Journal of Genetics Volume 62, Number 3 June 1976

SIMULATION OF ALTERNATIVE GENETIC CONTROL SYSTEMS FOR *AEDES AEGYPTI* IN OUTDOOR CAGES AND WITH A COMPUTER

C. F. CURTIS[1], N. LORIMER[2,9], K. S. RAI[3,9], S. G. SUGUNA[4], D. K. UPPAL[5], S. J. KAZMI[6], E. HALLINAN[7,9] and K. DIETZ[8,9]
WHO/ICMR Research Unit on Genetic Control of Mosquitos,
New Delhi, India

ABSTRACT

Cycling populations of *A. aegypti* of wild origin were established in outdoor cages. Releases were then made for 32-43 days of either males carrying chromosome translocations or males of the sex ratio distorter type. The translocation caused a maximum of 50% sterility, but this declined rapidly after termination of releases. The distorter males depressed the proportion of females among the pupae produced in the cage to a minimum of 35% and the distortion of sex ratio persisted for 13 weeks following termination of releases. It was possible to simulate the effects of the releases with a computer. Simulations were also made of standard release schedules of three types of genetic material. A strain carrying both sex ratio distortion and a translocation gave the most effective population suppression.

INTRODUCTION

Aedes aegypti is the vector of several medically important arboviruses such as the agents of yellow fever, dengue and chikungunya. An attempt at the genetic control of an isolated population of this species was planned in India and the se-

Present addresses:

1 London School of Hygiene and Tropical Medicine, Keppel St, London WC1E 7HT U.K.
2 North Central Forest Experimental Station, St Paul, Minnesota, U. S. A.
3 Department of Biology, University of Notre Dame, Indiana, U. S. A.
4 V. C. R. C., 22 Sham Nath Marg, Delhi 110 054, India.
5 S. G. T. B., Khalsa College, Delhi 110 006, India.
6 V. C. R. C., Lenin Street, Pondicherry, India.
7 Allentown College of St Francis de Sales, Center Valley, Pa, U. S. A.
8 H. S. M., W. H. O., Geneva, Switzerland.
9 Part of the work done on production of the translocation strains at the University of Notre Dame received partial support from ERDA through Contract E(11-1)-78. This is ERDA document no. COO-38-1037.

lection of the most suitable genetic system had to be made from among the several which are available. Much work has been done on chromosome translocation in A. aegypti (Rai, McDonald and Asman 1970; Rai and McDonald 1971; Rai, Grover and Suguna 1973; Rai, Lorimer and Hallinan, 1974) and translocations viable in the homozygous state are now available (Lorimer, Hallinan and Rai, 1972). This presents the possibility of mass rearing two translocations in the form of two pure breeding homozygous colonies and crossing them to produce highly sterile double translocation heterozygotes for release (Uppal, Curtis and Rai, 1974). Among the relatively few progeny which such males would produce, most would themselves carry translocations and therefore would be partially sterile. In A. aegypti there is a meiotic drive system causing sex ratio distortion in favour of males due to a factor at, or very close to, the M (male determining) gene (Hickey and Craig, 1966, a, b; Hickey, 1970). This gene would assist in the economical mass rearing of males for release and, in addition, matings by released distorter males would cause a reduction in the biting population and in the egg laying potential of the wild population. All of the males produced from these matings would themselves carry the distorter gene so that the distorter gene would be expected to spread in the wild population after release. However, a form of the m allele causing resistance to distortion exists in many wild populations and this would prevent the indefinite spread of distortion through a population, but would not prevent the released males themselves from causing distortion because these males would be bred from homozygous distortion sensitive mothers.

Genetic control systems have frequently been assessed in cages, but most of these experiments have been short term tests in which young adults have been mixed to determine their competitiveness. However, where prolonged releases are made into a continuously breeding population and especially where there is inheritance of the released genetic factors, there are several complications to be considered. These include variation in competitiveness with age, competition between the various aquatic stages of the released and wild genotypes and changes in ratio of released to wild males in the later stages of the release programme due to the population suppressing effect of the initial releases, moderated by density dependent regulation. To try to take account of these factors, continuously cycling populations of A. aegypti from the wild Sonepat population were established in outdoor cages. The population size was limited by controlling the number of eggs sustaining the cage population and there was a known level of density dependent regulation at the aquatic stages. Releases of translocated and distorter males were made every day for 32-43 days and the sterilizing, or sex ratio distorting, effect was monitored during and after the release period.

