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PROJECT STATEMENT

A. PROJECT SUMMARY

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

New or Extension: New Project

Contractor and Address: University of Notre Dame, Notre Dame, Indiana 46556

Principal Investigator(s):

Dr. George B. Craig, Jr.
Director, Vector Biology Laboratory
Professor of Biology

Dr. Karamjit S. Rai
Director, Mosquito Biology Training Prog.
Professor of Biology

Duration: Three Years

Total Estimated Cost: $258,900
Funding by Fiscal Years: FY 1971 - $86,300
1972 - 86,300
1973 - 86,300

Project Manager: B. L. Long, TA/OST

2. Narrative

Scientists from the University of Notre Dame's Vector Biology Laboratory will undertake a three-year program of research on population dynamics and reproductive biology of Aedes aegypti. This work is preliminary to field trials for genetic control of this mosquito. Certain critical biological questions must be answered and production and release techniques must be developed. Research will concentrate on two types of populations, those occurring in villages in close association with man and those in feral, sylvan situations far from human habitation. A number of promising techniques for genetic suppression of field populations will be investigated. Special attention will be given to the introduction of partial sterility through chromosomal translocations, a method first developed for this species at Notre Dame, whereby a latent, lethal and self-perpetuating "Achilles' Heel" is bred through successive generations of this mosquito.

A. aegypti should serve as a model system to demonstrate whether this type of biological control is feasible with other mosquitoes. Much of the genetics, physiology and bionomics of this species are already known; mass production is simple; and a variety of genetic mechanisms for modifying its genetic composition have been demonstrated, a combination not yet available for other mosquitoes. Development of an effective genetic technique would provide a non-chemical alternative or supplement for controlling A. aegypti, currently a pervasive and significant disease vector responsible for transmitting Yellow Fever, Dengue and Asian Hemorrhagic Fever in epidemic pro-

portions throughout large areas of the developing world. Equally important, a successful program will both stimulate and guide similar research on biological control of other mosquitoes, including the malaria-transmitting Anopheles.

The project will be administratively centered at the new International Centre for Insect Physiology and Ecology in Nairobi, Kenya, with a majority of the research at a field station in an area of high mosquito density, probably in the Malindi-Mombasa area.

B. EXPANDED NARRATIVE STATEMENT

1. (a) Background

Mosquito-borne diseases such as malaria, filariasis and yellow fever continue to plague vast areas of the world, particularly the developing nations of the tropics and subtropics. Twenty years ago with the appearance of long-lasting synthetic organic insecticides, such as DDT, control or even eradication of the major insect disease vectors was contemplated. However, the development of insecticidal-resistance, the discovery of behavioral characteristics of insects which impede control, and growing public concern over the ecological impact of chemical pesticides have seriously modified this optimistic outlook and vector control has again become one of the most important problems confronting health authorities.

During the last decade, many advances have been made in research on the control of insects by genetic manipulation of populations. Genetic techniques have a number of significant advantages over traditional chemical or source-reduction (drainage of water bodies) methods of mosquito control. Genetically-transformed male mosquitoes will seek out residual niches and populations which cannot be detected by conventional methods. Long-term economic advantages may result since the recurring costs of insecticide applications will either be eliminated or greatly reduced. And substitution of target-specific genetic techniques for nonspecific chemical insecticides will eliminate the environmental problems caused by biological magnification through the food chain of persistent chemicals such as DDT.

The best known of available genetic techniques is the "sterile male" method based on the use of radiation or chemicals to induce sterility. It was first applied successfully to the screw-worm in 1954 and, since 1962, to at least eight other insect species either in the laboratory or in field trials. However, attempts to apply the sterile male technique to mosquitoes have not proved successful and interest in biological control of this insect waned considerably after 1965.

Other genetic transformation techniques are known and may be adaptable for large-scale mosquito vector control. These include cytoplasmic incompatibility, hybrid sterility, meiotic drive, distorted sex ratios, and lethal factors. In 1968, the release by WHO of males of a genetically incompatible strain resulted in the elimination of a small population of Culex fatigans mosquitoes from an isolated village in Burma. As a consequence, interest in biological control techniques has been reawakened.

Among the numerous possible candidate mosquito species on which to base a concerted, integrated program of genetic control, Aedes aegypti is of high priority interest. 1/ This mosquito is, first of all, a pervasive and significant disease vector responsible for transmitting many fatal and debilitating viral diseases of man in epidemic proportions -- Yellow Fever, Chikyngunya, Bunyamwera, Bwamba, and Zika in Africa; Yellow Fever in South America and dengue (Break-bone Fever) in the Caribbean region; and Asian Hemorrhagic Fever. The latter has been called the most serious public health problem in Southeast Asia because neither protective vaccines, curative drugs, nor adequate vector control methods are available (WHO Symposium, Bull. WHO, Vol. 36, 1967).

Due in large part to the work of the University of Notre Dame's Vector Biology Laboratory, which serves as the WHO International Reference Center on Aedes mosquitoes, much is known about the physiology, behavior, bionomics, and genetic makeup of A. aegypti. This mosquito can be mass-produced simply and economically. Most important the Notre Dame laboratory has found at least seven different genetic mechanisms which could be used in control programs directed against this mosquito. This collective wealth of scientific information and technical and institutional capability does not exist at present for any other mosquito. If genetic control of A. aegypti can be shown to be feasible, then impetus will be given to the behavioral and genetic research necessary for supporting similar biological control programs for more complex and lessunderstood species such as the malaria-transmitting Anopheles.

