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REPORT TO THE RESEARCH ADVISORY COMMITTEE (RAC)

Subject: Status Of AID/CSD-3159, "Ecological Studies On Aedes aegypti Mosquitoes Preliminary To Genetic Control" (University Of Notre Dame) -- And Recommended Action

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A. Background

This project was approved by RAC in April 1971 for a three-year period at a funding level of \$258,900. It has now run approximately 2 1/2 years and will terminate this coming June if no action is taken to extend the effort.

RAC members may recall that the original University of Notre Dame proposal submitted to it requested a 5-year, \$986,000 project designed to establish both the technical and economic feasibility of genetic control of A. aegypti under East African conditions. The reduced version eventually approved by RAC restricted the research to investigations of the behavior of native A. aegypti populations; the techniques of developing and mass-producing in the laboratory genetically-crippled mosquitoes which would be competitive in the natural environment; and strategies and methods for large-scale releases of transformed mosquitoes. The intent was to limit the project to the "ecological studies" required to lay the basis for subsequent full-scale field testing of the most promising genetic control measures if the research data and overall state-of-the-art of biological insect control warranted continuation of the project beyond the first three years. The RAC decision was based largely on the results of a technical review (requested by RAC in December 1970) which involved experts from, among others, WHO and the USDA. The panel recommended a "go slow" approach given the long-term, high-risk nature of research in this field, and the much larger WHO project on A. aegypti in India.

Monitoring and evaluation of the project over the past 2 1/2 years has been carried out through reviews of progress reports submitted by the contractor (at 6-month intervals); correspondence with a field monitor in USAID/Kenya; and reports by individual TA/OST staff and consultants who have visited the Mombasa field station during trips to East Africa. In addition, a detailed field

evaluation was carried in April 1973, by a three-man team which included M. Rechcigl, AID Research Office; D. Adams, RAC member; and Rajinder Pal, World Health Organization, Geneva. Following the field evaluation, Messrs. Rechcigl, Adams, the TA/OST project manager, and several outside experts met in Washington with the University of Notre Dame principal investigator, George Craig, to consider the recommendations of the field team and to review the future design and implementation of the project.

Shortly after that meeting (in May, 1973), Dr. Craig indicated that he was preparing to request an extension of the project to include a field demonstration phase, given the progress the Notre Dame team was making on the ecological research. He requested that an early decision be made by AID on whether it intended to continue the project because he must provide his four-man field team ample time to arrange for new positions should the work in Kenya be terminated.

AID-TA/OST responded by stating that it would attempt to expedite a decision on extension. However, the contractor was informed that he must first demonstrate his positive response to the request by the AID evaluation team for strengthened project administration and preparation of a detailed work plan, and also for additional research data which appeared to be critical in terms indicating whether an extension of the project was warranted.

In August, 1973, the principal investigator submitted a detailed PERT Plan which covered the third year of the project, plus one additional year; and noted that specific steps had been taken to improve project management. The documentation accompanying the PERT Plan set forth a total 6-year research effort (i.e., three years beyond the existing contract). This expanded program included the following:

- Years 1 and 2: Ecology, site preparation
- Years 3 and 4: Population replacement via translocation homozygotes
- Years 5 and 6: Population suppression via double heterozygotes or by a homozygote carrying a conditional lethal.

Last September, the TA/OST project manager met with personnel from WHO/Geneva (James Wright and Rajinder Pal) and from the IAEA in Vienna (Donald Lindquist) to obtain their perspectives on

whether the AID project should be continued. All three individuals had prior knowledge of the Notre Dame work, and their own institutions are involved in conducting related research. Although they cited problems and shortcomings associated with the Notre Dame project, they were unanimous in recommending that it not be terminated before the ecological work and preliminary field trials are completed.

At the end of 1973, all the ecological data from the Kenya investigations that one would desire to support a decision on project extension was still not available. A particular deficiency was Notre Dame's lack of success with development of the desired type of viable, genetically-crippled mosquito (i.e., translocation homozygote) for field testing. However, it was apparent that further delay (which would preclude a final decision by AID much before the June 1974 termination date) would jeopardize the contractor's ability to keep his key, experienced people associated with the project in any follow-on phase.

On January 14 and 15, 1974, a technical review of the project was held in Washington to assess its status; determine if all the objectives of the three-year contract would be met by the end of June; and evaluate whether the anticipated results coupled with the contractor's program design for years 4, 5 and 6 justify an extension. Members of the review panel were:

- (1) Dr. Germain LeBreque, Insects Affecting Man Research Laboratory, U.S. Department of Agriculture, Gainesville, Florida (past director of the WHO genetic control project in India);
- (2) Dr. D. F. Matzinger, Department of Genetics, North Carolina State University, Raleigh, North Carolina (expert in insect population genetics and statistics);
- (3) Dr. Alan Donaldson, School of Public Health, University of Illinois at the Medical Center, Chicago, Illinois (past director of the Center for Disease Control, Atlanta, Georgia, and involved in restructuring original Notre Dame proposal);
- (4) Dr. David Adams, Coastal Zone Resources Corporation, Wilmington, North Carolina (member of RAC and participant in site evaluation in Kenya last spring);

- (5) Dr. W. S. Bailey, Chief, Parasitology and Medical Entomology Branch, NIH (his office is funding Notre Dame's domestic research in this area and has under consideration a 5-year extension of the approximately \$100,000/yr. grant);
- (6) Dr. Donald King, Department of State (entomologist who participated in earlier review of this project); and
- (7) Dr. John Stivers, AID, Office of Health.

The 1 1/2-day review included a thorough briefing by Dr. Craig and the project field director, Walter Hausermann, on research activities, progress and proposed future plans, followed by detailed questioning by the panel.

NOTE: The remainder of this report (which describes progress to date and makes specific recommendations regarding the future of the project) is based largely on the findings and conclusions of last month's review.

B. Project Results To Date

Attachment A describes the Scope of Work of the research project as it appears in the contract with Notre Dame (AID/CSD-3159). Attachment B is an up-to-date summary of accomplishments prepared by the contractor for the technical review meeting on January 14. The six-month progress reports provided detailed information on the various research activities and results.