To help to evaluate the cage experiments and also to explore the possible usefulness of other systems which have not yet been tested in cages, a computer model was set up and the effects of various strategies have been simulated. As in the cage experiments, the computer model population had overlapping generations and a specified level of density dependent regulation.

METHODS AND MATERIALS

Cage Experiments

Three outdoor cages were used: Cage A for translocation releases, Cage B as a control without releases and Cage C for releases of distorter males. Cages 5.6 x 3.3 x 2.1 meters were fitted with double doors to minimise escape and entry of mosquitos. Each cage contained a thatched brick hut in which four pans were kept, each containing one litre of water. Every day, 10 eggs were added to pan No. 1, 20 to No. 2, 30 to No. 3 and 40 to No. 4. Initially the eggs came from a stock recently collected in the town of Sonepat (Haryana State, India), but when sufficient eggs began to be laid by the adults in each cage these were used to provide the input for the same cage, so that separate cycling populations were established in each cage. On alternate days 40 mg. of larval food (60% powdered dog biscuits, 40% yeast) was added to each rearing pan and the rearing water was changed every fourth day, taking care that the young larvae were not lost in the process. The number and sex of the pupae produced from each pan were recorded daily and they were placed in separate containers for emergence into the cage. Pads with 1% glucose solution were available in the cage and two caged chickens were provided as a source of blood meal daily. In each cage five black jar ovitraps (Fay and Eliason, 1966) were set. Egg papers were collected from these on alternate days and the eggs were conditioned for three days (Fay, 1966). Sufficient newly conditioned eggs were counted out to provide the daily input of eggs to the rearing pans and the remainder were hatched by placing in de-oxygenated water for 24 hours to determine hatchability. With the eggs from cages A and B hatchability was determined by counting the number of hatched eggs and the total number of eggs (excluding unembryonated ones). Additional data on sterility induced by the translocation releases was obtained by capturing females in cages A and B, egging them individually in the laboratory and determining whether the eggs showed partial or normal hatch. After hatching the eggs from cages B and C, the larvae were reared in the laboratory and the sex of the pupae produced was determined. This provided supplementary data to those from the rearing pans in the cages, on the extent of sex ratio distortion induced by the releases in Cage C.

The cage population were initiated during May-June 1973 when there was almost no rain and day time temperatures reached 46°C. Releases were started in August, when average daily temperatures were about 33°C and there was a total of 455 mm of rainfall. The populations were allowed to continue to breed after termination of releases and that in cage C was continued until late December when the night temperature dropped to approximately 1°C. Mosquito breeding and development had virtually ceased under these conditions.

The translocations used were those described by Lorimer, Hallinan and Rai (1972) which involve chromosomes I/III and II/III, and are referred to as T1 and T2, respectively, in this paper. The translocations were induced in the ROCK genome and made homozygous. They were subsequently backcrossed three times to the Delhi wild type stock and re-isolated. Originally the intention was to cross

members of pure homozygous T1 and T2 colonies thus yielding double heterozygotes, which are highly sterile, for release. However, when members of the T1 and T2 colonies were crossed, the progeny were found to have a mean sterility of only 50% and this was explained when both the T1 and T2 colonies were found to be contaminated with non-translocated chromosomes. Observations on the extent of contamination of the two colonies led to the following estimate of the composition of the release material: 22% double heterozygote, 54% single heterozygote, 24% non-translocated.

The sex ratio distorter stock carried a distorter M^D gene from a Trinidad stock*. This gene was combined with Indian genome by five backcrosses to females of the Delhi wild type strain. From this backcrossed material a pure distorter line was derived from a single pair mating which showed sex ratio distortion and gave male progeny all of which showed distortion, thus proving that all the m alleles incorporated into the line were of the m^d, distortion sensitive, type. Initially the stock had a mean of about 14% females but towards the end of the experiment this was found to have risen to about 20%.

Both the translocation and distorter material for release was reared in the laboratory using the same larval diet as in the out door cages. Eggs were hatched daily so as to provide, as far as possible, a steady daily supply of young male adults for daily release into the cages. All females were removed prior to release.