(b) Project Description

A three-year research project involving field studies and concurrent laboratory experimentation will be carried out in East Africa by the staff of the University of Notre Dame's Vector Biology Laboratory to determine whether genetic control of Aedes aegypti is feasible. Field studies of the ecology of natural populations will be undertaken, with emphasis on population dynamics and reproductive biology. Special attention will be given to the solution of certain technical requirements for genetic control involving mass production and release of mosquitoes which are to be competitive with natural populations. Concurrently, genetics of East African populations will be studied in the laboratory in order to discover which among several currently available genetic mechanisms would be most useful for control.

The field studies will be conducted in a region with a dense population of A. aegypti, probably along the shore of the Indian Ocean. Numerous villages will be surveyed and approximately 12-15 sites will be chosen for year-round census of the mosquito population. The absolute number (not relative number) of mosquitoes present will be followed throughout the year to determine natural fluctuation. Special attention will be given to limiting factors and the biotic potential; i.e., potential rate of increase. Age structure and dispersal habits will also be assayed.

1/ Knipling, E. F., et al, 1968; Genetic Control of Insects of Public Health Importance, Bull. Wld. Hlth. Org. pp. 421-438.

Field studies on reproductive biology will be conducted at the test villages. Factors for attention include those which would determine the success or failure of a genetic control program, i.e., do the released males have a reasonable opportunity to inseminate field females? Methods and techniques will be developed to improve that opportunity. This requires laboratory and field studies on fitness and competitive mating ability of released males, as well as development of improved methods for mass production and release.

Strains of A. aegypti bearing potential genetic "Achille's Heels" will be synthesized in the ICIPE laboratories at Nairobi and at Notre Dame. Deleterious factors such as sex-ratio-distorters and sterility-inducing chromosome translocations would be incorporated into a genetic background from the test area. These strains would then be mass-produced. Preliminary field trials involving released males would be conducted and the amount of population suppression would be measured. Special attention would be given to the possibility of reinvasion by jungle forms of A. aegypti following eradication of the domestic forms.

The Aedes aegypti mosquito is an ideal insect species for such a project because of its importance as a disease vector; mass production is simple and economical; the genetics, physiology and bionomics are known; and numerous genetic control mechanisms are available (viz., dominant lethality, chromosomal, translocations, sex ratio distorters, conditional lethals, genes for inability to transmit disease). Further, unlike many mosquitoes, this species is accessible because of its highly domestic habitat.

East Africa is selected as the site for this research because of (1) the importance and abundance of Aedes aegypti in the region; (2) the presence of both domestic and wild forms of the species (which will provide information on whether jungle populations will feed back following eradication of the domestic form); (3) support available from the International Center for Insect Ecology and Physiology in Nairobi, Kenya (which will be the administrative center) and the East African Community of Institutes; (4) presence of a WHO Aedes Research Unit in Dar-es-Salaam which has conducted bionomic studies since 1968; (5) the availability of a top level U.S. research team with strong East African interest and experience; (6) preliminary field work by WHO and the East African Institute for Malaria and Vector-Borne Diseases in Amani, Tanzania; (7) the desirability of training East Africans in modern medical entomology; and (8) access to ecologically-isolated field populations for experimental manipulation, i.e., both mainland villages and islands off the coast. In addition, there appears to be some scientific advantage in carrying out the investigations in the native habitat of the insect which subsequently spread to other continents.

Throughout all phases of the project, opportunities for spinning off behavioral and genetic data to other mosquito species will be sought. Some subsidiary research on closely-related mosquito disease vectors will be conducted, particularly on A. simpsoni (a Yellow Fever vector which has just this year been colonized by a former Notre Dame student).

Specifically, the objectives of the projects are to:

(a) discover whether the ecology and behavior of Aedes aegypti make this mosquito susceptible to genetic control;

(b) develop technical methods which could be applied in a genetic control program;

(c) provide the scientific basis and operational spring board for future field trials;

(d) contribute to development of a model for genetic control of other insect pests and disease vectors which should stimulate additional research on techniques which are highly selective and environmentally safe;

(e) support a major research component of the International Center for Insect Ecology and Physiology (ICIPE) which should help to trigger financial and scientific assistance for this institution from a variety of potential donors;

(f) provide training opportunities for African scientists and technicians, as well as a mechanism for immediately focusing selected African post-doctorates in medicine and genetics from Notre Dame and other U.S. universities on major African problems in which they will eventually assume leadership roles;

(g) serve as a mechanism for coordinating and strengthening the East African Community by affiliation with both research institutes and universities.

2. Significance to A.I.D. Objectives

The proposed research project addresses a number of A.I.D. objectives since it:

(a) aimed toward eradicating a primary agent of several major diseases afflecting the developing world;

(b) is responsive to the growing interest of the Administration and A.I.D. for nonchemical pest control measures;

(c) promises to have worldwide application to health problems outside East Africa;

(d) has a significant institution-building element by virtue of being administratively centered at the newly-established International Center for Insect Ecology and Physiology and will cooporate with other Institutes of the East African Community;

(e) reinforces and complements efforts of international agencies, such as WHO, PAHO, and UNDP; and

(f) will provide training for African scientists and technicians both in the laboratory and in the field. In addition, the project will involve direct participation by top U.S. scientists and will serve as a model for genetic control techniques which may be readily transferable to other insect pests in Africa as well as in other regions.

It should be noted that while diseases transmitted by mosquitoes such as the Anopheles may be of higher priority from a health point of view, the importance of A. aegypti as a disease vector is unquestionable (independent of the fact that current knowledge of this species makes it a logical starting point for an economic feasibility study of genetic control of mosquitoes). Hemorrhagic fever in cities of Southeast Asia causes 10-30% mortality and no immunization or theraputic agents are available. Protection is accomplished only through control of A. aegypti, as in the case of dengue which is another debilitating disease which plagues many areas of the developing world.

