tested, the double heterozygotes did as well or better than Delhi males. This may be due to heterosis. The sterility of the double heterozygotes is approximately 75%.

Homozygotes in Delhi Stock: Forty-five translocations induced in the Delhi stock have been tested for homozygosis. Of these, two T(2:3)12M and T(1:3)6L have yielded viable homozygotes. Their fertility is 59% and 55% respectively when sib-mated and 82% and 88% respectively when outcrossed with Delhi stock.

All these four translocation homozygotes will be made available for evaluation against African populations.

Scientists from the University of Notre Dame are currently in Delhi and based on laboratory and field population cage studies, they are attempting to identify two or more suitable translocation homozygotes for releases in one experimental village and the most promising double heterozygote among them in another village. The choice of translocations for field releases will depend on which give the most fertile homozygotes and the most sterile single and double heterozygotes. Releases of two types of homozygotes would be more efficient if the homozygotes are fully competitive, but if not, the releases of double heterozygotes would be more effective.

By December 1973, the most suitable translocation combinations would have been already identified from actual field releases at the W.H.O. Unit in Delhi. The same with appropriate modifications could be tested for population control potential in Mombasa. From preliminary indications, the two homozygotes in the Delhi field stock and the double heterozygote among them may be more suitable for releases in Mombasa.

B. Two suitable translocation heterozygotes isolated from Mombasa could themselves be crossed to produce double translocation heterozygotes (with approximately 75% sterility). These could in turn be released in field populations in Mombasa.

The mass production of double heterozygotes from two semisterile translocation heterozygote stocks would be a little more difficult. However, in view of the relatively small size of target populations in coastal villages of Mombasa, this should not present much of a problem during the evaluation phase of our field releases.

2. Coordination with the W.H.O. India Project
(Point E, page 3, last paragraph, of Mr. Long's PAR)

The entire planning and execution of the A. aegypti project at the W.H.O. Unit in Delhi has been and is being done by Prof. K. S. Rai, one of the Principle Investigators of the U.S. AID Mombasa project. During the last three years, Rai has visited this project and spent one week to three months on seven different occasions. On one of these occasions, he spent a week in Mombasa on his return from Delhi. Two graduate students from his laboratory are currently in Delhi (N. Lorimer and E. Hallinan) making field releases of the translocation homozygotes.

Thus the coordination with the Delhi Unit is already ideal. Nevertheless, in order to strengthen it even further, it is proposed that:

A) on their return from Delhi next October, E. Hallinan and N. Lorimer spend a week or so in Mombasa.
B) Dr. P. T. McDonald visit the Delhi Unit in August 1973 while field releases of translocation stocks are underway.
C) The strategy of field releases in Mombasa is planned based on the results of translocation releases in Delhi. Prof. Rai will coordinate this aspect between the Delhi and the Mombasa Units.

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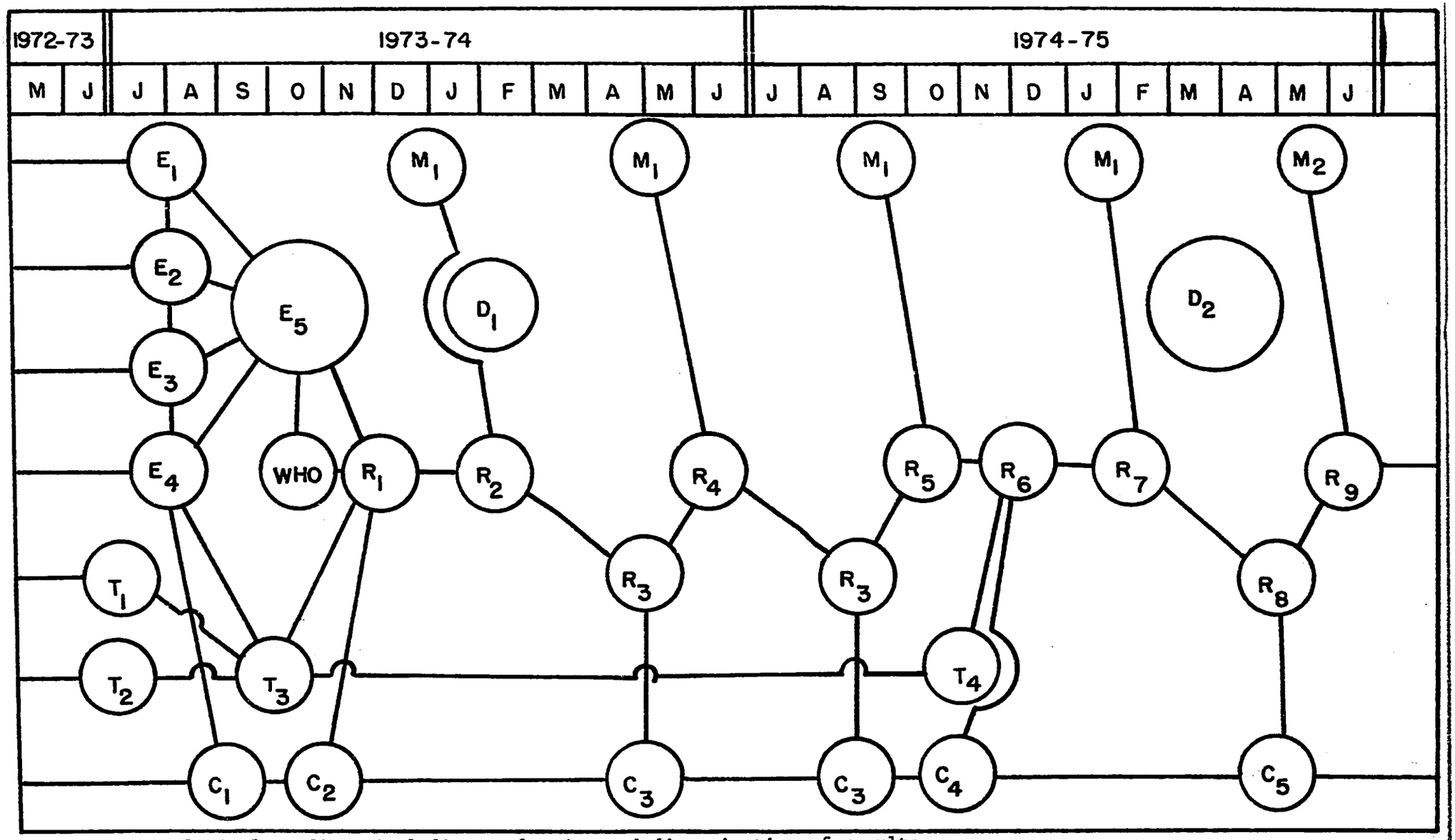
ANNEX 18. MBU Project outline for years 3 (1973-74) and 4 (1974-75), Plan A