Major achievements to date fall into the following categories:

- (1) Establishment of Laboratory and Field Site - The Notre Dame team has established its research headquarters in a rented 14-room house on the shore at Nyali, just north of Mombasa, Kenya. The structure now quarters laboratories, insectaries, mass-rearing rooms, a library, and living space for visiting scientists. It has evolved into a significant research facility on Africa's East Coast, and has recently been designated by the International Center for Insect Physiology and Ecology as its "Coastal Research Centre".

Notre Dame has kept on location in Mombasa three highly-regarded research scientists headed by Walter Hausermann

(Attachment C). The AID field evaluation team, and virtually every other visitor to the laboratory, has commended the scientific competence of the team, and singled out the rapport and excellent working relationships they have established with the natives in whose villages they work. Gaining access to individual living units in some twenty villages to periodically examine even the household water jars is, in itself, impressive.

The field site is located in the Rabai district west of Mombasa, a 20-minute drive from the laboratory. It involves some 20 small villages with an average of 10-25 buildings and a mosquito population of about 1000 (+ 500) adult A. aegypti. Distances between selected villages are adequate to ensure each center is an "ecological island" with no mixing of domestic mosquito populations. Several members of the technical review panel observed that this type of field site should provide an excellent natural laboratory for the conduct of a wide variety of health-related investigations (particularly since it is in proximity to the Coastal Laboratory and because so much will be known about the basic ecology and native mosquito populations.

- (2) Mosquito Population Structure and Dynamics - Baseline data on absolute numbers of A. aegypti in the test villages have been obtained, and studies of the migration between indoor and sylvan niches have been completed. The contractor's last progress report provides details of these studies and the results, and also of related investigations of mosquito reproduction, insemination, dispersal, and gene flow between indoor and sylvan populations.

Much of this work has been carried out through the use of native mosquitoes which are marked in the laboratory, released, and then recaptured. New techniques for trapping and for rapid isozyme analysis have been developed and are routinely employed.

Before field testing of genetically-transformed mosquitoes can be initiated, additional ecological studies must be carried out to obtain more information about dispersal, gene flow between domestic and sylvan populations, and age-structure-fecundity relationships (which requires computer modelling). Work is proceeding in these areas and most (but not all) should be completed by July, 1974.

- (3) Genetic Control Requirements - While the major thrust of the project has been to gain an understanding of mosquito population structure and dynamics (i.e., "ecological studies"), the contractor was also asked to undertake work on technical requirements for genetic control (see Attachment A). Concurrent work in both areas was judged essential if, by the end of year 3, a decision was to be made on whether actual field testing of genetically-transformed mosquitoes should be initiated.

The genetic work has taken place both at the University of Notre Dame in Indiana and at the Mombasa laboratory. It has included research to obtain and mass-produce chromosomal translocations, and investigations of release strategy, including timing and numbers. Research in this area has lagged behind the ecological investigations. However, this was predictable given the greater difficulty of the genetic work and the fact that initial emphasis was placed on the ecological relationships.

Virtually no progress has been made on the genetic engineering component because of the difficulty in producing a promising genetically-transformed African mosquito on which to experiment. The contractor has initially planned to obtain an induced translocation in the "homozygous" condition and base the first field trials on the release of this strain. In theory, the homozygotes should breed with and eventually replace the native domestic species. However, the homozygote must be created through a complicated process of genetic selection from a large population of mosquitoes which have had certain genes "translocated" through radiation treatment in the laboratory. To date, over 100 different translocations have been recovered but only one African homozygote has been produced. The contractor reported to the technical review panel in January that he had just learned that the fitness characteristics of the single homozygote are unsatisfactory and additional research must be carried out. There is consequently no evidence yet that a homozygote capable of competing with native African A. aegypti can be developed and mass produced.

In place of the homozygote mosquito, the contractor now plans to release double heterozygote translocations (which are available). Unlike the former, the double heterozygote will not replace the native population, but should theoretically

reduce and suppress it by imparting sterility. Though the contractor's second choice, the use of heterozygotes is a technique currently being advocated by WHO in India and by many other experts (e.g., Laven in Germany), and represents a potentially promising and untested approach. Thus, this change in plan was not judged by the technical review panel to be "settling for something less" given the unavailability of a homozygote strain, since the work plan called for testing heterozygote translocations in a later stage.

The mass production investigations are continuing. Facilities have been constructed and initial trials with large-scale mosquito rearing have been successful. Work on release strategies is also continuing but has suffered to date from lack of progress on the computer modelling techniques. The technical review panel indicated that a model should be available for predicting the impact of the initial field releases, and then be upgraded by feedback of results prior to further releases.

- (4) Institution Building In Africa - All individual reviewers and review panels have indicated that the project's major achievement to date may be its contribution to stimulating and strengthening African science and technology.

One of the principal AID objectives in supporting the research was to contribute to the development of the International Center for Insect Physiology and Ecology (ICIPE) in Nairobi. In early 1971, this Center was nothing more than a concept, since it lacked financial support. AID financing for the Notre Dame project (which was deliberately tied administratively to ICIPE but separated operationally) was the first infusion of funds into one of ICIPE's five major research areas. The ICIPE director, Thomas Odhiambo, has indicated that this enabled him to obtain additional funding from other potential contributors, each of whom seemed to be waiting for somebody else to take the first step. Today, 22 institutions throughout the world are contributing funds, staff, or equipment. The major support is coming from the UNDP which last January awarded ICIPE a four-year grant of \$3 million. The AID project continues to be, for all practical purposes, the only U.S. contribution to the Center.

Attachment D is a summary prepared by the principal investigator of the project's linkages in East Africa. In addition, 12 Kenyans are employed as laboratory and field assistants. Dr. Craig has made a particular point of his linkages with African institutions, and of the contribution the AID project has made to training and education in the region. He quotes Dr. J. D. Roberts, Chief of the Kenya Medical Department, Division of Vector-Borne Disease, as stating, "Craig and his colleagues have reawakened and put new life into the field of medical entomology in East Africa".

- (5) Project Spinoffs - Attachment B includes a list of "Miscellaneous Spinoffs" from the project that the contractor believes are significant. The technical review panel did not voice any opinion on the value of these. However it was concerned that their further pursuit and development would divert limited manpower and financial resources away from the principal objectives of the project. The panel urged that future spinoffs and peripheral activities be pursued with supplemental (non-AID) sources of support.