A. aegypti has long been referred to as the "Yellow Fever Mosquito" in the tropics and sub-tropics where it represents a major vector for this disease. Recent outbreaks of Yellow Fever and Dengue demonstrate man's failure to effectively cope with this disease vector problem despite heavy investments of manpower and funds for eradication programs. In Africa, epidemics of Yellow Fever continue to sweep through large areas. In 1962, 100,000 cases of Yellow Fever with 30,000 deaths occurred in Ethiopia. In 1966, 5,000 cases suddenly appeared in Senegal and there is a current outbreak in Nigeria and Upper Volta.

Dr. Hippolyte Aye, Minister of Population and Public Health of the Ivory Coast and President of the World Health Assembly addressed this issue in his Presidential Address before the 23rd Assembly this year. He observed that:

> "I am sorry, on the other hand, that I cannot be optimistic on the subject of Yellow Fever. During the last few months of 1969 five countries of West Africa experienced epidemic outbreaks of Yellow Fever in various rural areas. It is difficult to gauge their exact extent but the disease killed certainly hundreds and perhaps even thousands of people. Thanks to an emergency vaccination programme and also owing to the natural decrease in the number of mosquitoes during the dry season, the epidemic died down. But there is still a danger that the rainy season, which begins in July, will reactivate the foci. In order to make the threatened region safe from further epidemic outbreaks of Yellow Fever effective vaccination and vector-reduction programmes must be organized on a long-term basis. If timely action is to be taken external aid is necessary." 2/

2/ World Health Organization Chronicle July 19, 1970, Vol. 24, No. 7, pg. 292.

A hemisphere-wide Aedes aegypti eradication program under the aegis of the Pan American Health Organization appears to have failed as the vector has reappeared in Panama, Honduras, El Salvador, Brazil and Mexico after supposed eradication ten years ago. During 1964-1969, the U.S. Public Health Service spent $65 million for an insecticide-based campaign in the S.E. United States (in support of the PAHO Program) which was abandoned last year as a failure. At that time, the Director, National Communicable Disease Center, U.S.P.H.S., pointed out that, "Global species eradication through mechanical and chemical means alone is doubtful ... the A. aegypti shows an uncanny ability to adapt to new and changing environments." He asked, therefore, "What are the potentials of biological and genetic control?" 3/

It is of interest that the U.S. continues to contribute through the UNDP to insecticidal control of A. aegypti in countries such as Jamaica even though many experts feel that such programs are doomed to failure because of the evolution of resistant strains of the mosquito.

3. Relation to Existing Knowledge

As the WHO Reference Centre for Aedes mosquitoes, the Vector Biology Laboratory of the University of Notre Dame has ready access to essentially all published literature and data on A. aegypti. Possibly as much as 80 percent of the existing knowledge of the genetics of A. aegypti comes from research workers associated with the Notre Dame facility. Appendix B lists relevant publications by staff of the Vector Biology Laboratory, exclusive of Dr. Milan Trpis, who recently joined the staff from the WHO East Africa Aedes Research Unit in Dar-es-Salaam, and who has published extensively on tropical mosquitoes including A. aegypti.

The results of past research have provided a basic understanding of the bionomics, behavior, physiology and genetics of A. aegypti mosquitoes. Genetically, this is by far the best known mosquito and seven proven techniques are available to modify the insect to induce either complete or partial sterility. There is ample field evidence that genetic control will work in Culex mosquitoes. (USDA with chemosterilants in Florida, Laven and WHO in Burma -- see page 9). For A. aegypti, Notre Dame scientists have shown that released males will disperse and mate with field females, 4/ and that mass production methods are available. The unanswered question is whether one or several of the existing genetic techniques can be applied successfully and economically to control or eradicate A. aegypti populations under natural conditions. The investigators propose an approach to this question with a program of basic research on dynamics of a specific field population. After three years, the groundwork for an operational trial of genetic control should be established.

3/ Sencer, D.J., 1969, Health Protection in a Shrinking World, Amer. J. Trop. Med. Hyg., Vol. 18, No. 3, pp. 341-345.

4/ Fay and Craig, 1969, Mosq. News 29(1): 121-127;
Bond, Craig, and Fay, 1970, Mosq. News 30(3): 394-402.
Hausermann, Fay and Hacker, 1971, Mosq. News 31(1): in press.

4. Relation to Other Research

Aedes aegypti has been studied in the laboratory since 1880 and, today, more than 150 institutions within the U.S. alone are conducting biological investigations with the species, the majority concerned with insecticide development.

Far fewer institutions are engaged in research on mosquito genetics. In addition to the University of Notre Dame, they include:

--Department of Tropical Public Health, School of Public Health, School of Public Health, Harvard University (Dr. Andrew Spielman)

--University of California, Davis, California (Dr. G. A. H. McClelland)

--School of Public Health, UCLA (Dr. A.R. Barr)

--Division of International Medicine, School of Medicine, University of Maryland (Dr. H. C. Barnett)

--Florida Entomological Research Center, Vero Beach, Florida (Dr. M. Provost)

--School of Tropical Medicine, University of Liverpool (Dr. W. W. MacDonald)

--Department of Zoology, University of Illinois, Urbana (Dr. J. B. Kitzmiller)

-London School of Hygiene and Tropical Medicine (Dr. G. Davidson)

--Institut fur Genetik, Gutenberg Universitat, Mainz, Germany (Prof. H. Laven)

To this day most studies of mosquito genetics are carried out to investigate disease transmission, insect behavior, effectiveness of insecticides, or genetic characteristics and anomalies applicable to human systems. It was not until 1967, for example, that University of Notre Dame scientists discovered that A. aegypti females are inseminated only once, a point of obvious importance for genetic control. Recently, there has been great interest in biological control of mosquitoes. Within the past three years, many institutions have initiated programs on mosquito pathogens, predators, hormones, as well as on mosquito genetics. Many applied control agencies now recognize, perhaps belatedly, that such biological methods are urgently needed to supplant insecticides. In the few instances where A. aegypti have been the subject of major biological studies, the investigations have been "science-oriented" rather than directed to practical control techniques.