Computer model

Curtis and Robinson (1971) simulated the population genetics of double translocations in a population model with discrete generations. However, the present population model was based on the more realistic assumption of overlapping generations, all variables being updated daily. For this purpose it is necessary to keep account of the numbers of surviving females of each genotype which have mated with males of each genotype.

In the T1/T2 double heterozygote, cross-overs can occur in the differential segment between the two translocation breakpoints on chromosome III. Thus, the T1 and T2 can exist in the repulsion and the coupling combinations and hence a total of 10 genotypes are possible in each sex, i.e. there are 100 possible types of mating. The ratios of the types of viable progeny from each mating were written into the computer programme, based on the Mendelian rules, neglecting the possibility of complementation between chromosomally unbalanced gametes giving viable zygotes. The frequency of crossing over between the translocations and the fertility of the various matings were left as variables to be specified for any particular translocation. In simulating the behaviour of double translocations, no account was taken of the sex linkage of the T1 translocation, because it was assumed that, in practice, both males and females of a sex linked trans-

*Supplied by Dr. R. Wood, Genetics Unit, Department of Zoology, University of Manchester, United Kingdom.

location homozygote stock would be used for double heterozygote production, so that equal numbers of the M and m linked version of the translocation would be released.

A separate sub-model was used to simulate the sex ratio distortion system. Following the terminology of Hickey and Craig (1966, a) there are four male genotypes: M^Dm^d (distorter males) and M^dm^d, M^dm^D and M^Dm^D (all non-distorters). There are three female genotypes: m^dm^d, m^dm^D and m^Dm^D. The extent of sex ratio distortion in matings by M^Dm^d males and the frequency of m^d and m^D in the wild population were left as variables to the specified for each computer run. The model can simulate the combination of sex ratio distortion with one translocation, and it leaves the fertility of the translocation genotypes and the amount of recombination between the distorter gene and the translocation as variables to be specified. As a result of recombination, there are a total of 16 male and 10 female genotypes, i.e. 160 types of mating.

The model of the dynamics of the wild population assumed a constant daily mortality at all adult ages. Values of 43.9% and 16.9% per day were assumed for the male and female loss rates. These values generate populations with the same mean age at male mating and at egg laying as were observed for A. aegypti in outdoor cages (R. Reuben, personal communication). A constant 4 day period between egg laying and hatching and a 7 day period of aquatic life were assumed. The probability of larval survival is inversely related to larval population density and the strength of this density dependent regulatory mechanism is a variable to be specified for each run.

RESULTS

The behaviour of the population prior to the releases

For the cage population in the absence of releases, Table 1(a) shows the relationship of pupal output to egg input in the four rearing pans in the outdoor cages. There is a fairly clear inverse relationship of larval survival to input density. However, the density dependence relationship was considerably less steep than was intended, perhaps because too much food was given to create conditions of intense competition at the higher densities.

The yield of eggs in the ovitraps was extremely variable from cage to cage and week to week. It was hoped that when the rate of emergence of female adults was reduced as a result of the releases, the oviposition rate would decline, and the intention was then to reduce proportionately the input of eggs into the rearing pans. However, the oviposition rate prior to releases was so variable that it became clear that a correlation of reduction in oviposition to reduction in number of females emerging would not be demonstrated unless female emergence was reduced very drastically. Therefore, the practice was initiated of introducing 100 eggs per day from the ovitraps into the rearing pans, regardless of fluctuations in number of eggs laid.

Table 1. Density dependence of larval survival in the four rearing pans - the number of pupae produced (number of puape produced per input of eggs in parentheses).

	Pan No. 1 (100 eggs/day)	Pan No. 2 (20 eggs/day)	Pan No. 3 (30 eggs/day)	Pan No. 4 (40 eggs/day)
(a) The period before releases (10 weeks for A & B, 4 weeks for C)				
Cage A	573 (.82)	1052 (.75)	1468 (.70)	1859 (.66)
Cage B	557 (.80)	993 (.71)	1567 (.75)	2038 (.73)
Cage C	234 (.84)	423 (.76)	583 (.69)	782 (.70)
(b) The period when pupal output of Cage A was depressed[a] (3 weeks) and when sex distortion in Cage C was greatest[aa] (5 weeks)				
Cage A	76 (.36)	129 (.31)	205 (.33)	394 (.47)
Cage B	157 (.75)	303 (.72)	410 (.65)	592 (.70)
Cage C	282 (.81)	521 (.74)	704 (.67)	1092 (.78)

a i.e., the difference between A & B was greater than 150 pupae.

aa i.e., the difference between number of males and females was greater than 100.