Code	Activity	Timing
E_1	Environmental setting: Description of coastal environment with regard to factors (climatic, biotic, human) determining A. aegypti habitats	July 1971 - August 1973 (ongoing monitoring of climate until end of project)
E_2	A. aegypti as response system: Gene pool, gene frequencies, population structure, reproductive potential	Jan 72 - Oct 73
E_3	Response variable: Distribution and seasonal abundance, absolute population size and dispersal, survival and reproduction	July 71 - Aug 73
E_4	Behavioral characteristics affecting genetic manipulations through releases (habitat selection, selection of mating site and time, host selection)	July 72 - June 73
E_5	Systems diagram: A. aegypti populations on the Kenya Coast	Aug - Oct 73
T_1	Production of translocation heterozygotes and homozygotes in Mombasa	Dec 71 - July 73
T_2	Production of translocation heterozygotes and homozygotes at Univ. Notre Dame	Continuous
T_3	Selection of translocation homozygotes for replacement of native populations. Africanization of translocation stock for releases. Behavioral tests of release strains for habitat selection and selection of mating site and time	July - Nov 73
T_4	Selection of double translocation heterozygotes for population suppression. Africanization of translocation stocks, behavioral tests of released males for habitat selection and mating efficiency in the field	July - Nov 73
R_1	Select release strategy for population replacement based on the A. aegypti system and computer simulation of releases	Nov - Dec 73
R_2	Release of translocation homozygotes in hot, dry season	Jan - Mar 74
R_3	Evaluate release results and redefine strategy after each release	Apr - May 74

Code	Activity	Timing
R_4	Release of trans. homozygotes, rainy season	May - July 74
R_5	Release of translocation homozygotes in season of intermittent rains	Sept - Nov 74
R_6	Select release strategy for population suppression by double translocation heterozygotes based on the A. aegypti system and computer simulation	Nov - Dec 74
R_7	Release in dry season	Jan - Mar 75
R_8	Evaluate release results and redefine release strategy	Apr - May 75
R_9	Release in rainy season	May - July 75
C_1	Analyze ecological data by computer	June 72 - June 74
C_2	Develop computer model to select release strategies for population replacement by translocation homozygotes	Oct 73
C_3	Analyze release results, refine computer model to improve release strategy	Apr- May 74 Aug - Sept 74
C_4	Develop computer model to select release strategies for population suppression by double translocation heterozygotes	Oct - Nov 74
C_5	Analyze results of release for population suppression, refine computer model to improve release strategy	Apr - May 75
M_1	Mass production for release of translocation homozygotes	Dec 73 - Mar 74 Apr - Jul 74
M_2	Mass production for release of double translocation heterozygotes	Aug - Nov 74
D_1	Demonstration of techniques used in ecological studies, mass production and releases to DVBD personnel (Kenya Medical Dept.) and to Municipal Health Authorities of Mombasa. Involvement of selected personnel of these institutions in the actual release work	Continuous
D_2	Dissemination of ecological findings, release techniques and release results to interested parties	Feb 75 onwards
WHO	Visit to WHO Unit in Delhi for exchange of information on genetic control	Aug 73

MBU: Project outlines for 1974/75

Activity network plotted with time



Codes: E = Ecological studies, including evaluation and dissemination of results.
T = Production of translocations and Africanization of release strains.
C = Computer analysis of ecological & release studies; modeling & simulation in preparation of releases.
R = Release studies: Strategy, actual releases and assessment of impact.
M = Mass rearing. D = Dissemination of information gained to date.
WHO = Correlation and information exchange with WHO project in India.

MBU Project outline for years 3 (73-74) and 4(74-75), Plan B

PERT Plan prepared in July, 1973, by Drs. Craig, Crovello, Hausermann, McDonald and Fanara at the MBU laboratory, Mombasa, Kenya.

After extensive discussion among all personnel and guided by the purposes of the current AID-Notre Dame Research Contract (No. AID/csd-3159), the following PERT plan evolved. It is more difficult to PERT a project of this sort as compared to one dealing primarily with materials, transport and economic aspects. For example, we cannot say positively that Africanization of translocation stocks will take only two months. Only by ongoing observations and checking in each generation of rearing can one be sure of an adequate degree of Africanization. Nevertheless, we believe the following PERT plan provides a feasible and reasonably optimal approach which should achieve the goals for years 3 and 4 of the contract.

Explanation of the PERT Plan

The Plan proceeds chronologically from left to right on the chart. Horizontally, it is divided into three sections: Administration, Work to be Done at Mombasa (MBU), and Work to be Done at Notre Dame. The following guidelines should make the PERT Plan more understandable:

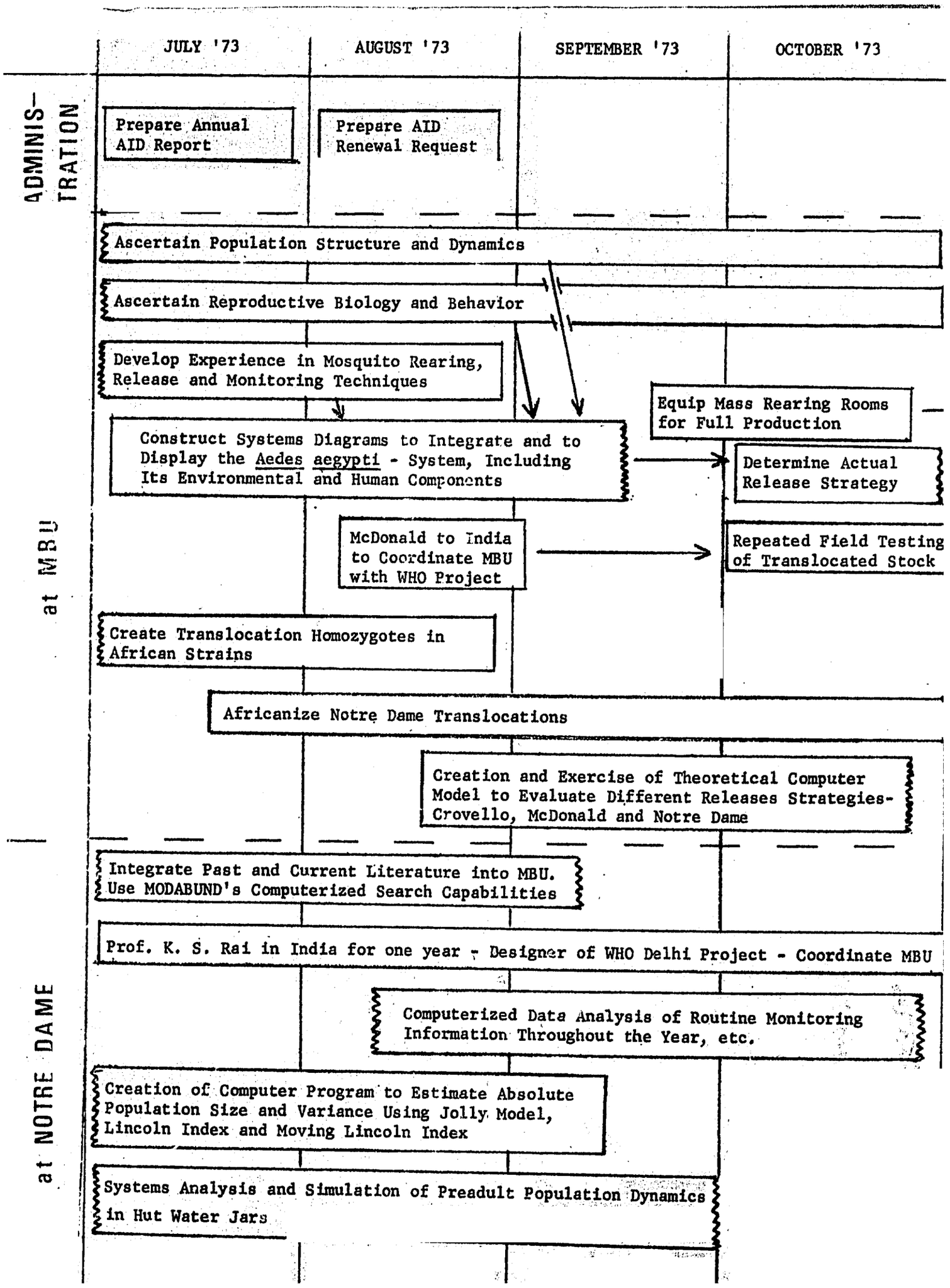
1. Each activity is contained in a rectangular box.

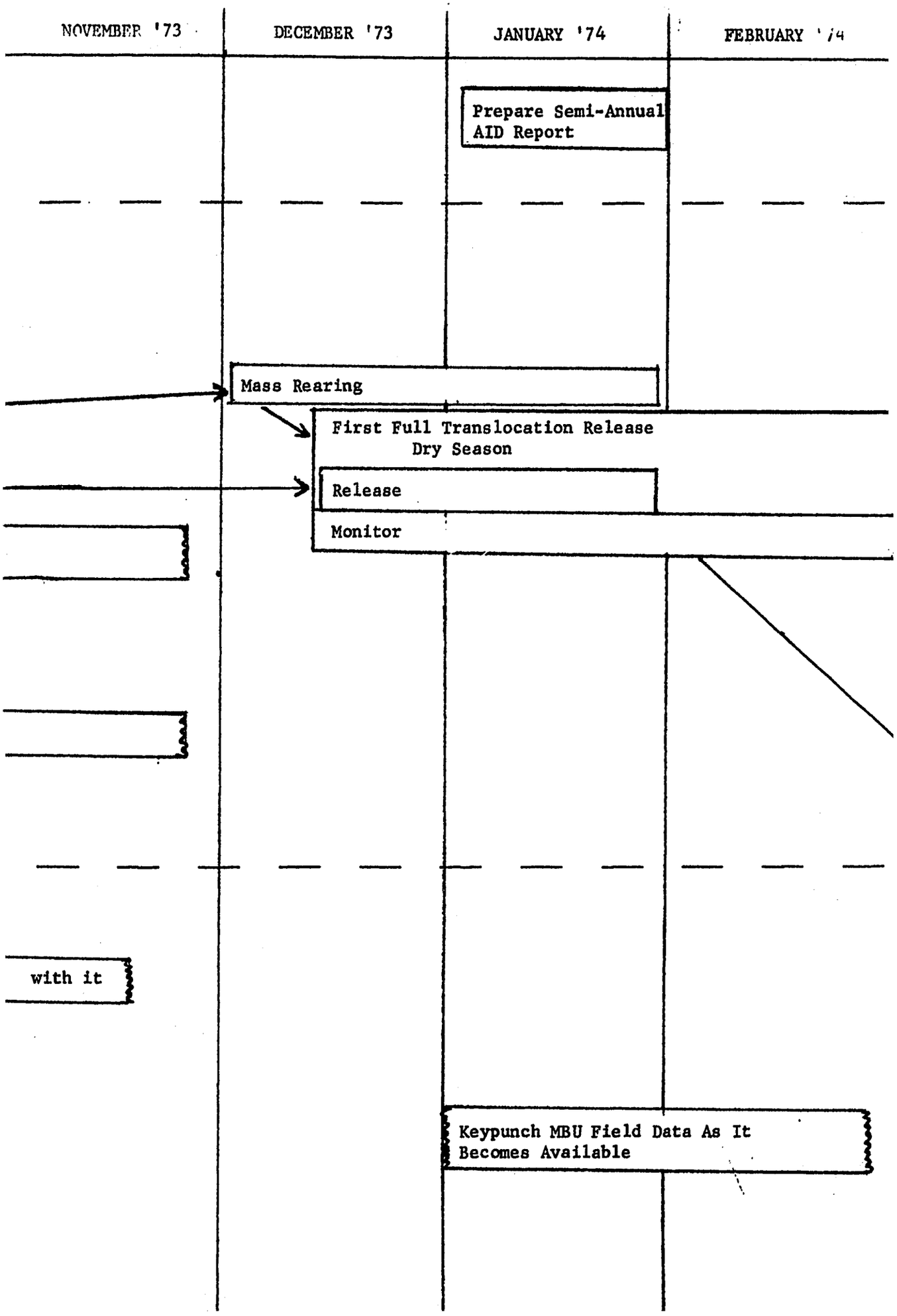
2. Because of the complexity of the project and the high number of activities, it is impossible to include all connecting arrows between activities. Readers should know which activities are prerequisites for others.