A U.S. Public Health Service program in Florida in 1961 has provided the only real field test of a biological technique for the eradication of A. aegypti. This program, based on release of radiation-sterilized males, failed completely, probably because of radiation overdoses which affected the mosquitoes' behavior pattern. Thereafter, interest in genetic control waned until there was a rebirth of interest in the late 1960's.

A WHO study of genetic control of culicine mosquitoes is underway in India supported by P. L. 480 funds. The Indian study is focused on Culex mosquitoes, with Anopheles stephensi and A. aegypti programmed for secondary attention. Indications are the relatively low A. aegypti populations near Delhi, the center for the study, will make field studies more difficult. Moreover, unlike Africa, India lacks nondomesticated forms of A. aegypti; thus, the threat of jungle forms to urban eradication cannot be studied. Finally, the Indian project will concentrate on chemosterilization with less effort on alternative genetic mechanisms. Professors Craig and Rai are serving as consultants to the WHO Indian Project and Craig participated in the design of the project. This will permit coordination of the two efforts to prevent unnecessary duplication and insure full utilization of discoveries.

The WHO also operates an Aedes Research Unit in Dar-es-Salaam, Tanzania. In operation since 1968, this Unit has accumulated a great deal of important information on bionomics and distribution of Aedes mosquitoes in East Africa. However, the facility has recently switched its major focus to the utilization of chemical insecticides. The Notre Dame ICIPE project would make every effort to coordinate with the Dar Unit of WHO. In this respect, it is important to note that all three of the principal scientists at the Dar Unit in 1968-70 were associated with Notre Dame at one time; moreover, a current associate member of the Dar Unit, Dr. W. Kilama, received his Ph.D. from Notre Dame in 1970.

The following table gives the history of field trials of applied genetic controls to mosquitoes:

Published Date	Place	Insect	Organization	Results & Agent
1962	Florida	Aedes aegypti	USPHS	Fail - Radiation
1962	Florida	Anopheles quad.	USDA	Fail - Radiation
1963	India	Culex pipiens	Health Ministry	Fail - Radiation
1965	California	Culex tarsalis	State Dept. Publ. Health	Fail - Chemosterilant
1967	Burma	Culex pipiens	H. Laven and WHO	Success - Genetic Incompatibility
1969	Florida	Culex pipiens	USDA	Success - Chemosterilant

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(Table continued)

Published Date	Place	Insect	Organization	Results & Agent
1969	Upper Volta	Anopheles gambiae	C. Davidson and WHO	Incomplete - hybrid sterility
1970	India	Culex Aedes Anopheles	WHO	Incomplete - radiation, chemicals, genetics
1970	France	Culex pipiens	H. Laven & French Govt.	Incomplete - chromosome translocation

The University of Notre Dame, supported largely by NIH and AEC, have conducted fundamental research on A. aegypti for over a decade. The following table shows research support for the Vector Biology Laboratory during 1971:

NIH	- Genetic and Reproductive Biology of Aedes Mosquitoes (Craig)	$100,000
NIH	- Training Grant, Mosquito Biology: Genetic, Organismic, Environmental (Rai)	40,000
USPHS	- (National Communicable Disease Center)-Biochemical Basis of Mutants for Sterility in A. aegypti (M. Fuchs)	31,000
AEC	- Radiation-induced Translocations in Aedes aegypti (Rai)	33,000
WHO	- International Reference Centre for Aedes (Craig)	under negotiation
John Muir Foundation	- Support of field survey in Africa for one year	20,000
Total		$224,000

A number of research breakthroughs by Notre Dame scientists have been made over the past five years which have set the stage for a full field test of genetic control techniques such as is herein proposed. These include discovery that:

(1) Laboratory-reared males will inseminate field females as indicated by transference of a genetic marker from released males to natural populations (Fay and Craig, 1968);

(2) Insemination occurs only once (Craig, 1967);

(3) Females are not receptive to insemination until 42-50 hours after emergence which gives released males ample time to compete (Gwadz and Craig, 1968); and

(4) A number of mechanisms can be used to bring about genetic transformations: sterility through chromosomal translocations (Rai, 1968); sterility through hybrid breakdown in the cross between A. aegypti and A. mascarensis (Hartberg and Craig, 1968); sterility through treatment of virgin females with the monogamy-inducing pheromone, matrone (Craig, 1967); and distortion of sex-ratio with a meiotic drive factor (Hickey and Craig, 1966).

Building on this fundamental knowledge, the proposed project will provide for basic ecological studies followed by field tests of mosquitoes altered by several different techniques. Methods for inserting these altered mosquitoes into natural populations will be developed so that at the end of three years, the time should be ripe for full-scale operational trials.