C. Performance Evaluation

The AID project management office (TA/OST), basing its evaluation on contractor progress reports, the field evaluation, and the January technical review, considers the performance to date to be generally satisfactory. While all original research objectives will not be achieved by the end of June (the contract termination date), the Notre Dame team has put forth a conscientious, imaginative and productive effort. By the end of FY'74, it will have advanced the state of knowledge about the ecological aspects of A. aegypti in East Africa to the point where, with a modest additional effort, a sound basis will have been laid for field testing of a variety of mosquito control techniques. The technical review panel noted that the ecological data alone appeared to be worth the research investment (although appreciating the fact that AID could not justify investment of research funds on such limited benefits). The principal research shortcoming has been the failure to move as far ahead as anticipated with the genetic translocations (in particular the lack of success with isolating viable homozygote strains of A. aegypti). However, as pointed out by the technical review panel, it is almost impossible to contract for achievement of what are largely basic research objectives during a fixed period of time.

The AID project office also believes that the establishment of the Mombasa laboratory and field site, and the many new linkages with East African institutions, are noteworthy accomplishments -- and the direct result of the personable, dedicated and competent field staff that Notre Dame has had on location.

The project has had significant weaknesses, however, and these were highlighted in the report by the AID field evaluation team (April 1973). In particular, the report noted, "The overall project is poorly designed and poorly managed. It is being conducted under the philosophy that each investigator may independently pursue his interest with little overall effort to contribute to coordinated project objectives. Direction from Craig and backup by the Notre Dame facilities are noteworthy by their absence and inefficiency. As a result, each of the field investigators is pursuing his individual studies, with inadequate managerial and logistic support".

The Notre Dame principal investigator, George Craig, strongly disagreed with this view. He maintained that, particularly during the initial stages of the project, it was necessary to give his team members relatively free rein to pursue the most promising research directions but within a broad framework on which all were in agreement. He also argued that ICIPE and Notre Dame policy require that the team members have a reasonable amount of freedom for individual basic research, especially since they were being paid on a postdoctoral salary scale. Craig consistently has stated that the research project milestones and objectives are being met and the effort has not been diminished by his team's project goals or diversion to peripheral activities".

However, after the recommendations of the field evaluation team were discussed with Dr. Craig, he indicated that he would initiate steps to insure that all concerned with the project at Notre Dame and in Mombasa were clear on overall objectives, individual responsibilities, integrated project design, and scheduling requirements. Craig then visited Mombasa and, in consultation with his staff, prepared the aforementioned PERT Plan for the remainder of the project. He maintains that the Plan has been, and will be, followed from that point in time.

It is difficult to determine exactly what impact management deficiencies have had on the project, and even whether the existence of a PERT Plan has actually accomplished any tightening

up of the effort. The Notre Dame field team appears to have consistently worked extremely hard, and the diversity of tasks involved in the overall project may have naturally precluded a closely integrated approach during the early stages. However, TA/OST has sensed that the orientation of the university workers toward individualized, basic research has been a tangible constraint against their commitment to the preferred (by AID) coherent, all-out, problem-solving approach to the task. However, given the complexity of the research problem, the difficulties of working in an unfamiliar socio-cultural environment, and the relatively modest investment of \$86,300 per year (compared to other insect control research investigations), TA/OST is hard-pressed to think of a single institution that could have carried out the task as well as has the present contractor. The AID project management office believes that many of the problems encountered in the project result from its underfunding -- the fact that virtually all components have been pursued with a "bare-bones" budget.

In addition to the issue of the Notre Dame approach to project management, two other problems were highlighted by the field evaluation team. The first is the failure of the contractor to develop a close working relationship with the large WHO project on A. aegypti in India. WHO personnel maintain that all cooperation (which has been very limited) has thus far come from their initiative; the Notre Dame principal investigator claims just the opposite is true, and cites the fact that Dr. Rai of the Notre Dame team is also involved in the India project. Dr. Craig further states that liaison has been hampered by lack of sufficient travel funds in the budget, an oversight he wishes to correct in any project extension. From the very outset of the project, AID has stressed the need to link the Mombasa effort to the larger India project, particularly since we originally justified the Notre Dame contract as a U.S. input into a larger international effort spearheaded by WHO. The contractor was certainly aware from pre-contract discussions that AID could not justify funding a duplicative, parallel effort given the existence of the WHO project. AID's decision to move ahead with the project was taken only after detailed discussions with WHO and Notre Dame personnel to lay the basis for continual exchange of data and personnel. The fact that this hasn't happened represents a major consideration in determining whether the Notre Dame effort should be continued.

The other shortcoming identified by the AID field evaluation team is Notre Dame's failure to involve a systems analyst to the extent required. A member of the University was identified in the Notre Dame proposal as the systems analysis specialist (see Attachment C), but this later became a non-budgeted position and

the individual found that other commitments precluded him from contributing much to the project to date. Consequently, the work on modelling of population response to fluctuations, release strategies, and age-structure-fecundity relationships has lagged. Last month, the technical review panel also cited this as the major shortcoming in the ecological work, and recommended that the gap be promptly filled.

In summary, while the contractor's performance has been generally satisfactory, the difficulty of solving some of the genetic translocation problems combined with aforementioned deficiencies in project management, linkages with WHO, and inputs in the systems analysis and modelling areas means that all objective will not be achieved by the contract termination date (July 1974). Under present plans, the field trials scheduled for the third year will be restricted to a single small release in March or April involving four (rather than six) villages.

D. Recommendations And Justification

The AID project office concurs with the principal recommendation of last month's technical review panel on the issue of whether the project should be continued, and herewith submits the recommendation to the RAC for review and approval:

RECOMMENDATION -- The Notre Dame project should be continued one additional year to enable the ecological studies to be completed, and to provide for two releases of double-heterozygote strains (one each in the wet and dry seasons). These releases should be thoroughly analyzed, and comprehensive documentation prepared covering the total 4-year effort. No peripheral studies, including further work to obtain homozygote strains, should be carried out under the extension, and the total team effort should be focused on the ecological studies and the field evaluation of the best available genetic translocation (i.e., double heterozygote). To support the ecological studies and analysis of the releases, a systems analyst should be employed under the new contract. In addition, sufficient funds should be made available to ensure that Notre Dame is able to establish and maintain close working relationships with WHO and the India project.