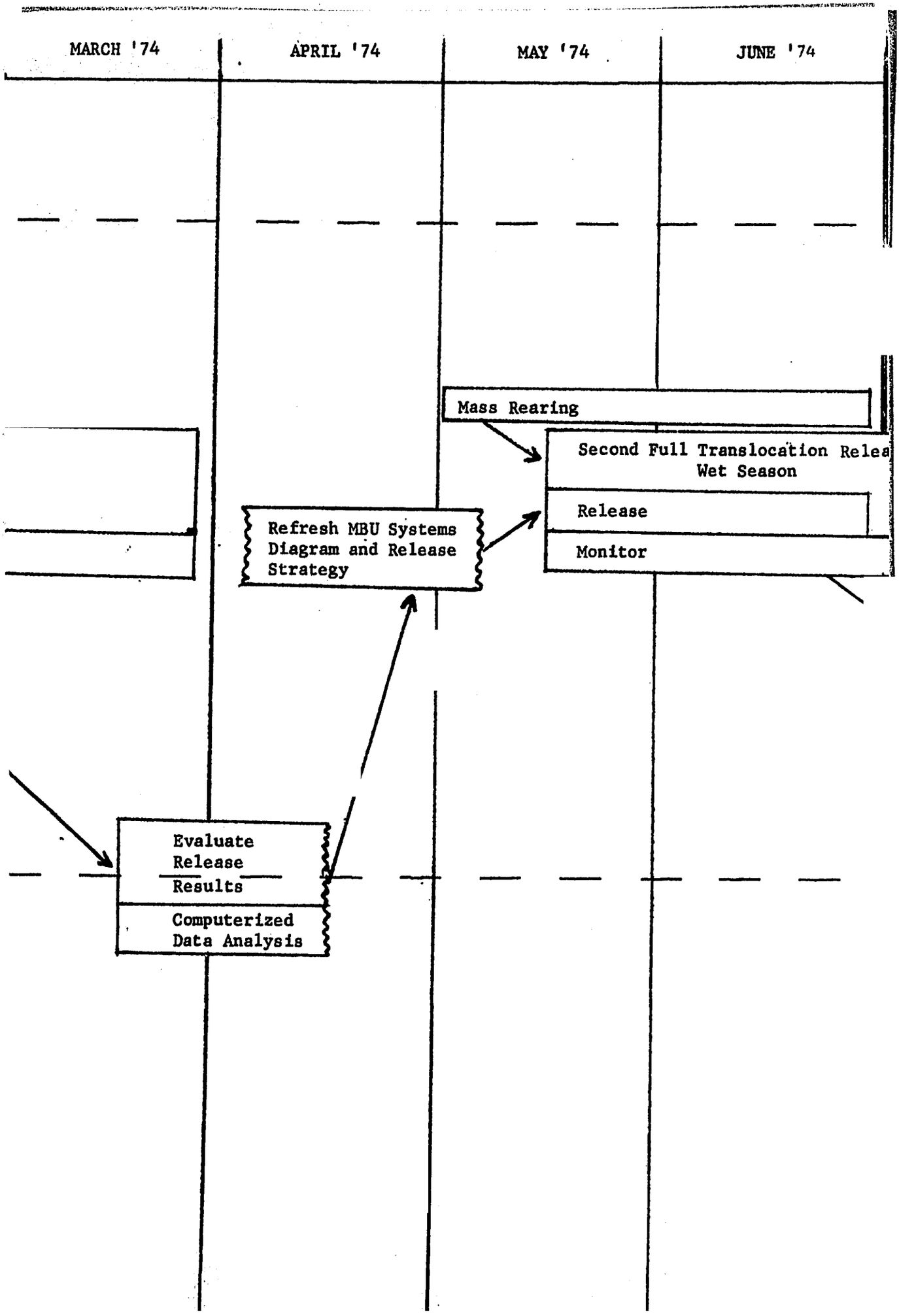
3. To make the PERT Plan more readable, some activities which extend over a long period of time are so indicated by a wavy vertical line. Such a line at the beginning of a box indicates that the activity is already under way. Similarly, a line at the end of a box indicates that the activity continues over future months.

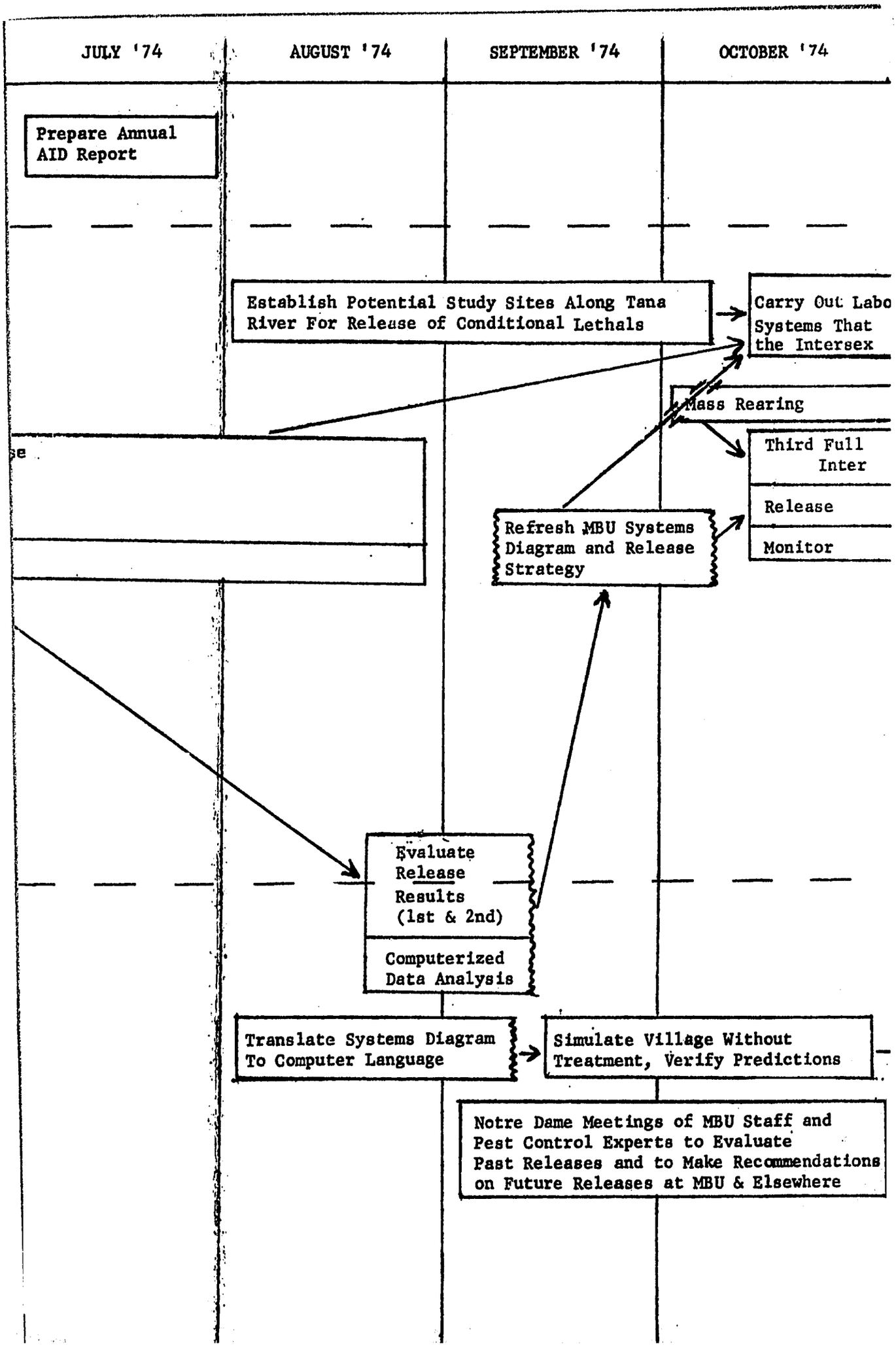
4. Activities found on the horizontal dividing line between MBU and Notre Dame is one that involves substantial work both at MBU and at Notre Dame.