5. Proposed Work Plan

(a) Scope of Work

The project will initially involve field studies of the ecology and population dynamics of the East African variety of A. aegypti 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
Site selection - geographic and local (island, village, forest)
Procurement - personnel, equipment; design of lab and production facility
Preliminary research on bionomics
At UND & 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.
Lab-tool up for mass production
Lab-mosquito bionomics, esp. product evaluation from mass production
Field and lab - Release technology - when, where, how many?
At UND & ICIPE: Genetic mechanisms and cage trials

FY 1973: Field ecology - Year-long census in designated trial sites
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

For later research, if indications are favorable:
Practical field releases - island and village biotopes
Field comparison of efficacy of genetic mechanisms: chromosome translocations, chemosterilants, etc.
Ongoing genetic and bionomic studies
Evaluation, stressing financial feasibility

The project will be administratively based in Nairobi, Kenya, at the International Center for Insect Physiology and Ecology (sited at the Chiromo Campus of the University College). ICIPE will provide administrative and logistic support, and an affiliation with physiologists, chemists, and ecologists required for certain aspects of the project. Laboratory research on mating pheromones and on strain variation in reproductive competitive ability will be conducted in Nairobi. In addition, ICIPE will develop a program to bring in consultants, using as an entree its access to the best scientific talent in the world in this field.

The major research effort will be centered at a field site which remains to be chosen. The most important requirement is availability of a dense population of Aedes aegypti. Preliminary survey has limited the list to the following possibilities:

1. Mombasa-Malindi region, coast of Kenya
2. Tanga-Amani region, coast of Tanzania
3. Kisumu region, Kenya, bordering Lake Victoria
4. South of Mt. Kenya-Embu-Ft. Hall district

At present, there is a strong preference for the Mombasa-Malindi region because of the following characteristics:

1. Heavy year-round population of A. aegypti.
2. Proximity of domestic (house-breeding) and feral forms of A. aegypti
3. Availability of many semi-isolated villages and islands with separate and discrete populations of A. aegypti.
4. Accessibility, good roads and housing; availability of space at a field station in Malindi kept by the Zoology Dept. of University College, Nairobi.
5. Availability of an African staff in the area trained in entomological methods.
6. Accessible to Nairobi by railroad or 6-hour drive.
7. Extensive research on A. aegypti bionomics conducted in the past by Kenya Medical Dept.; current project by K.M.D. on mosquitoes and arboviruses in Malindi.

Dr. Walter Hausermann of UND has been in Kenya gathering data on site selection since 1 January 1971. A definitive decision on site selection will be made by Drs. Hausermann and Craig during 15-30 May 1971, following attendance of Dr. Craig at an ICIPE meeting in Nairobi.

During the duration of the project supportive research funded outside of the project (principally by NIH and AEC) will be undertaken at the University of Notre Dame. This will consist of:

1. Development of genetic mechanisms, especially chromosome translocations and chemosterilant-induced sterility.

2. Construction of genetic stocks for use at the field site (in conjunction with ICIPE-Nairobi).

3. Continued research on genetics, reproductive biology, population dynamics of Aedes aegypti.

4. Computer simulation to develop predictive models and strategies for release programs.

5. Training of East African and other personnel in modern medical entomology and genetics in preparation for participation at the field site.

The research will be carried out under the overall direction of Dr. George Craig and Dr. K. S. Rai. Dr. Craig will be in Kenya for a full year beginning next September on sabbatical leave and Dr. Rai will follow in 1972 and Dr. Trpis in 1973. Drs. Craig, Rai and Trpis all expect to spend significant parts of each of the three years at the field site. The initial field survey will be directed by Dr. W. Haeusermann who is presently in East Africa. It is anticipated that the University of Notre Dame scientists will work closely with scientists at the University Colleges in Dar-es-Salaam and Nairobi, the WHO Aedes Research Unit in Dar, and the East African Institute for Malaria and Vector-borne Diseases in Amani. The Director of the latter institution has volunteered space and equipment. Cooperation of the East African Institute of Virus Research in Uganda will also be sought.

East African scientists and students will be involved in both the laboratory and field studies. In addition, the University of Notre Dame will train African students in the U.S. for subsequent work on the project. One post-doctorate and two pre-doctorate students at Notre Dame have already been identified for participation, and there will be a yearly increase in participation by East Africans, as students now in training at UND complete their degree work. African Ph.D.-level scientists will be added wherever possible.

(b) Program of Work

The specifics of the proposed activities are identified in the description of the "Research Phases" in the preceding section, 5(a). The project involves essentially three closely-related and overlapping phases (1) field studies of A. aegypti bionomics and population dynamics; (2) laboratory research on

reproduction and genetic mechanisms, and (3) field testing of the behavior of altered males. Evaluation of alternative genetic techniques by release of males during trials and village tests will be initiated in the third year.

6. Research Methodology

Experimentation will be designed to develop answers to the following questions for specific populations:

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.

2. How does real population size fluctuate over a 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?

5. What is the relation of domestic and feral populations? How much gene flow between them?

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?

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?

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 on 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?

Most of these questions can be answered with research methods already available. Population studies will depend heavily on the "capture-mark-release-recapture" method (the Lincoln Index) used widely in ecological studies. Genetic markers in released males will be used extensively to follow reproductive biology. This method was first developed in a joint project between Notre Dame and the U.S. Public Health Service. Primary tool for census will be the ovitrap of the U.S. Public Health Service. Other tools will include the black trap for adults (Fay-USPHS) and the more conventional biting, resting and sweeping collections.