The decision to recommend termination of the project after one additional year (i.e., in June 1975) was based on the following considerations. Research conducted both in Mombasa and India to date on A. aegypti genetic control suggests that

if the three year extension requested by Notre Dame was approved and all objectives achieved, the most one would be able to say at the end is that the particular genetic control techniques are applicable to certain specialized populations in certain East African villages. The applicability to other mosquito populations in other parts of the world would have to await further ecological studies and field releases, although the basis established in Kenya would greatly facilitate these efforts. Proving out the universality of a single technique or a complex of methodologies, may well be a costly, extremely long-term, open-ended quest. Under the best of circumstances, it appears that operational control programs based on genetic techniques will require sizeable investments over a long period of time. The most prudent approach for AID seems to be to ensure that the ecological work initiated by Notre Dame in the Rabai villages is completed, and that the best available genetic control technique is adequately tested. If the control technique is successful, it would recommend itself to WHO and other experimenters throughout the world for testing in other locales, and also become available for possible utilization should A. aegypti vector-borne diseases appear in East Africa. If the genetic control field releases are unsuccessful, the double heterozygote approach in this particular ecosystem will have been subjected to a good test, and future research can focus on development of other tools. In addition, the availability of comprehensive baseline ecological data for the village "islands" should facilitate future research on mosquito-borne diseases supported by other institutions. Several members of the technical review panel noted that the ongoing project should probably have been funded by a research-oriented institution such as NIH with AID reserving its investment for the development and application of any successful techniques which emerged -- although not all panelist concurred in this view.

The technical review panel was unanimous in recommending against terminating the project before the ecological studies and one or two field releases are completed. This same position is advocated by WHO and IAEA experts. All argue that termination at the end of this June will leave uncompleted much valuable work that may be impossible for any other contractor to pick up and complete, and certainly only at much greater cost. Further, a one-year extension would enable Notre Dame to work out a method for transferring the results (and possibly personnel) of the Mombasa experiment to the India project if work in Mombasa is to be completely stopped -- and also for Notre Dame to pursue alternative sources of funding to continue its work in East Africa.

Possibilities for the latter should be good since it is difficult to imagine that ICIPE, for example, would allow the Coastal Laboratory at Mombasa to be closed down, particularly since Dr. Craig has noted ICIPE's desire to utilize the laboratory for work in other areas besides A. aegypti. Also, A. aegypti is one of five disease vectors which ICIPE has selected for program concentration. Thus far ICIPE has not had to concern itself with funding the A. aegypti component because of AID's support to George Craig. AID's withdrawal will obviously change this situation. Although Dr. Craig has indicated that he doesn't believe ICIPE would probably make a concerted effort to provide support, possibly using UNDP funds. Another possibility is that WHO would use the laboratory in India, and thereby give a broader dimension to its project -- particularly if the ecological baseline in the East African villages is established.

It should be noted that AID withdrawal from the Notre Dame project either in 1974 or 1975 will leave ICIPE with no direct funding from U.S. institutions. Three years ago, the U.S. scientific community, particularly the National Academy of Sciences, the White House Office of Science and Technology, and the State Department's Science Bureau strongly advocated AID support of ICIPE. Whether this feeling still exists is unknown, but the issue of U.S. support for ICIPE should be considered prior to AID's termination of the Notre Dame project.

TA/OST discussed a one-year, close-out extension with the Notre Dame principal investigator immediately following last month's technical review. Dr. Craig indicated his desire and willingness to proceed in accordance with the specifics of the recommendation to RAC set forth at the beginning of this section (i.e., completion of the ecological studies; a wet and dry-season release of double heterozygote strains; employment of a systems analyst to work on release design and predictive modelling; thorough documentation of all work; and close liaison with WHO to ensure exchange of results). The contractor has now submitted a proposal to accomplish these objectives (Attachment E). Note that it is budgeted at \$154,150 for the one-year extension. Both TA/OST and the TA/Office of Health have reviewed the proposal and agree that: (1) its content and work plan are directly responsive to the recommendations of the technical review panel; and (2) the requested funding level is reasonable. On this basis we are herewith submitting for RAC consideration the recommendation for a one-year extension of the A. aegypti project being carried out by the University of Notre Dame.

- Attachments:
- A. Contract Work Scope
 - B. Contractor's Summary of Accomplishments
(through 12/73)
 - C. Notre Dame Project Staff
 - D. Project Linkages with African Institutions
 - E. Notre Dame Proposal for 1-year extension

Other Available Documentation (in AID/TA/OST)

- Contractor Progress Reports (4)
- PERT Plan and Documentation for 6-year Project
- Report by AID Field Evaluation Team (April 1973)
- ICIPE Mandate and Programs

SCOPE OF WORK (Extract From Contract
AID-CSD-3159)

- A. The project will initially involve field studies of the ecology and population dynamics of the East African variety of A. gambiae and concurrent investigations of mass production and genetic modification: During the second year, experiments to identify proper release methods and mating habits will be undertaken. Finally, preliminary field testing to compare the effectiveness of alternative genetic controls will be initiated in the third year. The phasing of the research follows:

Research Phases

- FY 1971: Field survey, biotope designation
2 Site selection - geographic and local (island, village, forest)
Procurement - personnel, equipment; design of lab and production facility
Preliminary research on bionomics
At Contractor facility & ICIPE - Insert genetic mechanisms into background of E. African mosquito populations. Study mating competitiveness of different synthetic strains.
- FY 1972: Mapping of field sites, including bionomics of designated populations.
3 Lab-tool up for mass production (see notes 5/20/71)
Lab-mosquito bionomics, esp. product evaluation from mass production.
Field and lab - Release technology - when, where, how many?
At Contractor facility & ICIPE: Genetic mechanisms and cage trials.
- FY 1973: Field ecology - Year-long census in designated trial sites.
4 Mass production facility fully operational.
Field evaluation of mass-produced mosquitoes - acceptability, survival, dispersal, mating, competitiveness.
Release technology - large-scale experiments on effective numbers, mating, site and time.