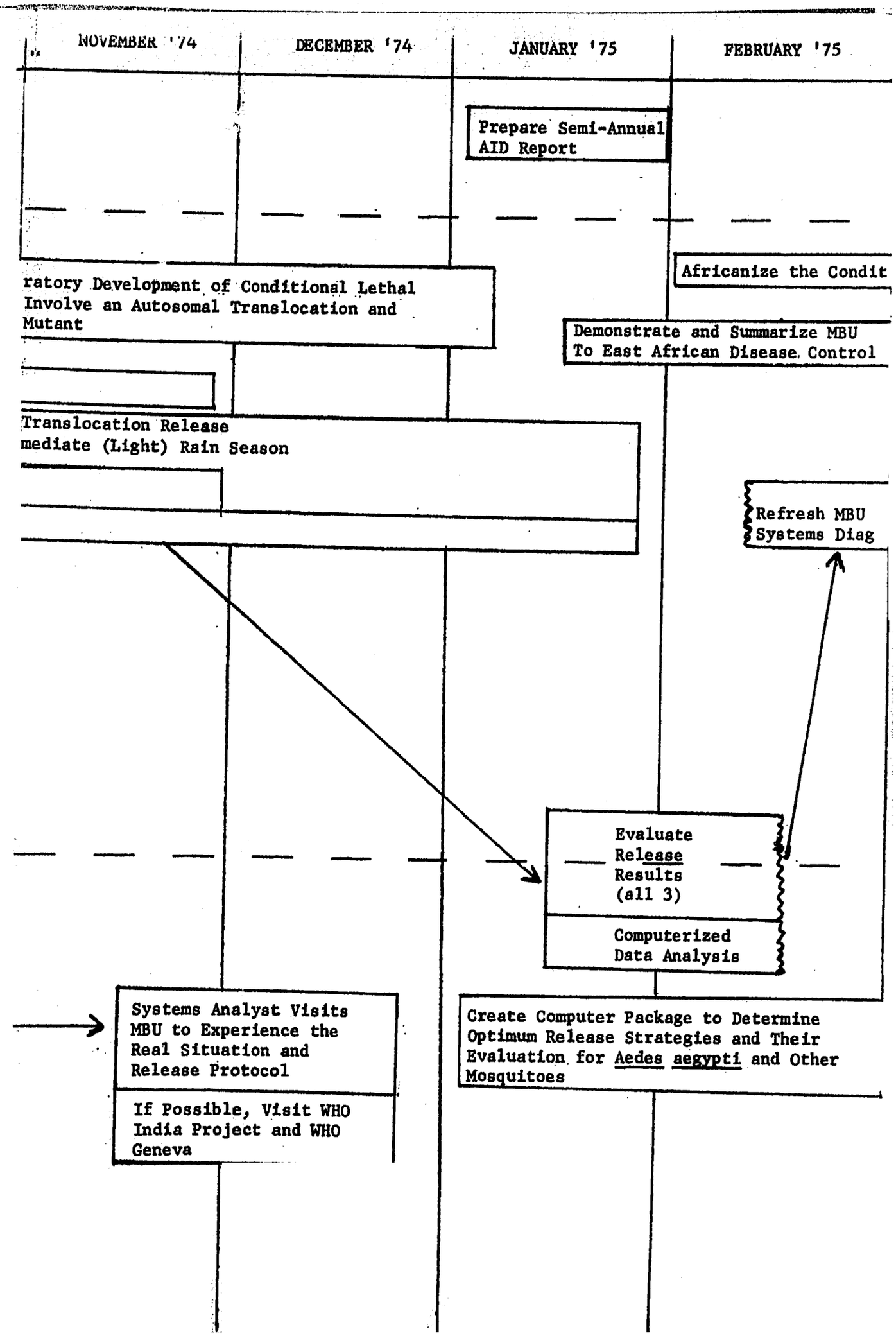
5. Following the PERT Plan, a prose explanation of each activity is given, together with responsible personnel. These descriptions are arranged by the month in which an activity starts. Readers should be aware that routine activities (e.g., vehicle maintenance) are not included in the PERT Plan, although these activities involve a substantial part of each month.