7. Research Competence

The Vector Biology Laboratory of the University of Notre Dame is among the world's leading research institutions on genetic control of mosquitoes and serves as the WHO International Reference Centre for Aedes mosquitoes. (Possibly as much as 80 percent of the existing knowledge of the genetics of A. aegypti comes from research workers associated with Notre Dame.) Research is currently supported at about $200,000 annually by NIH, AEC, NSF, IAEA, and WHO. Some 30 scientists, technicians, and students are affiliated with the Laboratory, including 5, full-time, senior faculty members. The Laboratory has extensive experience with A. aegypti in the area of genetics, cytogenetics, biochemistry, physiology, population biology, and disease transmission. Over the past 10 years, scientists of the Vector Control Laboratory have published over 100 papers on Aedes mosquito (Appendix B). Essentially all of the genetic mechanisms proposed for control of A. aegypti were developed at Notre Dame. An NIH-sponsored training program will provide an ongoing supply of well-trained scientists for this program. (See also Section 3 of this proposal).

Professor George Craig, co-director of the project, is one of the leading U.S. insect geneticists and its leading authority on the genetics of A. aegypti. His selection as a research director of International Centre for Insect Ecology and Physiology (ICIPE) in Nairobi is indicative of his international standing. Professor K.S. Rai is Director of the Mosquito Biology Training Program at the University of Notre Dame with extensive experience in mosquito cytogenetics and mutagen-induced sterility. Moreover, he was responsible for the development of the chromosomal translocations which will be used in this project. Dr. Milan Trpis has worked and published extensively on mosquito bionomics and field collection and testing, and was affiliated with the East African Aedes Research Unit, WHO, Dar-es-Salaam as ecologist/entomologist from 1967-1970. Dr. Walter

Hausermann is scheduled to be project Field Director for the entire project period, 1971-1973. Dr. Hausermann worked on bionomics of blackflies in Tanzania (1965-1968) and on reproductive biology of mosquitoes at Notre Dame (1968-1970). He has been in Kenya since January, 1971, collecting data necessary for selection of a field site. Hausermann will supply continuity to the field program.

Personnel involved in the project, excluding East African scientists and students who will be selected for participation once the project is initiated, include:

I. Personnel - University of Notre Dame

Dr. Walter Haeusermann - Assistant Faculty Fellow, UND. Field Ecology. Worked at Swiss Tropical Institute, Ifakara, Tanzania, 1965-68 and at U.N.D. 1967-70. Began research at ICIPE, Nairobi in 1971. To serve as Field Director of this project.

Prof. G. B. Craig - Professor, UND. ICIPE Research Director and Director, Vector Biology Laboratory. Will spend year of sabbatical leave in East Africa. Basic direction of project. Will have primary responsibility for studies on reproductive biology.

Prof. K. S. Rai - Professor, UND. Director, Mosquito Biology Training Program, UND. Will spend year of sabbatical leave in East Africa. Co-director of project. Will have primary responsibility for studies on genetic control mechanisms, especially translocations.

Dr. Milan Trpis - Assistant Faculty Fellow, UND. Ecology of mosquitoes. Will have primary responsibility for studies on population dynamics. Ecologist with WHO Unit in Dar, 1968-70.

Dr. Paul McDonald - Post-doctoral Fellow, UND; translocation co-developer. Field trials with translocation. Will work at the field site, beginning 1971.

Prof. T. Crovello, UND faculty, biostatistician, population dynamics, computer simulation through entire program.

Prof. W. Hickey, faculty UND (SMC), population dynamics and genetics, entire program.

Dr. Paul Rodriguez, Post-doctoral Associate, UND. May work at field site, 1972. Research on genetics of ability to transmit disease.

Other post-doctorals, University of Notre Dame and possibly, Harvard University, on mosquito behavior and endocrinology.

II. Personnel - East Africa

Dr. Wenceslaus Kilama, Lecturer in Parasitology, Dept. Public Health, Medical School, University of Dar-es-Salaam. Ph.D., Notre Dame, 1970. Available to participate in this project (when his University is not in session) and to furnish students to work in the project. Dr. Kilama is an authority on genetics of ability to transmit disease in A. aegypti.

Dr. Louis Mukwaya, East African Institute for Arbovirus Disease, Entebbe, Uganda. In residence at Notre Dame, Summer, 1968. Also, G. B. Craig was a member of his doctoral committee at Makerere University, 1970. Dr. Mukwaya is an authority on genetics of host choice in Aedes aegypti. Will be asked to serve as Consultant.

Dr. Graham White, East African Institute for Vector-Borne Disease, Amani, Tanzania. In residence at Notre Dame, summer, 1968. Authority on chemosterilization of mosquitoes and on anopheline bionomics and field population assay. Will be asked to serve as a Consultant.

Personnel of the WHO Aedes Research Unit in Dar-es-Salaam will be asked for advice and consultation, especially in regard to the use of insecticides for barrier strips and for initial population reduction.

III. The following Africans are now in training at the Vector Biology Laboratory, U. Notre Dame and may be expected to participate in the project later on:

Dr. Emmanuel Igbokwe, Postdoctoral Research Associate, Nigeria (Ph.D.- Queens U., Canada). Authority on electrophoresisfor analysis of genetic variability.

Mr. V. K. Prashar, Ph.D. candidate, on leave from Kenya Medical Department.

Mr. Jack Oyugi, B.Sc., Makerere U., Uganda; begins Ph.D. studies at Notre Dame in September.

IV. Other Consultants from U.S.A.:

Dr. Eugene Gerberg, Director, Insect Control & Research, Inc. Authority on mass production of mosquitoes. Former Acting Chief, WHO Aedes Research Unit at Dar.

Dr. G. A. H. McClelland, Assoc. Prof. Entomology, U. Calif., Davis. Former Chief, WHO Aedes Research Unit at Dar, 1969-70. Postdoctoral Associate at Notre Dame, 1963-64. Authority on genetics and bionomics of Aedes aegypti. Has worked extensively on A. aegypti in the Malindi area, 1957-1960, while employed by the Virus Institute at Entebbe.