The release of translocated males

The number of translocated males released into cage A are shown in Table 2. The numbers released may be related to a mean weekly emergence in the cage of male pupae of about 250, so that the overall average release ratio was about 3:1. The fertility of eggs laid in cage A and in the control cage B is shown also in Table 2. Fertility in cage A decreased to its minimum two weeks after the termination of releases and thereafter rose rapidly back towards the control level. The fertility from cage B was uniformly very high, so there is no doubt that the sterility in cage A was an effect of the releases of translocations. Females captured from cage A and egged individually showed an increasing proportion that laid partially sterile eggs up to a maximum of 13 out of 16 in the week releases were terminated. From a sample of 28 females showing partial sterility, a total of 119 sons were reared in the laboratory and tested for fertility. From 25 of these partially sterile females, all or some of the sons showed inheritance of the partial sterility, thus proving that the partial sterility was due to the released sex linked or autosomal translocations. Three of the partially sterile females gave all fully fertile sons; this could be explained if the females had mated to males carrying m linked T1 translocations or, in these cases, there might have been no involvement of translocations. Only in one week were any partially sterile females found in cage B and sons obtained from these were all fully fertile. Thus almost all cases of partial sterility in cage A can be attributed to mating by a translocated male and/or to the fact that the females themselves had inherited a translocation. During the period when egg fertility was at its minimum, the pupal yield in cage A was considerably depressed. In this period in cage A there was no relationship of larval survival to egg input density (Table 1b), suggesting that the partial sterility reduced the density of larvae below that at which they compete with each other, even at the highest egg input densities. However, the contemporary data from cage B (Table 1b) show such a weak density dependence relationship that the difference in the relationship in cage A and B is not conclusive.

The release of sex ratio distorter males

Releases of distorter males into cage C were initiated one week after those of translocation males into A and Table 3 shows the numbers of distorter males released. The mean release ratio, relative to an approximate mean weekly emergence of male pupae of 250, was about 2:1. The data in Table 3 on the proportion of females among pupae produced in the cage and on the proportion of females among eggs collected in the cage and reared in the laboratory showed some distortion of the sex ratio from 2 weeks after initiation of releases. Distortion continued to appear for 13 weeks after the termination of releases, at which time the cage population could no longer be maintained because of cold weather.

An important parameter in determining the extent of persistance and spreading of sex ratio distortion in a population after release, is the relative frequency of the m^D (distortion resistant) and m^d (distortion sensitive) alleles in the target

Table 2. Data on the cage experiment with translocated males.

Week	No. of males released into Cage A	Eggs from ovitraps				Captured females				Total Pupal Yield	
		Experimental Cage A		Control Cage B		Experimental Cage A		Control Cage B			
		Total counted	Percent fertility	Total counted	Percent fertility	Total Egged	Percent partially sterile	Total egged	Percent partially sterile	Cage A	Cage B
1	271	0	-	0	-	0	-	0	-	528	574
2	834	940	72	500	98	10	30	12	0	521	541
3	891	464	74	100	100	17	24	10	0	461	491
4	405	300	67	200	100	24	58	18	6	440	534
5	1094	1394	57	450	98	22	50	18	0	445	519
6	1350	2368	57	200	97	14	57	20	0	324	476
7	440	1046	62	300	98	16	81	16	0	330	528
8	0	0	-	0	-	0	-	0	-	211	476
9	0	120	50	100	100	13	39	13	0	261	359
10	0	800	75	200	99	13	23	16	0	345	481
11	0	500	91	100	99	17	24	12	0	311	312

Table 3. Data from the cage experiment with distorter males.