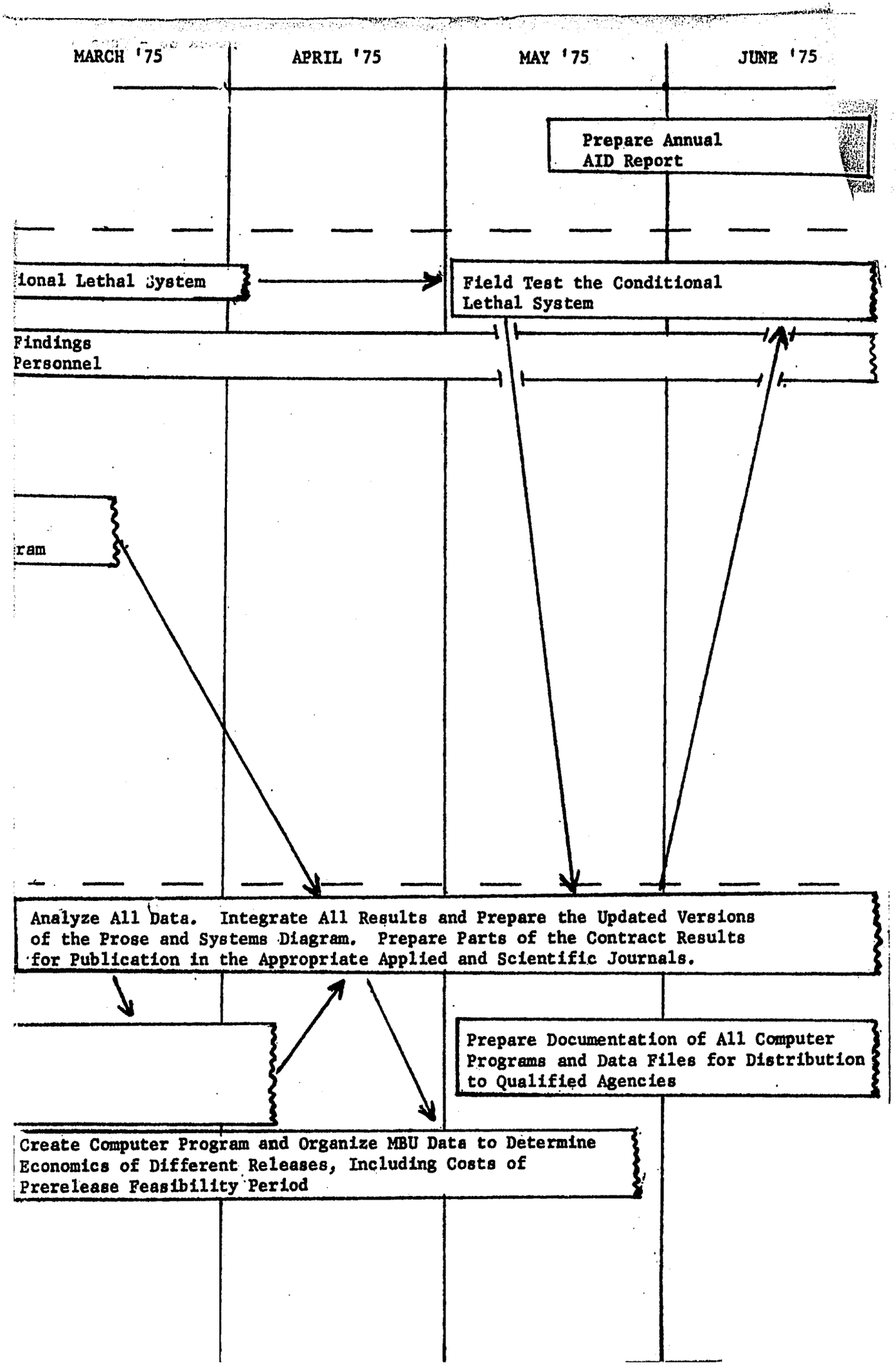

JULY '73
AUGUST '73
SEPTEMBER '73
OCTOBER '73
ADMINIS-TRATION
Prepare Annual AID Report
Prepare AID Renewal Request
Ascertain Population Structure and Dynamics
Ascertain Reproductive Biology and Behavior
Develop Experience in Mosquito Rearing, Release and Monitoring Techniques
Equip Mass Rearing Rooms for Full Production
Construct Systems Diagrams to Integrate and to Display the Aedes aegypti - System, Including Its Environmental and Human Components
Determine Actual Release Strategy
McDonald to India to Coordinate MBU with WHO Project
Repeated Field Testing of Translocated Stock
at MBU
Create Translocation Homozygotes in African Strains
Africanize Notre Dame Translocations
Creation and Exercise of Theoretical Computer Model to Evaluate Different Releases Strategies- Crovello, McDonald and Notre Dame
Integrate Past and Current Literature into MBU. Use MODABUND's Computerized Search Capabilities
Prof. K. S. Rai in India for one year - Designer of WHO Delhi Project - Coordinate MBU
Computerized Data Analysis of Routine Monitoring Information Throughout the Year, etc.
Creation of Computer Program to Estimate Absolute Population Size and Variance Using Jolly Model, Lincoln Index and Moving Lincoln Index
Systems Analysis and Simulation of Preadult Population Dynamics in Hut Water Jars
at NOTRE DAME


NOVEMBER '73
DECEMBER '73
JANUARY '74
FEBRUARY '74
Prepare Semi-Annual AID Report
Mass Rearing
First Full Translocation Release
Dry Season
Release
Monitor
with it
Keypunch MBU Field Data As It Becomes Available


MARCH '74
APRIL '74
MAY '74
JUNE '74
Mass Rearing
Second Full Translocation Relea
Wet Season
Release
Monitor
Refresh MBU Systems Diagram and Release Strategy
Evaluate Release Results
Computerized Data Analysis


JULY '74
AUGUST '74
SEPTEMBER '74
OCTOBER '74
Prepare Annual AID Report
Establish Potential Study Sites Along Tana River For Release of Conditional Lethals
Carry Out Labo
Systems That
the Intersex
Mass Rearing
Third Full
Inter
Release
Monitor
Refresh MBU Systems Diagram and Release Strategy
Evaluate Release Results (1st & 2nd)
Computerized Data Analysis
Translate Systems Diagram To Computer Language
Simulate Village Without Treatment, Verify Predictions
Notre Dame Meetings of MBU Staff and Pest Control Experts to Evaluate Past Releases and to Make Recommendations on Future Releases at MBU & Elsewhere