- B. The foregoing Research will be designed to develop answers to the following questions:

I. POPULATION STRUCTURE

1. What is the real number of mosquitoes in a local population? Previous estimates, using trapping to give relative numbers, are of little value. (see notes 5/20/71)
2. How does real population size fluctuate over a year?
(Family study over 1/2 year)

3. What is the age structure of the population? What is the rate of productivity of females? Note that most genetic control methods are aimed at the virgin female.
4. How much movement occurs into and out of the population?
(Very different. Flying distance is \approx 500 meters, sometimes 1000 meters / 1 hour)
5. What is the relation of domestic and feral populations? How much gene flow between them?
↳ (not known yet)
6. What are the limiting factors that prevent increase? If the population is artificially decreased, how fast will it recover, i.e. what is the biotic potential?

II. REPRODUCTIVE BIOLOGY

1. Where does the female get inseminated in the field? How much dispersal from the breeding site?
r(24-48 hours)
2. How old is the female at insemination? Laboratory studies show a minimum of 2-3 days is required. What does she do between emergence and insemination?
after 2-3 days; 1st insemination after 3 days
3. How long does a released male remain effectively competitive? What is the attrition rate on released males?
4. What genetic and environmental factors govern mating success in the field?

III. TECHNICAL REQUIREMENTS FOR GENETIC CONTROL

1. What can be done by genetic engineering to improve the mosquitoes to be released? Can heterosis or breeding for specific fitness traits improve either the capacity for mass production or field competitiveness of males?
2. Do genetic control mechanisms (chromosome translocations, sex ratio distorters, chemosterilization) affect competitiveness of males?
3. How should mosquitoes be released? We need to know: When? Where? How many?
4. Can insecticides be used, either in an integrated program to improve the odds for released males or to create barrier strips which prevent reinfestation?

Progress in Meeting Objectives - AID/csd-3159

As of 1 January 1974 (30 months operation)

Project 1 (Population Fluctuation): Completed. Baseline data on absolute numbers in selected villages on an annual basis have been obtained. About 20 villages potentially useful for experimental releases have been identified and are being monitored.

Project 2 (Life Tables): Three-fourths completed. Age-structure+fecundity data have been accumulated and computer modeling is under way. More information on dispersal is needed. Current emphasis is on capture-mark-release-recapture experiments and on de-faunating villages of their mosquito population to follow rate of reinfestation.

Projects 3 and 4 (indoor and sylvan niches): Completed. There is no direct migration between indoor and sylvan niches. In the wet season, a third "peridomestic niche" appears; no such niche is evident in the dry season. Sylvan populations are more humidity-sensitive and crash-adapted. Biting behavior of sylvan strains is very different, with little attraction to man. Standard trapping methods are unsatisfactory.

Project 5 (Gene flow between indoor and sylvan populations): Half completed. Although it is too early for conclusions, it seems that gene flow is zero in dry season and of minor long-term significance in wet. Current emphasis is on this project. Addition of the new isozyme population geneticist contributed markedly to this project.

Project 6 (Chromosomal translocations): Project two-thirds completed. Seventy-two induced translocations have been recovered and one homozygote has been produced; however, its' fitness characteristics are unsatisfactory. Use of double heterozygotes and of radiation-sterilized males also under consideration.

Project 7 (Genetic engineering): Little progress. Test system not yet available.

Project 8 (Mass production): Project active. Facilities constructed and methods under development. Some large-scale rearing has been successful.

Project 9 (Release strategy): Project initiated and currently active. Radiation-sterilized (under Nitrogen) males are being released to test timing and requirement for numbers.

Miscellaneous spinoff:

1. Aedes aegypti has been demonstrated to be a potential vector of filariasis in East Africa.
2. Three potential virus vectors (A. metallicus, A. heischii, A. adersi) were colonized for the first time.
3. Ecological methods used by MBU are being applied against Aedes triseriatus, primary vector of California Encephalitis in the middle west.
4. Our laboratory at Notre Dame is preparing release of a predatory East African mosquito, Toxorhynchites, for biological control of A. triseriatus in Michigan.

Liaison and Linkages in East Africa

I. In ICIPE:

1. Craig, as a Research Director, serves in planning and development.
2. Hausermann serves on Resident Research Council, meeting monthly.
3. Approximately US\$8,000 per year goes to ICIPE in overhead.
4. Joint research projects with Nairobi-based ICIPE Research Fellows:
 - a. W. Wood and D. Fanara - treehole extraction for oviposition attract.
 - b. D. Elder and Hausermann - isolation of ♀ sex pheromone
5. Designation of MBU as ICIPE Coastal Research Station, eventual hope of ICIPE to buy property and make permanent.
6. Use of MBU as base lab for insect studies of ICIPE scientists: Schneider, Meinwald, Wood, Smith, Scheltes, DeWilde, Elder, Leahy, Gebreyessus, etc.
7. MBU is ICIPE component with closest linkage to economic entomology, control.
8. In April of 1974, MBU to serve as host for annual meeting of ICIPE Advisory Council, with representatives from WHO, IAEA, FAO, CIMMYT, IRRI, IITA, etc.
9. Future use of MBU for other ICIPE projects:
 - a. Predatory mosquitoes - P. Corbet, Canada
 - b. Genetics of Culex and Anopheles - J. Mouchet and E. Boesiger, ORSTOM, Fr.
 - c. Termite pheromones - M. Luscher, Switzerland

II. University College, Nairobi

1. Seminars given at University College by Notre Dame personnel - Rai, Trpis, Crovello, Fanara, Hausermann, McDonald, Craig
2. Joint project between Fanara and Dr. Canute Khuzala, Lecturer in Entomology, on stratification of populations of Aedes simsoni on Mount Kenya.
3. Joint project on mutant analysis and isozyme polymorphism with Professor Reuben Olembo, Head, Botany Dept. Note that Prof. Olembo spent three weeks in September, 1973, at Notre Dame, training in mosquito genetics.
4. Graduate student in genetics from Univ. College to be in residence at MBU, Spring, 1974.