Dr. Keith Hartberg, Assistant Professor, Arthropodology Institute, Georgia Southern College, Statesboro, Ga. Ph.D. Notre Dame, 1968. Geneticist with WHO Aedes Research Unit in Dar, 1968-1970.

Phasing of personnel on location in Africa is as follows:

1971-72 - Haeusermann, Craig, McDonald, additional postdoctoral
part-time: Rai, Kilama, Gerberg

1972-73 - Haeusermann, Rai, Igbokwe, McDonald, additional postdoctoral
part-time: Craig, Trpis

1973-74 - Haeusermann, Trpis, Igbokwe, Prashar, additional postdoctoral
part-time: Craig, Rai

8. Contribution to Institution Building

Foreign institutions which will be involved in the research effort include:

(a) International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, will provide administrative and logistical support; one of the scientists in this project will conduct research on reproductive physiology in the ICIPE laboratories; ICIPE will also provide consultative support from its research directors in genetics, physiology and chemistry.

(b) The East African Community would be asked to provide consultative support from its institutes, especially in Amani, Tanzania (Malaria and Vector-Borne Disease) and in Entebbe, Uganda (Arbovirus Disease).

(c) World Health Organization Aedes Research Unit, Dar-es-Salaam, Tanzania, would provide consultative support.

(d) University College, Dar-es-Salaam, Tanzania, would provide consultative support.

The on-site involvement of leading U.S. scientists will enhance the stature and capabilities of the East African Community of Institutes and the University College. The opportunity to work with the Notre Dame team on a comprehensive, integrated program of laboratory research and field testing will be invaluable to East African scientists and students. The sharing of information and experiences should also contribute significantly to the ongoing research activities of the African institutions in related areas, such as malaria control -- as will the laboratory equipment and mass production facilities to be acquired by ICIPE during the course of the project.

The research will also supplement the work of the WHO Aedes Unit in Dar-es-Salaam which is now focusing on insecticidal approaches to mosquito control. It will likewise make a major contribution to launching the research program of the new International Center for Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya -- the administrative base for the project.

ICIPE is a newly-formed international organization for basic research on insects, sponsored by a worldwide consortium of national academies of science and directed by Dr. Thomas Odhiambo, Dean of Agriculture, University College, Nairobi. The Governing Board includes V. Wigglesworth, acknowledged to be

the foremost entomologist in the world; E. Knipling, Chief of Entomology, USDA, who directed the research program that led to control of the screw-worm; and Professor Carroll Williams of Harvard University, probably the most distinguished insect endocrinologist in the world. Dr. George Craig of Notre Dame is a Research Director of Insect Ecology and Genetics of the Centre. All of the ten research directors from the U.K. and U.S. are F.R.S. or members of the N.A.S. and/or American Academy Arts and Sciences, respectively. ICIPE is to be an international center of excellence. Its goal is to gather the best talent to conduct basic research with insects of economic importance in East Africa. Five areas of specialization have been selected: Tsetse Flies, Ticks, Termites, Armyworms, and Aedes aegypti mosquitoes. By focusing on one of the basic areas of ICIPE interest, this research project will have the full support and assistance of the directorship of the Center and should stimulate funding for other ICIPE research components (a number of international and national lending institutions have expressed interest but have not yet committed funds). However, it should be noted that this project is not contingent upon ICIPE funding.

9. Utilization Plans

The results of the research will be published in scientific journals when appropriate throughout the course of the project. The close cooperation of Notre Dame scientists, ICIPE, the WHO Aedes Unit, and East African counterparts will insure rapid exchange of data and will enhance opportunities for spin-off into ongoing and proposed mosquito research around the world.

If this research leads to a feasible method of genetic control, the information will provide the basis for an operational control program conducted by a health-oriented institution such as the World Health Organization. (An informal inquiry to WHO verified this interest). In addition, a success would give encouragement and direction for similar research on more complex mosquito disease vectors such as the Anopheles.

10. Budget Analysis

Funding requirements from A.I.D. are $258,900 for the three-year project:

Three-Year Funding Requirements 1/

FY 1971	$ 86,300
FY 1972	86,300
FY 1973	86,300
Total 3 years	$258,900

1/ Includes 16% University of Notre Dame overhead

First Year Budget

(In U.S.$)

A.	**Personnel**	36,000
	(1) Scientists (includes salary, medical insurance, housing allowance)	
	Field Director 12,000	
	Postdoctorals (2) 18,000	
	Notre Dame Scientists . . . . . Funded by other sources*	
	(2) Supporting (field and laboratory helpers, following local salary scale. . . 6,000	
B.	**Travel**	10,000
	a. Overseas - 5 trips, 1200/trip 6,000	
	b. Local travel and field living costs . . 4,000 (includes vehicle maintenance)	
C.	**Equipment**	13,000
	Vehicles (2 Toyota Land Rovers) 8,000	
	Microscopes (3 Stereo). 2,000	
	Environ. Control. Apparatus 2,000	
	Miscellaneous 1,000	
D.	Expendable Supplies	4,000
E.	Building rental and maintenance at field site	4,000
F.	Administrative Costs (Money paid to ICIPE for purchasing, record-keeping, importing, etc.-10%)	7,400
	TOTAL	74,400
	Overhead - 16% University of Notre Dame (Standard Off-campus rate)	11,900
		86,300

First year	86,300
Second year	86,300
Third year	86,300
	258,900

Note: In second and third year, expenditure for equipment will be reduced and expenditure for personnel will be increased, specifically by adding additional scientists.

*Includes Craig, Rai and Trpis who hold faculty appointments at Notre Dame (Trpis is Research Faculty) and are salaried by the University and/or by other research grants and contracts.

