U. S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

PROGRESS REPORT

SCIENTIFIC ACTIVITIES OVERSEAS
SPECIAL FOREIGN CURRENCY RESEARCH PROGRAM

1. DATE OF AGREEMENT	2. FOREIGN RESEARCH AGREEMENT NO.	
5 December 1972	01-325-2	
3. TYPE OF REPORT	4. REPORT PERIOD	
	FROM	TO
Annual	1.4.73	31.3.74

5. NAME OF INSTITUTION

World Health Organization, Geneva

6. PROJECT TITLE

Feasibility Studies on the Genetic Control of Mosquitos in India (IR-0529)

7. SIGNIFICANT FINDINGS *(If any)* The 8th Technical Planning and Review Group which met in November 1973 reviewed the progress of the Unit during the first phase of its programme of work. During the past four years, work has concentrated on Culex fatigans and Aedes aegypti and an unparalleled body of knowledge on the ecology and on the biological and technical problems of genetic control of these species has accrued. The major achievements of the Unit are summarized in Annex 1.

8. SUMMARY OF PROGRESS *(Give concise summary of progress for this report period. If additional space if required use PHS-3567 (10-60), Continuation Sheet.)*

During the year under review, two field trials on the genetic control of C. fatigans were carried out in the villages around Delhi as follows:-

1. Releases of Sterilized Males (see map attached)

A daily release averaging from 150,000 to 300,000 chemosterilized males was made in the target area of Dhulsiras from 15 February to 31 July 1973. A maximum sterility of 87.7% was reached in the target population.

2. Releases of (IS-31B) Males with Incompatible Cytoplasm and a Male Linked Translocation Complex (see map attached)

A daily release averaging 40,000 males was made in the target area of Gummanhera from August to November 1973. The highest sterility reached in the target population was 67.7%.

In both the experiments, a high percentage of sterility was not achieved for an extended period and there was no evidence of population suppression because of massive infiltration of mosquitos from adjoining areas, in spite of the fact that a barrier zone extending to 3 km was established around the target area. Contrary to the previous observation, C. fatigans was found in these experiments to fly beyond 11 kms.

Continued.....

9. SIGNATURE OF PRINCIPAL INVESTIGATOR

10. SIGNATURE OF DIRECTOR OF RESEARCH INSTITUTION

Studies on Other Integrated Strains of C. fatigans Combining Cytoplasmic Incompatibility and Translocations

Experiments have continued with three new integrated strains: IS-315, IS-328 and IS-335. An average sterility of 80% has been observed in them as compared to 50 - 70% sterility in IS-31B. (In this strain there has been a gradual reduction of sterility and, furthermore, partial compatibility with the Delhi strain has also been observed. Both the phenomena are under investigation).

Experiments on Population Replacement

The release of sufficient males and females of a strain cytoplasmically incompatible with the target population is expected to lead to complete replacement by the released strain. The potential advantages of replacement by a strain selected for its incapacity to transmit filaria have been emphasized by computer simulations demonstrating the impossibility of effective population suppression by any genetic method where immigration and density dependent regulation have strong influences.

A series of cage experiments have been carried out with the Delhi strain and the IS-31B strain. These experiments have shown that elimination of the Delhi strain was possible when it was in a majority provided the initial ratio of the released males was strongly biased towards IS-31B. This system would be desirable in a field release programme to minimize the temporary increase in biting population (females) which would be required to initiate the replacement process. Simultaneously, a search is being made for a refractory strain of C. fatigans to Wuchereria bancrofti for the replacement experiments.

Rearing of C. fatigans under Ambient Conditions

In order to reduce the expenditure in mass production of C. fatigans, rearing of this species under ambient conditions was investigated. Although it is clear that the species can be reared under ambient conditions from April to September in the north Indian climate, the pupal yield is only about 50% as compared to over 90% under controlled conditions of temperature and humidity. Furthermore, in other months, the larval development time is prolonged up to 26 days. Therefore, the rearing of C. fatigans under ambient conditions would not be feasible in northern India.

Field Studies on C. fatigans

The 8th Technical Planning and Review Group has recommended that further release experiments should now be carried out in urban areas in Delhi, as well as in a filaria endemic area of India. The townships of Faridabad and Palwal near Delhi are being surveyed and a site in a filaria endemic area is under selection. Studies are also in progress on new methodology for population measurement and dynamics, which include the testing of newly designed trap boxes, dippers, etc. with a view to standardizing techniques to be used in the future.