III. Division of Vector-Borne Diseases, Kenya Medical Department

1. Generous cooperation of Dr. J.D. Roberts, Chief, DVBD.
Rent-free use of laboratories for first six months.
2. DVBD personnel assigned to MBU for training; Mr. P. Bebora for 6 months.
3. MBU personnel to be hired by DVBD after termination of contract.
4. Mr. George Oketch, Chief Field Entomologist, DVBD
 - a. ANCA Good Neighbor Program - 12 years, sponsored by G. Craig
 - b. Son has applied to Notre Dame for freshman class, fall, 1974
5. Mr. Vijay K. Prashar, Field Entomologist, DVBD
 - a. Year of training at Notre Dame on WHO Fellowship
 - b. Assigned to Galana River Survey with Hausermann
6. Mrs. E.C.C. van Someren, Mosquito Taxonomist, DVBD
MBU consultant; possible joint support (WHO and MBU) for monograph on "Mosquitoes of East Africa"

IV. Medical School, University College, Dar-es-Salaam, Tanzania

- Dr. Wenceslaus Kilama, Senior Lecturer in Medical Parasitology
- a. PhD in mosquito genetics, U. Notre Dame, 1970
 - b. Consultant to MBU on filariasis transmission by Aedes aegypti
 - c. Currently testing MBU strains against Wuchereria in man

V. East African Inst. for Arthropod-borne Virus Disease, Entebbe, Uganda

1. Dr. Louis Mukwaya had G. Craig as Advisor and External Examiner for PhD thesis on genetics of host choice in Aedes simsoni, Makerere University
2. Mr. Sylvester Sempala has Dr. Hausermann as PhD thesis advisor, Makerere U.

VI. East African Inst. for Malaria and Vector-borne Disease, Arusha, Tanzania

- Dr. Philip Wegesa, Director, as co-organizer of ICIPE Symposium (with G. Craig) on Genetic variability in Aedes as it affects disease transmission

Professional Staff--Contract No. AID/esd - 3159

I. At MBU, Mombasa

Dr. Walter Hausermann, Field Director (Chief, ICIPE Coastal Res. Station)
Responsible for over-all supervision, for population monitoring and
for indoor populations. Study area is Rabai villages.

Dr. Paul McDonald, Geneticist
Deputy Field Director, Responsible for isolation of translocations and
for genetic engineering. Preliminary experiments in Rabai villages.

Dr. Dean Fanara, Ecologist (terminates Dec. 73)
Responsible for sylvan populations. Study area is Changombe, with
Kombeni Forest and associated villages.

Dr. Fidelis Ogah, Population Geneticist (joins Sept. 73; paid by UNDP)
Biochemical identification of strains (Ph.D., Cornell; Nigerian)

Project also employs 12 Kenyans as subprofessionals. See Annex 2 of Annual
Report, year 1.

II. At Notre Dame

Professor George B. Craig, Jr., Principal Investigator.
Primary responsibility Research Director of ICIPE. Basic genetics
and reproductive physiology.

Professor K.S. Rai, Co-investigator.
Responsible for chromosomal engineering and for liaison with WHO
project in Delhi.

Assoc. Professor Theodore Crovello, Co-investigator.
Responsible for computing, modeling and experimental design.

Ass^{cc}. Faculty Fellow Milan Trpis.
Responsible for ecological studies, population dynamics, dispersal;
correlates data gained by WHO Aedes Unit, Dar-es-Salaam.

Research Assistant Raymond Russo, NIH Trainee
Population modeling under Prof. Crovello

Res. Assistant John Petersen, NIH Trainee,
Genetic basis of host choice (Prof. Craig)

III. Consultants

Dr. Eugene Gerberg, Insect Control and Research, Inc., Baltimore, Md.
Site selection.

Dr. W. Kilama, Senior Lecturer in Parasitology, Dar-es-Salaam, Tanzania,
filariasis

Mr. George Lawrence, General Accounting Office, U. of Notre Dame.
Accounting.

AID/csd - 3159

Project Review, 14 January 1974

1. Project Title: Ecological studies on Aedes aegypti in East Africa preliminary to genetic control.
2. Principal Investigators:
Professors G.B. Craig, Jr., K.S. Rai and T. Crovello
Department of Biology
University of Notre Dame
Notre Dame, Indiana 46556
3. Contract Period: 3 years, 1 July 1971 - 30 June 1974
4. A.I.D. Funding: Total - \$258,900; yearly \$86,300
5. Requested Renewal, Year 4:
 - a. Period: 1 year, 1 July 1974 - 30 June 1975
 - b. AID Funding: \$173,650*
6. Project Manager: B.L. Long, TA/OST
7. Narrative: After three years of preparative research on ecology a fourth year will be spent on field testing of population suppression by release of translocation heterozygote males. Two major releases will be made, one in the wet season and the other in the dry season. A systems analyst will be added to the team to computerize data and develop a systems diagram.

* TA/OST is in the process of careful review of the budget with the contractor. We believe that a 10-15 percent reduction is possible. We concur with Notre Dame's view that the earlier work was constrained by underfunding, and believe that an increase above prior years is justified.

Proposed Renewal, Year 4

1. Accomplishments

In 30 months of operation, the project located a site, staffed and equipped a laboratory, developed a vigorous program of field investigation in the Rabai area near Mombasa, accumulated base-line monitoring data on Aedes aegypti populations in villages, made good progress with life table preparations, identified the components of the indoor versus sylvan breeding problem isolated over 100 chromosome translocations, started mass production and genetic release technology and showed a possible new vector of human filariasis. Generally the progress has been better than we at Notre Dame expected. We are in harmony with the time table of the contract, except for the failure to date to produce satisfactory translocation homozygotes in African material. Unfortunately our first choice method for genetic control, the replacement or modification of existing populations through the release of translocation homozygotes has thus become impracticable and will very likely remain so for the coming year. The present laborious method to produce homozygotes has been sufficiently explored to state that it is unlikely to produce suitable homozygote strains in near future. New methods to obtain translocation homozygotes will have to be developed first.

In addition, MBU has been a keystone of ICIPE, giving both financial and administrative support at crucial times. MBU is ICIPE's only public health related project. Finally, MBU has established strong linkages to several East African research institutes and has contributed to the training of Africans. To quote Dr. J.D. Roberts, Chief of the Kenya Medical Department Division of Vector-Borne Diseases, "Craig and his colleagues have reawakened and put new life into the field of medical entomology in East Africa".

2. Request

We now request extension of the contract for an additional year from July 1974 to June 1975. This extension would allow us to thoroughly test one of the presently available approaches to genetic control. The method selected is best described as population suppression through release of translocation heterozygote males. The other available method would have been the release of radiation sterilized males, a field in which much progress has been made recently in reducing somatic damage due to sterilizing irradiation. We think however that by releasing males which are heterozygous for two translocations we will have an equally good chance to achieve population suppression.