Other support for the project is to be made available from research grants to University of Notre Dame Vector Biology Laboratory, amounting to approximately $200,000 per year. These funds will go for University of Notre Dame faculty scientist salaries; research in the U.S.; and training of participants from the U.S. (including African students) for subsequent work on the project in Africa. In addition, the John Muir Foundation Conservation Fund, has donated $20,000 as half support for two years for the initial field survey by Dr. W. Haeusermann. (See Section 4 of this proposal.)

Local African institutions will provide limited logistic support, including use of facilities, and several African scientists will contribute their services to the project. If ICIPE funds become available from other sources, these will go largely for post-doctoral salaries and to build up the Nairobi base laboratory -- both of which will contribute to this project.

Material included as Appendices

1. Publication List, Vector Biology Laboratory

2. Curriculum Vitae, George B. Craig, Jr.

3. Brochure of the Mosquito Biology Training Program, U. Notre Dame.

4. Stock List of the WHO International Reference Centre for Aedes, Univ. Notre Dame.

5. Reprint: Knipling, E. F., H. Laven, G. B. Craig, Jr., R. Pal, J. B. Kitzmiller, C. N. Smith and A. W. A. Brown. 1968. Genetic control of insects of public health importance. Bull. Wld. Hlth. Org. 33:421-438.

6. Reprint: Craig, G. B., Jr. 1969. Genetic control of mosquitoes: progress and prospects. Proc. Naz. Accad. Lincei 128:145-154.

11 Internal and External Reviews

The Notre Dame proposal was distributed in October to all A.I.D. regional bureaus and to more than twenty scientists and program administrators within and outside government. In addition, the TA/OST Director discussed the proposed project during visits to ICIPE, the WHO Aedes Unit, several East African Institutes, and A.I.D. missions during a recent trip to East Africa.

To date, comments have been received from the following institutions and offices:

National Science Foundation (Office of International Programs)
State Department (Office of Environmental Affairs)
Johns Hopkins University (School of Hygiene and Public Health)
NIH (Parasitology and Medical Entomology Branch)
NIH (Tropical Medicine and Parasitology Branch)
American Academy of Arts and Sciences
National Academy of Sciences (Rutgers University Review)
U.S.D.A. (Entomology Research Division)
University of Illinois (Department of Zoology)
International Centre for Insect Physiology and Ecology (Nairobi)
East Africa Office of Regional Activities (Nairobi)
WHO Aedes Research Unit (Dar-es-Salaam)
East African Community Research Institutions (Entebbe, Amani)
Economic Planning Ministry (Dar-es-Salaam)
A.I.D. (TA/H)
A.I.D. (NESA/TECH)
A.I.D. (AFR/TAC)
A.I.D. (EA/TECH)

Outside reviewers were in agreement that Dr. Craig and the team he proposes to utilize are the best in the world on A. aegypti genetics, and there was almost unanimous concurrence that the project objectives, scope of work, and proposed budget are sound and realistic. The African institutions consulted were very enthusiastic over the prospect of the Notre Dame team conducting a research project of this nature in East Africa.

Both the review comments from USDA and NIH suggested certain procedures which should be followed in the course of the project. The present proposal appears to incorporate these: specifically, the need to limit the tests initially to "restricted islands" (NIH); emphasis on ecology and population dynamics during the first two years (USDA); and involvement of experts with experience in field trials (USDA). In addition, we have been assured by Dr. Craig that his close involvement in the WHO India mosquito project will prevent duplication of effort (USDA).

Questions were raised by A.I.D. bureau personnel regarding the potential for transferring a successful control technique to other mosquitoes, particularly the Anopheles; and the possibility of NIH funding for the project. Dr. Craig and several outside reviewers were subsequently asked to comment specifically on these issues with the following conclusions:

(a) While it is impossible to state unequivocally the probability of transferring a successful technique, A. aegypti is the necessary first step toward large scale genetic control of mosquitoes based on existing knowledge and techniques of mosquito mass production and genetic transformation. If genetic control is economically feasible for A. aegypti, then there is a strong likelihood that the intensified research effort which will be triggered will establish similar controls for other mosquitoes and related insect vectors;

(b) Within present NIH funding limitations, and specific restrictions on support of research abroad which does not focus on health problems of direct concern to the U.S., there is virtually no possibility that NIH would fund this proposal in the foreseeable future.

12. Proposing Office General Evaluation

A.I.D. has a unique opportunity to involve top scientists from an outstanding U.S. research institution in seeking solutions to a problem of long-standing and significant concern to millions of people in the developing nations. The proposed research effort is manageable in five years and the results, regardless if a genetic control technique is found, will have major implications. If successful, the path will be opened to intensified applied research and eradication programs based on genetic control which will reduce reliance on chemical pesticides which are coming under increasing attack by environmental-conscious people. If genetic manipulation for control of A. aegypti is not feasible, researchers can direct their efforts in other directions with assurance that genetic methodologies have been given a thorough trial.

The fact that Dr. Craig will be available in East Africa next year on sabbatical and will devote himself to this project should be a major consideration. Also, the proposal provides A.I.D. with an excellent vehicle for assisting ICIPE get off the ground by supporting a major research activity within the framework of the institution, while at the same time preserving the project's independence in the event additional ICIPE funding does not materialize. Based on the significance of the problem, the present state-of-the-art of A. aegypti genetics, the availability of Dr. Craig to work in East Africa, the opportunity for scientific and financial support from the Vector Biology Laboratory at Notre Dame, and A.I.D.'s desire to assist ICIPE become established without supporting a general nondirected institution building program, it would seem that the timing is right for a project of this nature.