NOVEMBER '74
DECEMBER '74
JANUARY '75
FEBRUARY '75
Prepare Semi-Annual AID Report
Africanize the Condit
ratory Development of Conditional Lethal Involve an Autosomal Translocation and Mutant
Demonstrate and Summarize MBU To East African Disease Control
Translocation Release
mediate (Light) Rain Season
Refresh MBU Systems Diag
Evaluate Release Results (all 3)
Computerized Data Analysis
Systems Analyst Visits MBU to Experience the Real Situation and Release Protocol
If Possible, Visit WHO India Project and WHO Geneva
Create Computer Package to Determine Optimum Release Strategies and Their Evaluation for Aedes aegypti and Other Mosquitoes


MARCH '75
APRIL '75
MAY '75
JUNE '75
Prepare Annual AID Report
ional Lethal System
Field Test the Conditional Lethal System
Findings
Personnel
ram
Analyze All Data. Integrate All Results and Prepare the Updated Versions of the Prose and Systems Diagram. Prepare Parts of the Contract Results for Publication in the Appropriate Applied and Scientific Journals.
Prepare Documentation of All Computer Programs and Data Files for Distribution to Qualified Agencies
Create Computer Program and Organize MBU Data to Determine Economics of Different Releases, Including Costs of Prerelease Feasibility Period

Explanation of PERT Plan Activities, Plan B

July 1973 (Activities beginning or continuing)

1. Prepare Semi-Annual Report to AID: Craig, Hausermann
   Reports of the activities of each scientist are examined and integrated into the Semi-Annual Report, first at Mombasa and then at Notre Dame.

2. Ascertain Population Structure and Dynamics: Hausermann
   Populations in the Rabai study area can be divided into Domestic (indoor), Peri-domestic and Sylvan. Primary emphasis of this project is on Domestic. A village of 10-15 houses will have a population of about 1000 adults, with 50 new adults emerging each day. The amount of migration between villages is low. Thus, each village serves as a separate experimental unit.

3. Ascertain Reproductive Biology and Behavior: Hausermann
   Males disperse fairly uniformly through a village, whereas females accumulate in certain houses. A male can inseminate up to five females. Females are not open to insemination until three days after emergence; females are inseminated only once, for life. Therefore, projected releases must be on a daily basis and even coverage of males must be maintained.

4. Develop Experience in Mosquito Rearing, Release and Monitoring Techniques: Hausermann
   Capacity for sustained production of 40,000 males per day has been developed. Releases are planned for a central point, daily, in each village. Monitoring techniques include those already in use for studies of population dynamics - (1)landing-biting catches of adults, (2) pupal production in water pots, (3) egg production in clay jars (ovitraps).

5. Construct a Systems Diagram to Integrate and to Display the *Aedes aegypti* System, Including its Environmental and Human Components: Craig, Crovello, Hausermann
   This essential activity is an ongoing one for the remainder of the project. It can be considered the framework for understanding the mosquito system in Nature. As each new fact and insight emerges from the field work or the literature, it is added to the systems diagram.

6. Create Translocation Homozygotes in African Strains: McDonald
   In the past two years, 46 translocation heterozygotes have been produced and isolated. One of these is probably available as a homozygote (Annex 15). As a back-up, four other translocation homozygotes are also available (Annex 16), two from Notre Dame and two from Delhi.

7. Africanize Notre Dame Translocations: McDonald
   This involves crossing and then repeated backcrossing of the alien homozygote so that maximum genetic material will come from the village where it is to be released.

8. Integrate Past and Current Literature Into MBU Activities; Use MODABUND's Computerized Search Capabilities: Crovello
   Throughout the AID contract, participant scientists have available a unique access to the mosquito literature. The MOsquito DAta Bank of the University of Notre Dame, MODABUND, by September, 1973, will have files of over 25,000 mosquito literature references from over three decades that can be searched via computer. This has proven extremely valuable in searching older literature for both ecological and genetic data.

9. Prof. K.S.Rai in India for One Year: Rai
   Professor Rai will be in an excellent position to coordinate and integrate results of the WHO project in Delhi with MBU -Mombasa. Exchange visits of personnel between Delhi and Mombasa are planned.

10. Creation of Computer Program to Estimate Absolute Population Size and Variance: Crovello
    Jolly's Model, the Lincoln Index and a Moving Lincoln Index will be compared in order to determine absolute size of a village population. Without such information, realistic numbers for control release can only be guessed. Mark-recapture data from previous MBU activities in villages are used as imput.

11. Systems Analysis and Computer Simulation of Preadult Population Dynamics in Hut Water Jars: Crovello
    Based on field data, this component of the mosquito life history system is being analyzed in detail, since it appears to be the point at which most density-dependent factors operate. It also is a source of density-independent catastrophes caused by the fairly stochastic activities of the women who live in each house.

August 1973

12. Prepare AID Renewal Request: Craig
    The first three years of this project extended from July 1971 through June 1974. The renewal request, at a slightly higher level of funding, will extend from July 1974 through June 1977. The project is actually seen as three 2-year components: (a) site preparation, basic ecology, (b) population replacement by translocations, (c) population reduction by translocations and conditional lethals.

13. Creation and Exercise of Theoretical Computer Model to Evaluate Different Release Strategies: Crovello, McDonald
    We do not have the resources at this point to undertake a detailed computerized systems analysis of the mosquito system and of possible release effects (but see plans for July 1974 and following). Nevertheless, we can carry out a semiquantitative simulation to explore the relative effectiveness of different release strategies, e.g., when and how many to release, etc., and the effect of ecological factors such as rainfall pattern, water jar catastrophes, etc.

14. Computerized Data Analysis of Routine Monitoring Information Throughout the Year: Crovello
    Environmental and mosquito data from several villages are being key punched at Notre Dame. These data will be subjected to a suite of multivariate analyses, including periodicity analysis, to reveal insights into the population dynamics of the system and to provide a firmer basis on which to choose optimal release strategies.

September 1973

15. Visit to India to Coordinate MBU with the WHO-Delhi Project: McDonald
    This includes liaison with Prof. K.S.Rai and Notre Dame graduate students Hallinan and Lorimer now working on the *Aedes aegypti* Section of the WHO Unit for Genetic Control of Culicine Mosquitoes.

16. Equip Mass-Rearing Rooms for Full Production: McDonald
    No new types of apparatus are needed; we only need more of the apparatus already on hand.

October 1973

17. Determine Actual Release Strategy: Craig, Hausermann

18. Repeated Field Testing of Translocated Stock: McDonald
    Data needed on survival, dispersal, mating competitiveness of both sexes.