In addition to the one year extension of the present contract we also request enlargement of the project for the same period by adding the position of a systems analyst (biostatistician). The new team member will be based at the University of Notre Dame and will have to analyse the presently accumulated and the still incoming field data. In addition he will also develop and constantly revise a systems diagram for domestic A. aegypti populations in Rabai on the basis of which release strategies can be formulated and reviewed. A review of this plan is appended.

3. Approach

The project will be aimed at the suppression of domestic A. aegypti populations through release of males bearing two translocations in heterozygous conditions. These males will be partially sterile and confer this sterility to their viable offspring. We expect that through continuous daily releases of such males over several generations we will obtain total suppression of domestic A. aegypti populations comparable to the suppression achieved through conventional source reduction.

Our activities will be conducted in the Rabai District near Mombasa. Tests will be limited to selected villages of 15 to 30 houses and A. aegypti populations of not more than 1000 individuals.

The basic design for our release experiment requires 6 villages treated as follows:

- a. Two villages - No treatment except routine monitoring
- b. Two villages - Suppress population by conventional means, pyrethrum spray against adults, water pot scrub against larvae and eggs.
- c. Two villages - Suppress population through release of males which are heterozygous for two translocations.

The releases will be carried out daily over extended periods of 6 weeks to 2 months and will be monitored weekly by oviposition traps and landing-biting catches. Each release will be preceded by a short period of monitoring and followed up by the same monitoring procedure until the native A. aegypti population has reestablished itself on the pre-release level again.

The first release will be started in the middle of the dry season of 1974 in early March. It will continue at least to the end of the dry season expected in the middle of April. If the release is successful in the first six weeks, it will be extended through the rainy season.

The interim period from June to November 1974 will be used to make short term releases for testing various combinations of double heterozygotes and to complete the ecological investigations. Simultaneously the analysis of the results of the first release will be carried out in cooperation between Notre Dame and MSU.

In December 1974 the second dry season release will be initiated and carried through till March. With beginning of the rainy season in late April or May, the releases will be started again in a new set of villages. By the end of May or the beginning of June, the releases will end in order to leave enough time to observe the return of the native A. aegypti population and to terminate the project.

Time schedule for double heterozygote male releases for population suppression

Month	February			March			April			May		
	7	8	9	10	11	12	13	14	15	16	17	18
Week No.												
	Pre-Release	Release	Release	Release	Release	Release	Release	Release	Release	Post-Release	Post-Release	Post-Release
<u>Village Group I :</u>												
HAIRI MOYO	Monitoring of oviposition rates and fertility once a week	Daily releases of 1000 males Weekly monitoring of oviposition and fertility	Daily releases of 1000 males Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Weekly monitoring of oviposition and fertility (come back)	Weekly monitoring of oviposition and fertility (come back)	Weekly monitoring of oviposition and fertility (come back)
IBARANI												
MELE												
<u>Village Group II :</u>												
GANDINI I	Monitoring of oviposition rates and fertility once a week	Daily releases of 1500 males (bigger village) Weekly monitoring of oviposition and fertility	Daily releases of 1500 males (bigger village) Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Conventional control once each week through pyrethrum spray catch and cleaning of pots Weekly monitoring of oviposition and fertility	Weekly monitoring of oviposition and fertility (come back)	Weekly monitoring of oviposition and fertility (come back)	Weekly monitoring of oviposition and fertility (come back)
GANDINI II												
MA DENA												
<u>Village Group I :</u>	All villages of group I are fairly isolated. Immigration accounts for less than 20% of population											
<u>Village Group II :</u>	Villages are not well isolated. Immigration may at times account for up to 50% of population											

4. Proposed Budget: Year 4.
(in U.S.\$)

Expenditure	Yearly average years 1-3	Proposed year 4
AT NOTRE DAME		
1. Salaries & Benefits	33,000	45,000*
2. Supplies	3,500	1,000
3. Travel-Overseas	6,800	12,000
4. Indirect costs - UND (14% of S & W)	4,500	6,300
IN KENYA		
6. Salaries & Benefits	7,000	12,000
7. Housing Allowance	3,600	6,000
8. Rental - Lab space	4,000	6,000
9. Supplies	3,500	6,000
10. Travel in Kenya (E.A.) (+ vehicle maint.)	4,000	7,000
11. Capital	9,000	8,000
12. Indirect costs - ICIPE (25% of direct)	<u>7,400</u>	<u>11,250</u>
	38,500	56,250
TOTALS.....	<u>86,300</u>	<u>120,550</u>
Computerized Systems Analysis at U. Notre Dame (Crovello)	-	33,600
Grand Total:		154,150

* Personnel salaries - Project Leader, 18,000; Geneticist, 12,000; Ecologist - 12,000; Bookkeeper (half-time), 3,000.

5. Budget Comments

- a. The project has been hard hit by dollar devaluation and by galloping inflation in Kenya. Salaries and other budget items that were appropriate in 1970, at the time of original application, are now out of line.
- b. The position of the Project Leader is absolutely essential in Year 4. The role of this individual is changed from semi-independent researcher to team director, in order to accomplish the coordination and direction needed in the large releases. For this reason, the salary and benefits for this individual is raised from \$14,000 to \$18,000. The need to hold a first-class man in this position was acknowledged by the Review Committee.
- c. Travel-Overseas was raised to \$15,000 because the earlier yearly budget of \$6,800 proved to be grossly inadequate. Extensive use of consultants from Notre Dame and elsewhere is planned for year 4. Earlier reviews criticized this project for lack of liaison with WHO and other groups doing genetic control. The new travel budget will allow this liaison.
- d. Salaries in Kenya have been raised from \$7,000 to \$18,000 in year 4, reflecting the large amount of field work (monitoring) that will be required in connection with the releases. Services of an Administrator have been added to give the Project Leader more time to do science.
- e. Housing allowance for each of the three scientists has been increased from \$1,200 to \$2,000, reflecting the staggering increase in rents that has occurred on the Kenya coast. Rental of lab space has been increased for a similar reason; our landlord demands it.
- f. Supplies and travel in Kenya (including vehicle maintenance) are both increased to reflect greater activity by the larger staff that will be required for the final year.
- g. One additional vehicle is requested in capital equipment. The project now has three field vehicles, but one is always in the shops for repair. Automotive maintenance standards and competence of mechanics is very low on the coast. Other capital equipment required will be environmental control apparatus for mass production and insectary rooms.