Week	No. released into Cage C	Pupae reared from eggs laid in ovitraps					Pupae in Cage				
		Control Cage B		Experimental Cage C			Control Cage B		Experimental Cage C		
		No. Females	No. Males	No. Females	No. Males	Percent Female	No. Females	No. Males	No. Females	No. Males	Percent females
2	489	211	217	153	154	49.8	270	271	224	224	50.0
3	467	201	189	65	112	36.7	246	245	223	226	49.7
4	530	-	-	1	60	1.6	292	323	229	305	42.9
5	485	117	110	107	258	29.3	255	264	268	313	46.1
6	435	136	116	187	363	34.0	244	232	255	343	42.6
7	0	147	145	326	502	39.4	234	294	263	387	40.5
8	0	-	-	151	269	35.9	232	244	182	315	36.6
9	0	-	-	96	176	35.2	181	178	173	329	34.4
10	0	100	103	228	296	43.5	249	232	195	316	38.2
11	0		-	299	398	42.8	-	-	150	271	35.6
12	0	116	100	451	569	44.2	-	-	208	311	40.1
13	0	-	-	151	172	46.7	-	-	150	212	41.4
14	0	412	410	55	95	36.7	186	204	184	260	41.4
15	0	113	85	472	563	45.6	182	195	146	194	42.9
16	0	-	-	218	303	41.8	244	220	140	223	38.6
17	0	242	242	318	517	38.1	212	213	202	299	40.3
18	0	-	-	-	-	-	145	162	110	170	39.3
19	0	-	-	-	-	-	123	129	93	151	38.1

wild population. These genes can be identified in a female by mating to a distorter male and test mating a sample of the sons. If the female was m^dm^d all the sons will be distorters, if it was m^Dm^D all the sons will be non-distorter and if it was m^Dm^d there will be a segregation of distorter and non-distorter sons. A sample of 24 females from the cage population prior to the start of releases were classified as follows: $4m^Dm^D$, 12 m^Dm^d and 8 m^dm^d, giving an estimated m^d frequency of 62.5%. A sample of 31 females from the town of Sonepat, where the material originated, indicated an m^d frequency of 69.3% which conforms with data of R. Wood (personal communication) from collections in the same town. No M^D alleles have been found in wild males in Sonepat. At weeks 6, 18 and 19 after the start of the experiment, males from eggs laid in the cage were mated to females of the distorter line (which are known to be m^dm^d). The number of these individual matings which showed distortion were recorded and, from the matings without distortion, sons were test mated. When the original male captured from the cage had the distorter allele neutralized by the resistance allele (M^Dm^D), the sons show sex ratio distortion when mated. Those tested allowed the following classification of genotypes from the cage:

Week	M^Dm^d	M^Dm^D	M^dm^-	Total No. tested
6	17	10	7	34
18-19	8	6	4	18

Thus there was little apparent change in the gene frequencies over the 12-13 week period during which no releases were made.

Computer simulation

(a) Simulation of the cage experiments

Computer predictions of the fertility or proportion of female pupae in the field cage populations compared with the observed data in field cage A and C are shown in Fig. 1. The following values were used for the fertility and recombination in the translocation genotypes in cage A: T1T1 - 44%; T1T2 - 20%, T2T2 - 62%; 10% recombination between T1 and T2 (Lorimer et al, 1972; Rai et al, 1974; Lorimer and Hallinan, unpublished data). All genotypes were assumed to have full viability and mating competitiveness. For the translocation cage, A, the predictions and the observed data fit reasonably well. For the distorter cage, C, an initial frequency of 30% of the m^D gene in the wild population was assumed and the simulations were run specifying either (a) 14% or (b) 20% females from matings of the distorter males. In simulation (b) a larval viability of 81% of normal was attached to distorter males on the basis of limited data which indicate such reduced viability under outdoor conditions. There was a large discrepancy between simulation (a) and the observations, but simulation (b), with a weaker distortion and reduced viability of distorter males, agreed better with the observations. The simulation predicted a steady increase in the proportion of females produced after the termination of releases because of the evolution of an increasingly high m^D frequency under the selection pressure for this gene, which is