Studies on the Translocated Strains of A. aegypti

Four translocated strains of A. aegypti have been developed by the Unit in collaboration with the WHO Collaborating Centre on Aedes aegypti at the University of Notre Dame, USA: T1 and T3 strains have sex-linked translocations, whereas T2 and T4 strains have autosomal translocations. During the preliminary field release experiment at Sonepat last year, it was found that there was some contamination in T2 and T4 strains. It has been established that T2 autosomal translocation cannot be made homozygous apparently because of some factor in the Delhi genome which interacts with T2 causing lethality of the homozygotes. All attempts to establish a homozygous line of the T4 at the Unit have also been unsuccessful.

However, T1 and T3 strains have been established in pure homozygous state, and at the same time a sex ratio distorter gene has been isolated and successfully linked with T1 and T3 translocations. In the distorter T1 homozygous line, there is a strong sex ratio distortion (15 males to 1 female). Double heterozygotes have been made by crossing distorter T1 males to T3 females. These show 56% sterility and sex ratio distortion and appear to be promising material for field releases. The sensitivity of field populations of A. aegypti to the distorter gene is being studied.

Chemosterilization of A. aegypti

Preliminary studies on chemosterilization of the Delhi laboratory strain of A. aegypti have indicated that the males can be sterilized by treatment of pupae for three hours at a concentration of 0.6% thiotepa. Chemosterilized males compete fairly well with normal males for mating with wild females in both laboratory and field cage experiments.

Mass Rearing of A. aegypti

Accelerated efforts are being made on the development of mass-rearing procedures for A. aegypti. Investigations include studies on colony size, male/female ratio, number of larvae per tray and techniques for pupal separation. The Unit has developed the capability of producing more than 200,000 mosquitos per day.

Field Studies with A. aegypti

Field observations on relative density of A. aegypti in Sonepat area are being made to obtain data for one year. These have shown a decrease in density during winter months. Detailed maps of the target release area are under preparation before releases are started in 1975.

Investigations on Anopheles stephensi

Owing primarily to the concentration on C. fatigans and A. aegypti and because of lack of potential intrinsic genetic mechanisms in this species, relatively little work has been undertaken on An. stephensi. Crossing experiments with different strains from Asia and the Middle East were completed

by the WHO Collaborating Centre on Anophelines in the UK, but no genetic factors of potential value have been revealed. However, preliminary studies were made in Delhi on colonization techniques and sufficient competence was acquired in handling the species to allow a quick expansion of work during the next phase of the programme.

The 8th meeting of the Technical Planning and Review Group stressed the importance of An. stephensi as a vector of malaria especially in urban areas and indicated that a high priority should be given to this species during the next few years.

Plan of Work During the Second Phase of the Unit's Programme (see planning profile)

Culex fatigans

The studies on rural populations of C. fatigans will now be phased out and field trials will be carried out in urban areas around Delhi and subsequently in urban sites in filaria endemic areas. An intensive effort will be made to develop a competitive, well-marked strain of C. fatigans refractory to infection with Wuchereria bancrofti. Large-scale releases of the filaria refractory or other selected genetic strains will be made with continuous evaluation of the effects of the releases.

Aedes aegypti

Studies on A. aegypti have reached a stage where a large-scale genetic control experiment can be conducted and such a trial is planned for 1974/75 in the town of Sonepat. There will be continuous evaluation during 1975 and provision is also being made for ecological assessment of the results to continue during 1976.

Anopheles stephensi

Increasing attention will be paid to this species during the next few years, especially the development of sterilization techniques and the advance planning and ecological evaluation necessary for a field trial. Efforts will also be made to develop genetic strains of this species with translocations.

Summary and Evaluation of the Work So Far Carried Out

Culex fatigans

Techniques for the mass-rearing of C. fatigans have been developed to allow the production of approximately one million mosquitos per day. With additional facilities, this number could be increased to any desired level. A new technique for separating the sexes in the pupal stage has been perfected and 99.8% precision is routinely achieved.

Techniques for sterilizing male pupae, either by radiation or by chemicals, have been refined. With 0.60 to 0.65% thiotepa in a phosphate buffer, and a 3-hour exposure period, sterility of 99.9% in males is obtained. After exposure at 6 k R in a cobalt 60 source, both pupae and adults were effectively sterilized. Methods suited to the local conditions have been developed for marking, packing, transporting and releasing C. fatigans males. Quality control systems have been developed to test the material which is released and monitor both the level of sterility and the degree of contamination with females. Furthermore, the effects of sterilization, marking and transport are routinely measured in order to maintain a strict control over the quality of released mosquitos.