December 1973

19. Mass Rearing for First Release: McDonald

20. First Large-Scale Translocation Release: Hausermann, McDonald

21. Keypunch MBU Field Data as it Becomes Available: Crovello
    This has been and will continue to be an ongoing activity at Notre Dame. All computer-related projects must rely on accurate transformation of data into machine-readable form.

January 1974

22. Prepare Semi-Annual AID Report: Craig

March 1974

23. Evaluate Release Results: Hauserman, Crovello, Craig
    When post-release monitoring data are available, they will be keypunched and computer analyzed in various ways. The output will serve as a supplement to MBU personnel in their evaluation of the release results, the effects of the season of the year, etc.

April 1974

24. Refresh MBU Systems Diagram and Release Strategy: Hausermann, Crovello
    This is a continuous process whose goal is to maintain the most reliable and detailed summary of the *Aedes* aegypti system.

May 1974

25. Mass Rearing for Second Release: McDonald

26. Second Full Translocation Release: Hausermann, McDonald

August 1974

27. Prepare Annual AID Report: Craig

28. Establish Potential Study Sites for Release of Conditional Lethals: Hausermann, Craig
Release of the heat-sensitive mutant intersex (ix) is proposed. This recessive, autosomal factor is on linkage group 2. Larvae reared at temperatures of 30°C or above result in normal females but sterile, feminized males. This mutant would be released on a translocation homozygote in the cool season; hopefully and theoretically, it would spread through the population via the translocation. Then, in the hot season, the sterility gene would take effect, the males would be sterilized and the population would be eliminated. A study site with high water temperature would be needed; the Rabai Study Area would not be satisfactory but we are interested in the villages along the Tana River. Here there is nothing but indoor-breeding, domestic A. aegypti and the villages are surrounded by hot desert. Further investigation of this and other areas is required.

29. Translate Systems Diagram into Computer Language: Crovello
This is the first task of the systems analyst who will join the project at Notre Dame. He (or she) will probably be obtained from one of the ongoing International Biological Program Biome Projects, with preference given to someone from the crop and pest ecosystem programs. The systems analyst will work closely with Crovello and will have primary responsibility for all computerized activities at Notre Dame from July 1974 onward.

August-September 1974

30. Evaluate Release Results: Hausermann, Crovello, Craig
As in activity #23, but now the results of both the first and second releases will be integrated.

31. Refresh MBU Systems Diagram and Release Strategy: Hausermann, Crovello
As in activity #24, but based on results of two major releases.

32. Simulate a Village on the Computer and Verify Predictions: Crovello
We cannot analyze potential release strategies accurately until we have a reliable model of the unperturbed system.

33. Notre Dame Meeting of MBU Staff and Pest Control Experts to Evaluate Past Releases and to Make Recommendations on Future Releases at MBU and Elsewhere: Craig
This self-explanatory activity is essential for the efficient progress of MBU and also for the use of our results and computer programs by other agencies around the world.

34. Laboratory Development of Conditional Lethal Systems: McDonald, Craig, Rai
See Activity #28. This involves incorporation of the mutant intersex into an Africanized translocation homozygote. Considerable laboratory genetic manipulation will be required.

October 1974

35. Mass Rearing for Third Release: McDonald

36. Third Full Translocation Release: Hausermann, McDonald

37. Systems Analyst Visits MBU: Crovello
The Systems Analyst from Notre Dame must experience the real world of the Rabai villages and observe release protocol, in order to understand more completely what he is modelling.

December 1974

38. Demonstrate and Summarize MBU Findings to East African Disease Control Personnel: Craig, Hausermann
This would involve an invitational meeting with insect control personnel from the ministries of health of Kenya, Tanzania and Uganda, as well as personnel from East African Community laboratories such as Malaria & Vector-Borne Disease (Amani, Tanz.), Arbovirus Disease (Entebbe, Uganda) and Pesticides (Arusha, Tanz.).

39. Evaluate Release Results of All Three Releases: Hausermann, Crovello, Craig
As Activity #30, but now it will involve the integration of data from all three releases.

40. Create Computer Program Package: Crovello
The Systems Analyst will produce a free-standing program package to allow determination of optimum release strategies and their evaluation for Aedes aegypti and other mosquitoes. This package could be used on almost any third generation computer anywhere, for the purpose of gaining heuristic insights into the particular mosquito pest under study, and to evaluate effects of alternate release strategies.

January 1975

41. Prepare Semiannual AID Report: Craig

42. Africanize the Conditional Lethal System: McDonald
See Activity #28 and #34.

43. Refresh MBU Systems Diagram: Hausermann, Crovello
At this point, after the releases and the integration of the resultant data, MBU will have an accurate, detailed systems description useful to other mosquito biologists.

February 1975

44. Summing Up: Craig, Staff
Analyze all data, integrate all results and prepare the updated versions of the prose and systems diagram. Prepare parts of the contract results for publication in appropriate applied and scientific journals.

45. Create Computer Program and Organize MBU Data to Determine Economics of Different Releases, Including Costs of Prerelease Feasibility Studies: Craig, Crovello, Hausermann
This step will be most useful for feasibility estimates of future projects.

April 1975

46. Field Test the Conditional Lethal System: McDonald
Prepare for potential releases in Contract Years 5 & 6.

47. Prepare Documentation of All Computer Programs and Data Files For Distribution to Qualified Agencies and Personnel: Crovello
This is an important step in assuring that the results of the AID Contract are utilized as completely as possible by others.

May 1975

48. Prepare Annual AID Report: Craig

RESOLUTION OF PLANS A AND B, MBU YEARS 3 AND 4

1. Plans A and B are virtually identical for Year 3, July 73 - June 74. They differ only in style of presentation. Both plans are based on 6 years:
   Years 1 & 2: Basic ecology, site preparation
   Years 3 & 4: Population replacement (translocation homozygotes)
   Years 5 & 6: Population elimination
   Plan A - by double translocation heterozygotes
   Plan B - by a conditional lethal on a translocation homozygote

2. Both plans aim for population replacement in years 3 & 4, with a series of three release experiments in different seasons. Releases would be designed as follows:
   a. Two villages - no treatment, monitor as control
   b. Two villages - defaunate by pyrethrum spray & water pot scrub
   c. Two villages - release translocation homozygotes
   d. Two villages - defaunate, then release trans. homozygotes

3. The plans differ slightly in Year 4, depending on strategy selected for Years 5 and 6. This strategy, in turn, depends on results of releases in Years 3 and the early part of 4.