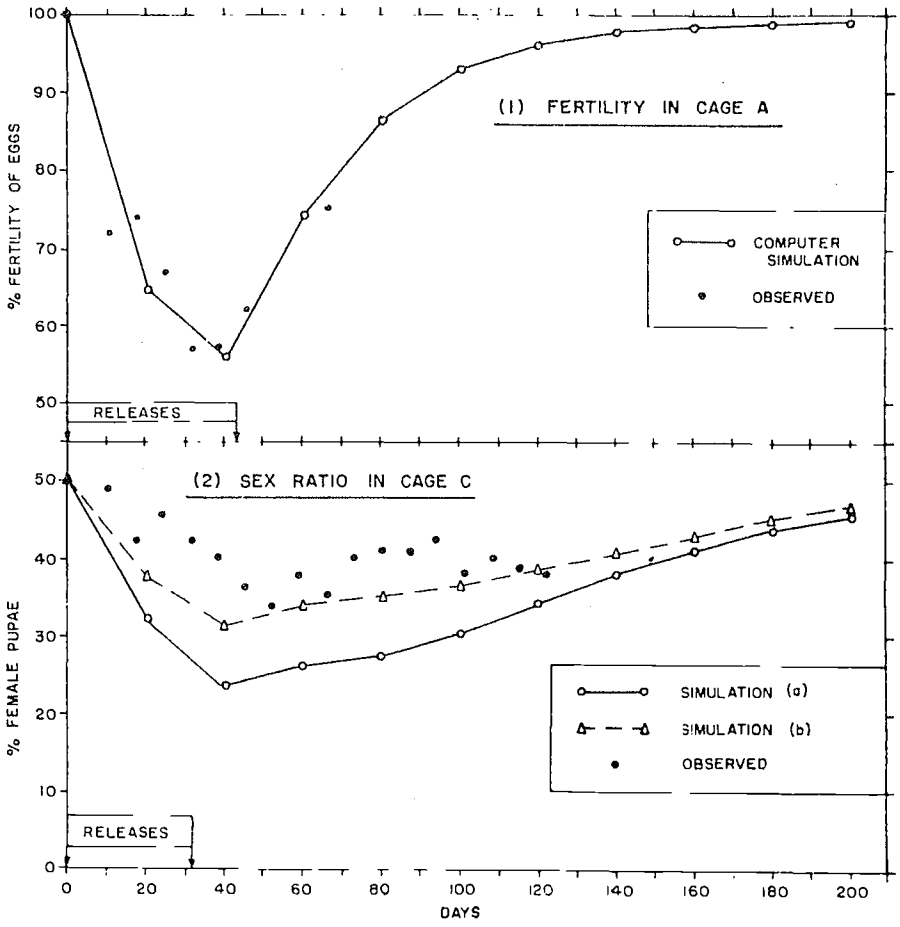

Fig. 1 The observed data on fertility in cage A (with translocation releases) and proportion of females among the pupae in cage C (with sex ratio distorter releases) in comparison with computer simulations. The assumptions on which the simulations are based are defined in the text.

created by the presence of the M^D gene in the population. The observed data did not show evidence of this effect in the 13 week period after releases were terminated. This may, at least in part, be explained by reduction in the rate of turnover of the population to a low level due to low temperatures in the later stages of the experiment.

(b) Simulation of possible control systems

Simulations of releases of three types of genetic material into a population, which has been assumed to have density dependent regulation leading to a maximum of three fold recovery potential per generation, are presented in Fig. 2. In each case a standard release pattern was tested, i.e. daily releases for 60 days of six times the initial daily emergence of males. The release males tested were: (a) distorter giving 14% females, (b) double translocation heterozygotes (T1T2), (c) distorter single translocation heterozygotes (Distorter T1+) made by crossing males of a distorter T1T1 homozygote stock to untranslocated distortion sensitive females. These males were assumed to produce 14% females and 50% sterility and to show 3% recombination between T1 and the sex locus. In each simulation it was assumed that 99.5% of the females were removed before the mosquitos were released (Singh, Brooks and Ansari, 1974). The values used for the fertility of the translocation genotypes were as specified above.

In each case the population was suppressed to a minimum some time after the termination of releases and it then started to recover. The recovery was due to density dependent regulation coupled with selective elimination of the translocation and/or selective increase in the m^D frequency. The distorter males succeeded in achieving a maximum of 88% population suppression only, but the other two systems achieved over 99% suppression and after the Distorter T1+ releases the population remained suppressed to the extent of more than 99% for about 80 days.