The ecology of C. fatigans has been studied qualitatively and quantitatively to a degree which has never before been attempted. Many new or poorly understood aspects of the population dynamics of the species have been clarified. Both immature stages and adults have been studied, special attention being given to the estimation of relative and absolute population size, sex ratios, seasonal loss rates and survival, dispersal, swarming and mating behaviour. In addition, the role of density-dependent factors in the regulation of populations has been studied in natural habitats. The ecological studies have also been extended to urban areas to obtain estimates of the parameters which will be relevant to subsequent release trials in towns and cities.

Strains of C. fatigans which are cytoplasmically incompatible with indigenous populations have been developed, reared on a large scale, and tested in the field. One such integrated strain, IS-31B, has been field-tested for the first time. Effective methods have been devised for testing the mating competitiveness of sterilized males and of females of selected genetic strains. The methods include laboratory cage tests, field cage tests and direct measurements in the field.

Twelve village-scale trials have been made to test the feasibility of genetic control methods under field conditions. The trials were as follows:-

Radiation sterilized males	2 trials
Chemosterilized males	6 trials
Incompatible males	1 trial
Integrated strain with cytoplasmic incompatibility and a translocation	3 trials

Males have been released daily or on alternative days for periods ranging from ten days to eight months. Evaluation of their effectiveness has been based primarily on the sterility rate of the egg-rafts laid in the target area and of the rafts laid by captured females. The maximum weekly sterility rates ranged from 30% to 95% in small-scale experiments of 1971, whereas in larger trials during 1974 and 1975, the sterility rates stabilized at about 60%.

The analysis and evaluation of the data from these field trials is still in progress, but already a realistic picture is emerging of the problems facing genetic control of urban populations of C. fatigans, and, at the same time, the experience gained from the studies in rural areas is suggesting some of the ways in which problems can be tackled and overcome.

Aedes aegypti

Methods for mass-rearing, automatic separation of pupae from larvae, and for the separation of male and female are in the course of being developed and adapted to local conditions. Methods for marking, packing, transporting and releasing A. aegypti males have been developed.

Ecological studies on immature stages and on adults have been in progress in the township of Sonepat for one year where field releases will be made. The parameters being measured are very similar to those listed above for C. fatigans.

Two sex-linked and two autosomal homozygous translocation strains were made available to the Unit by the University of Notre Dame, WHO Collaborating Centre. These have been maintained and their relative merits assessed in the laboratory. The hybrid double heterozygotes have also been studied in cage experiments prior to releasing them in field control trials. Marker strains and single translocation heterozygotes have also been utilized for small field releases. Methods have been designed to measure the mating competitiveness of any stocks of males which may be released.

Three field releases have been carried out:-

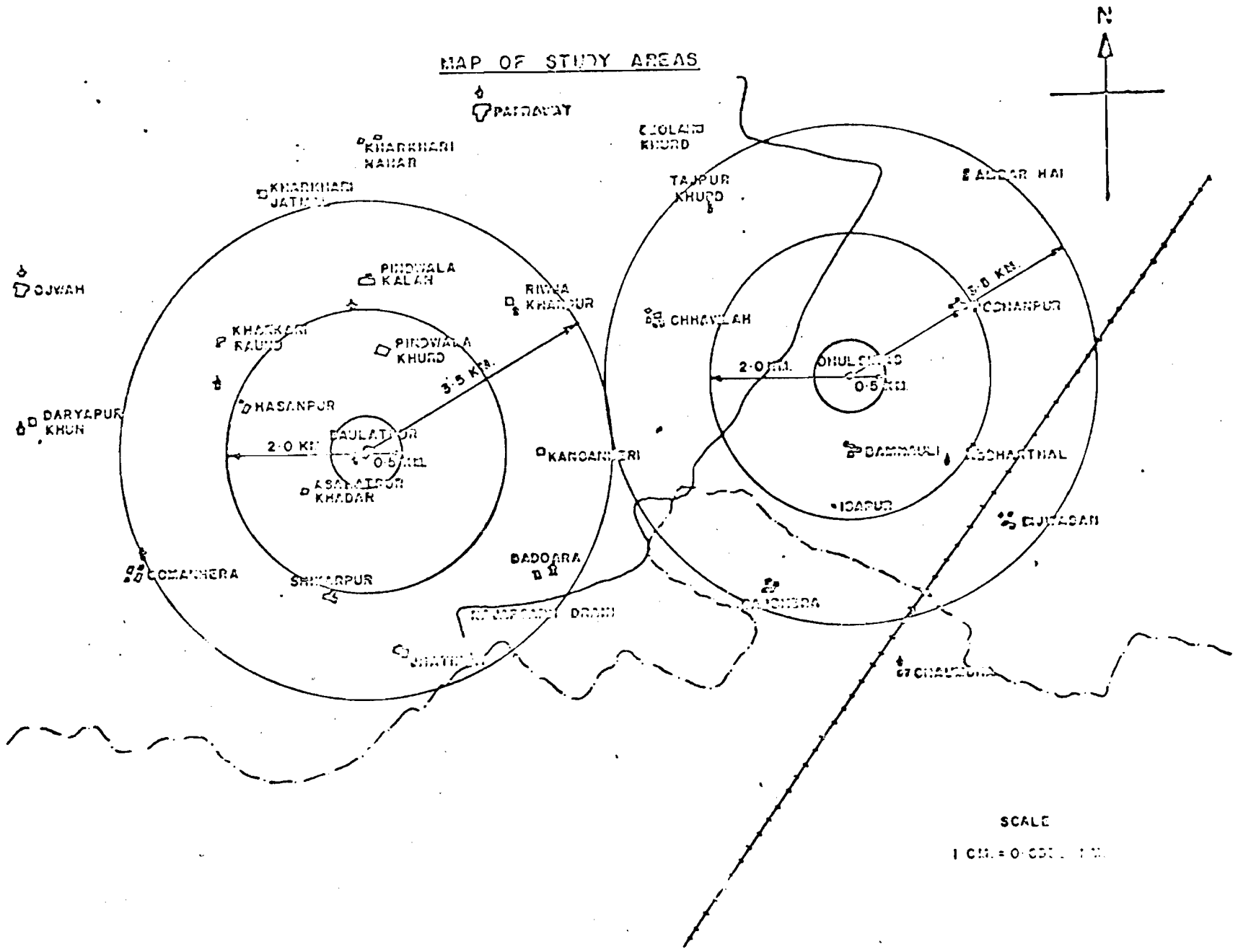
(i) in a tyre dump in Shastri Nagar, Delhi;