Overview

January 1974

Today many biological control programs are in progress around the world. Their goal is the same - to control an insect pest species by means that will not permanently harm the local ecosystem. Success has varied from project to project, but unfortunately, most have one drawback. Little thought is given to the integration of the findings and raw data collected from a given project with that of all others. As a result, each new program must start at the beginning more than it should. Even when the target species differs from one project to another, we ought to expect that some of the findings and data of one study can be applied to other studies, even if it is to compare such previous data with those of a current study to highlight the differences between the two populations. It is this view that we have adopted in the study of the yellow fever mosquito in Kenya - that our study is not to be an isolated one; rather our findings and data should be collected, processed and stored in such ways as to maximize their value for use in future projects, be they in mosquito control, or in the reduction of other pest organisms.

Computerized Systems Analysis

The most efficient means by which to make our findings available to others and simultaneously to make our own project most efficient is to utilize the capability of a large, modern computer and the systems analysis approach to pest control. This is the ongoing protocol of the International Biological Program's Crop Ecosystems Program, a series of large scale efforts aimed at the control of such agricultural pests as the cotton boll weevil.

Previous and Current Computer Usage

Computers have already been used extensively in our Kenya program. This includes their use in multivariate statistical analyses of results of individual field experiments and in supportive laboratory experiments at Notre Dame. The former are described in earlier reports while the latter involved a series of experiments to ascertain which physical and biological factors are important in determining larval survival in simulated village and hut water jars.

Computers were essential to carry out a systems analysis and simulation of preadult population dynamics in hut water jars. Such studies allow us to describe more concisely and to understand more fully the population dynamics of this essential life stage.

Currently, computers are being used to analyze routine year-round monitoring information of environmental and biological variables. Through multivariate periodicity analysis we hope to isolate "indicator" variables

which would permit us to predict seven to fourteen days in advance when we can expect large population buildups. Such knowledge is invaluable in developing efficient release strategies.

In addition, a probabilistic, theoretical computer model is being developed at Notre Dame to evaluate different release strategies that might be employed in Kenya trials. Such "computer-releases" are not only quicker than actual field releases, but they are more economical, less harmful and permit decisions about actual field release strategies to be made on a firmer basis.

Naturally, supporting functions such as the continued availability and expansion of MODABUND, the computerized Mosquito Data Bank of the University of Notre Dame, absolutely require the computer. Presently over 25,000 references can be scanned with little effort.

Future Project Uses

Future use of the computer will include ongoing aspects just described, but to really enhance the value of the entire project, both to itself and for others, we must move towards a rigorous phase of computerized systems analysis. This involves several aspects which we shall consider in turn.

1. Clearly state the purposes and desired results of the systems analysis. The purposes of the project have been stated several times in earlier reports. Specifically, we wish a systems analysis to integrate and describe the Kenya village system, to help us to determine optimal control strategies and to be able to be modified and expanded as new data become available. The major results desired will be listed below.
2. Continue to keypunch MSU field data and other information as it becomes available - This essential step will assure the use of such data not only by us but also by workers in other projects.
3. Continue to use and modify the theoretical, probability model to help to determine the best, ongoing experimental release strategies. Refresh systems diagram.
4. Translate detailed systems diagram into computer language (detailed Systems Description) and simulate a village without treatment to verify and correct predictions (Systems Exercise and Tuning).
5. Site visits to MSU by systems analyst to experience the real situation and actual release protocols under natural conditions.
6. Add a program module to the systems program to determine optimum release strategies and their evaluation for the Kenya village situation.
7. Attempt to evaluate the above model for use in Aedes aegypti control in other areas and for use in control projects involving other mosquitoes.

8. 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.

9. Create computer program and organize MBU data to determine economics of different releases, including costs of prerelease feasibility period.

10. Prepare documentation of all computer programs and data files for distribution to qualified agencies.

Required Personnel for Computerized Systems Analysis

The above task may appear simple and straightforward. It is to some degree, but readers must be aware that while computers are extremely fast in calculation, and can store and retrieve large amounts of data, the task of preparing the data and of instructing it to do what will be useful to an actual biological control project requires the skills and resources of several types of people. But the support available is such that we apparently must seek these capabilities in the same person - a biologist who is also a systems analyst. Such persons will be available from the IBP's Crop Pest Ecosystems Programs mentioned earlier. But we emphasize that working only with the resources of one systems analyst-biologist will hinder us in making the current project as valuable and useful as it could be. Such a second person would require an additional \$20,000-\$25,000 per year.

Finally, we realize fully the value of bringing together leading workers in biological control in workshops both at Notre Dame and in Kenya. Such useful sessions would require an additional \$5,000-\$15,000.

Budget For Computerized Systems Analysis
One Year Only

Personnel

Systems Analyst	\$12,000.00
Prof. Crovello (Partial summer salary)	2,000.00
Keypuncher-Typist ($\frac{1}{2}$ time)	<u>4,000.00</u>
	18,000.00

Benefits

Permanent Equipment Desk calculators, etc.	500.00
Computer Supplies	500.00
Travel	2,000.00
Publication	100.00
Computer Costs	<u>2,000.00</u>

Direct Costs 24,600.00

Indirect Costs 10,000.00
(57% of S & W)

TOTALS \$34,600.00

Our current mosquito rearing facilities are geared for the daily production of about 5000 adults or 2000 to 2500 males. All these males will be used for releases. The study villages will be selected such that their native A. aegypti populations will not surmount 1000 adults at any given day. The expected pupal output under these conditions will not in any case surmount 100 males per day. Aiming for a daily release of 1000 males, the ratio of release males to fresh native males should thus always be better than 10:1. A schedule for the first release is attached.

During the past three years, this project has had the full time services of 3 Ph D's. The team for year 4 will have the same composition. The major thrust for year 4 is the two releases and subsequent evaluation. Essentially all of the activity of the team will be devoted to this end. As in the past three years, scientists from Notre Dame will continue to work for various periods on this project, even though they are not paid by this project.