DISCUSSION

Both the translocation and distorter releases had detectable effects on the emergence rate of females in the cage populations, the effect being temporarily stronger with translocations but also dying away faster after releases were terminated. The experiments did not provide a definitive comparison of the two control systems for several reasons, notably: (a) the impurity of the translocation material, (b) the relatively small numbers released, (c) the variability of numbers of eggs collected in the cages, and (d) the weakness of the density dependence of larval survival. After remedying these defects it is believed that meaningful comparisons of the population suppressing efficiency of different systems will be possible from field cage data, supplemented with studies on dispersal, mating competitiveness and density dependent regulation in the wild. Ideally small scale comparative field studies on population suppression would be preferable because they would avoid the obvious artificiality of the cage system. However, experience in this Unit with Culex fatigans has indicated the difficulty

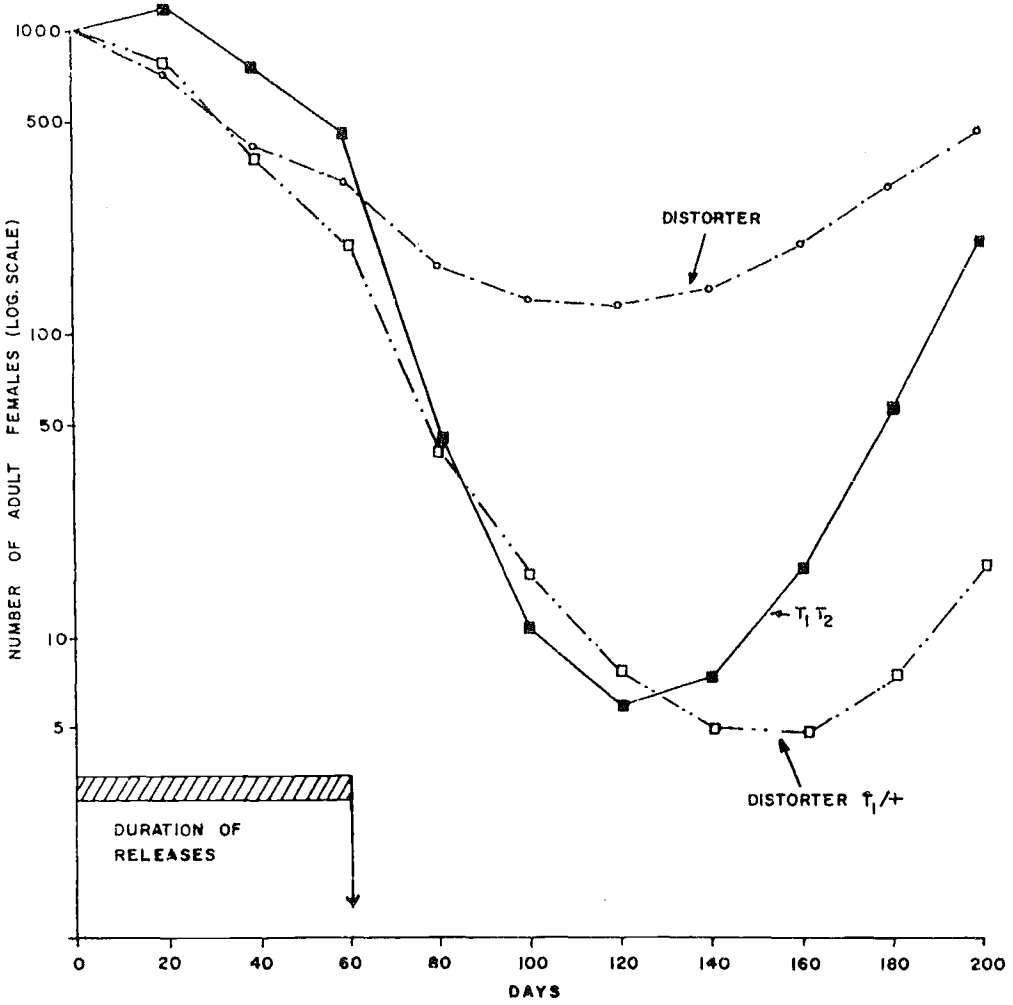

Fig. 2 The effects of standard release schedules of three types of genetic material. The assumptions on which the simulations are based are stated in the text.

of finding sites for such studies which are sufficiently isolated and comparable in their natural seasonal trends to yield conclusive comparisons of different genetic control systems.