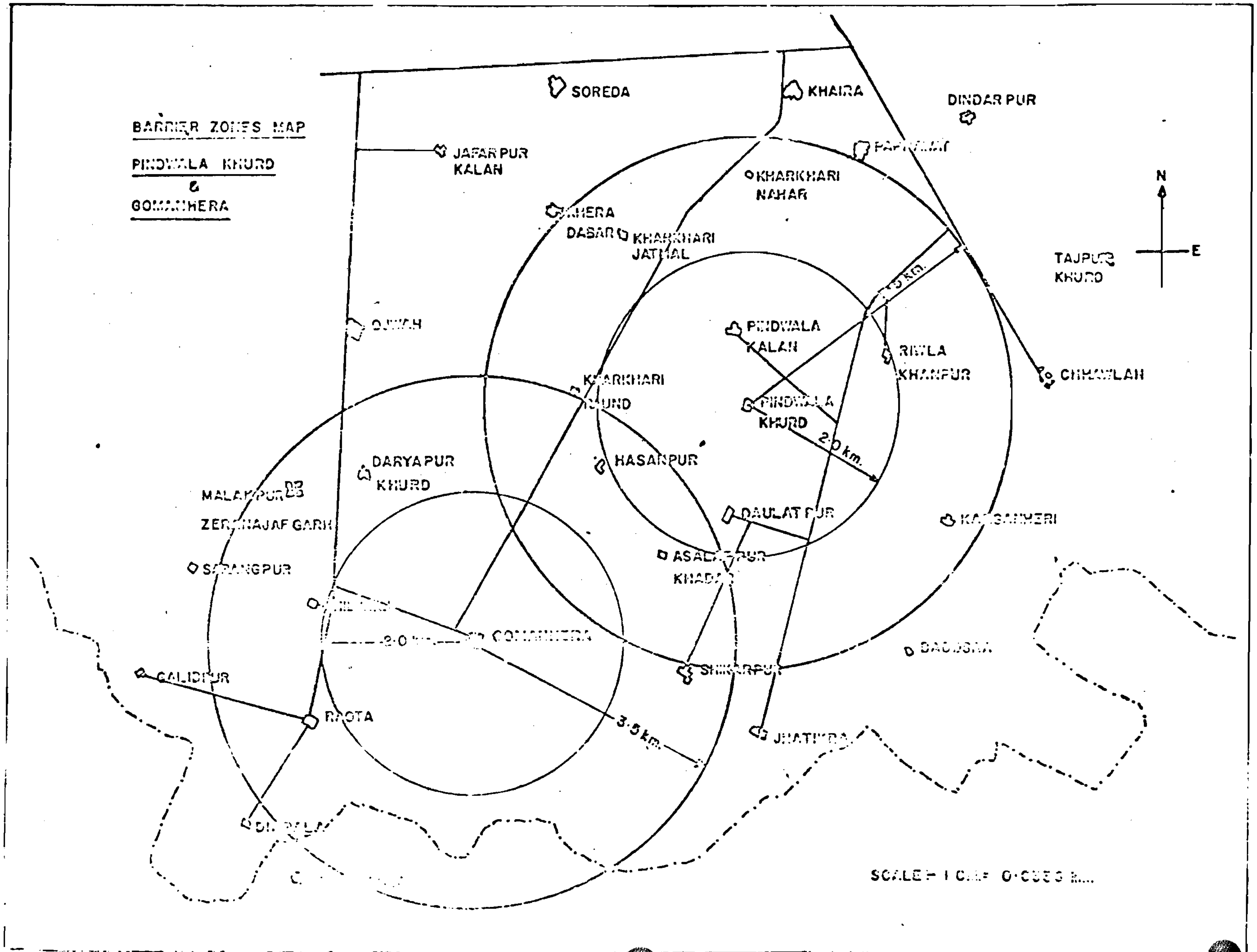
(ii) in a tyre dump in Model Bast, Delhi; and