The cage experiments and computer simulations confirmed intuitive expectations that the sex ratio distorter alone would be insufficiently powerful to control a population with a considerable frequency of the distortion resistant, m^D, gene. However, the computer simulation indicated considerable promise for a linked system of the distroter and a translocation. Recently the T1 translocation has been linked to the distorter and inter-actions have been found which will require modifications in the assumptions on which the simulation of DT1/+ in Fig.2 is based (Suguna and Curtis, 1974). Another I/III translocation, T3, has been established as a pure homozygous line and the T1/T3 double heterozygote without distortion (Uppal, Curtis and Rai, 1974) and with distortion (Suguna and Curtis, 1974) have been produced and these genotypes, together with chemosterilized males, were compared during 1974 for their ability to suppress populations in field cages (Curtis et al., 1976).

ACKNOWLEDGEMENTS

We are grateful to V.K. Soni, H.E. Joshi, J.K. Swamy, K. Ramesh and Ashok Kumar for their conscientious technical assistance and to A. Thomas for carrying out the computing.

We thank Dr R. Wood, Genetics Unit, Department of Zoology, University of Manchester for provision of a stock carrying the sex ratio distorter gene.

We are grateful to Dr G.D. Brooks for his support and encouragement.

REFERENCES

CURTIS, C.F. and ROBINSON, A.S. 1971. Computer simulation of the use of double translocations for pest control. Genetics, 69, 97-113.

CURTIS, C.F., GROVER, K.K., SUGUNA, S.G., UPPAL, D.K., DIETZ, K., AGARWAL, H.K. and KAZMI, S.J. 1976. Comparative field cage tests of the population suppressing efficiency of three genetic control systems for Aedes aegypti. Heredity, 36(1), 11-29.

FAY, R.W. 1964. The biology and bionomics of Aedes aegypti in the laboratory. Mosquito News, 24, 300-308.

FAY, R.W. and ELIASON, D.A. 1966. A preferred oviposition site as surveillance method for Aedes aegypti. Mosquito News, 26, 531-535.

HICKEY, W.A. and CRAIG, G.B. 1966. Genetic distortion of sex ratio in a mosquito Aedes aegypti. Genetics, 53, 1177-1196.

HICKEY, W. A. and CRAIG, G. B. 1966b. Distortion of sex ratio in populations of Aedes aegypti. Can. J. Genet. & Cytol., 8, 260-278.

HICKEY, W. A. 1970. Factors affecting the distortion of sex ratio in Aedes aegypti. J. Med. Ent., 7, 727-735.

LORIMER, N., HALLINAN, E. and RAI, K. S. 1972. Translocation homozygotes in the yellow fever mosquito Aedes aegypti. J. Hered., 63, 159-165.

McDONALD, P. T. and RAI, K. S. 1971. Population control potential of heterozygous translocations as determined by computer simulations. Bull. Wld. Hlth. Org., 44, 829-845.

RAI, K. S., GROVER, K. K. and SUGUNA, S. G. 1973. Genetic manipulation of the mosquito Aedes aegypti 1. Use of a genetic marker and a chromosomal translocation in field studies. Bull. Wld. Hlth. Org., 48, 49-56.

RAI, K. S., LORIMER, N. and HALLINAN, E. 1974. The present status of genetic methods for controlling Aedes aegypti. In: Whitten M. J. and Pal, R., eds, The use of genetics in insect control, North Holland, pp. 119-132.

RAI, K. S., McDONALD, P. T. and ASMAN Sr. M. 1970. Cytogenetics of two radiation induced sex linked translocations in the yellow fever mosquito Aedes aegypti. Genetics, 66, 635-651.

SINGH, K. R. P., BROOKS, G. D. and ANSARI, M. A. 1974. Mass rearing of mosquitos. J. Comm. Dis., 6, 121-126.

SUGUNA, S. G. and CURTIS, C. F. 1974. Sex ratio distorter strains in Aedes aegypti. J. Comm. Dis., 6, 102-105.

UPPAL, D. K., CURTIS, C. F. and RAI, K. S. 1974. A double translocation heterozygote in Aedes aegypti. J. Comm. Dis., 6, 98-101.