(iii) in an urban area of Sonepat, Haryana State.

In the first experiment, it was demonstrated that released mosquitos with a semi-dominant marker could inject the character into the wild population. In the second experiment, a heterozygous translocated strain was released, and sixteen days after termination of the release, 40.9% semi-sterility was observed in the wild females. The third experiment, at Sonepat in 1973, was designed to field test males which were double heterozygotes for translocations. Unfortunately, owing to the contamination of the stocks, the experiment had to be terminated after a few weeks. Nevertheless, despite this setback, 13% of the wild females were found to have been inseminated by the released translocated males.

A theoretical evaluation of alternative genetic control strategies has been conducted by computer simulation.


MAP OF STUDY AREAS
N
PATHANAT
KHARKHARI NAHAR
KHARKHARI JATIAN
OJWAH
PINDWALA KALAR
RINWA KHANPUR
KHARKARI RAJPUT
PINDWALA KHURD
HASANPUR
DARYAPUR KHUN
2·0 KM.
3·5 KM.
0·5 KM.
KANCHANHERA
SHIKARPUR
BADOARA
KANCHERI
DHANI
JHATIPA
TAJPUR KHURD
CHHAWLAH
5·0 KM.
CHANPUR
DAMMAULI
CHHARTHAL
JUNASAN
CHAUDHRA
SCALE


BARRIER ZONES MAP
PINDWALA KHURD
&
GOMANHERA
SOREDA
KHAIRA
DINDAR PUR
JAFAR PUR
KALAN
KHARKHARI
NAHAR
DABAR
KHARKHARI
JATMAL
N
E
TAJPUR
KHURD
PINDWALA
KALAN
KHANPUR
KHARKHARI
PINDWALA
KHURD
2.0 km.
HASANPUR
DARYA PUR
KHURD
DAULAT PUR
ZER NAJAF GARH
RAOTA
3.5 km.
2.0 km.

PLANNING PROFILE

WHO RESEARCH UNIT ON GENETIC CONTROL OF MOSQUITOS

IR-0529

ACTIVITY/PROJECT YEAR		FIVE July 1973 June 1974	SIX July 1974 June 1975	SEVEN July 1975 June 1976	EIGHT July 1976 June 1977	NINE July 1977 June 1978
Culex fatigans	URBAN AREAS AROUND PLANT	oooooooooooooo *** ***	oooooooooooooo *** ***			
	FILARIASIS ENDEMIC AREA	XXXXXXXXXXXX oooooooooooooooooo	XXXXX ----------	//////////////	oooooooooooo *** ***	oooooooooooooo IIIIIIIIIIII
Aedes aegypti	SONEPAT	oooooooooooooo ------///////	oooooooooooooo // IIIIIIIIIII	oooooooooooooo	(a) IIIIIIIIIIII	
An. stephensi	STUDY AREA	XXXXXXXXXXXXXXXX	oooooooooooooo ////////// ***	oooooooooooooo *** IIIIIIIIIIII	oooooooooooooo IIIIIIIIIIII	ooooo

KEYS:

SITE SELECTION AND EVALUATION	XXXXXXXXXXXX
BIOLOGICAL STUDIES	oooooooooooo
PREPARATION OF GENETIC STRAINS	----------
MASS REARING PREPARATIONS	//////////
FIELD RELEASES	*** ***
LARGE SCALE RELEASES	IIIIIIIIIIII

(a) Tentative, possibly in a new site