AN EXPERIMENTAL INVESTIGATION OF AUTOSOMAL TRANSLOCATIONS FOR INSECT PEST CONTROL: FITNESS EFFECTS AND MARKER-FREE ISOLATION TECHNIQUES.

BY

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ABSTRACT

Because of recent advances in genetic insect control theory, it has become important to investigate the fitness effects of, and isolation procedures for, homozygous autosomal translocations. I isolated 57 autosomal translocations in Drosophila melanogaster . Of these 21 were homozygous viable. From data obtained during the isolation of these translocations and from fitness tests and competition cage experiments, the following points can be made: (1) Between one in ten and one in one hundred homozygous viable laboratory produced translocations are likely to be of value in field tests of genetic insect control procedures. (2) Translocations which produce high levels of unbalanced gametes when heterozygous do not tend to be less fit in the homozygous state than others. Therefore screening procedures dependent only on reduced progeny production from translocation heterozygote parents should be satisfactory for the isolation of useable stocks. (3) Translocations whose breakpoints are very near the center of chromosomes tend to produce small progeny reduction in the heterozygous state, making these translocations useless as negative heterotic carriers of useful genes. (4) Those translocations which are the result of multiple break events tend to be less fit than simple double-break translocations and therefore should be discarded.

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INTRODUCTION

A number of genetic insect control techniques have been proposed in recent years. Several of the theoretically most promising methods involve the use of reciprocal translocations.

The main property of translocations which makes them potentially useful (for the control of insect pests) is that hybrids arrising from crosses with normal insects have reduced fecundity since as many as half of all gametes produced by the hybrids are genetically unbalanced.

It should therefore, be possible to use translocations to control insect pests in several different ways. Multiply translocated laboratory stocks, if released in large numbers into populations of pest insects should reduce the fecundity of the populations. Most proposed methods, however, involve the use of translocations linked to genes which, from man's point of view, are preferable to their alleles in pest populations. For these methods to work, translocation homozygotes must have fitness superior to heterozygotes under field conditions. If this is the case, and enough translocated insects are released into wild populations, negative heterosis will ensure that the translocation will replace the wild genotype. It is fortunate that crossing over is severely depressed in the vicinity of translocation points. This means that translocations can be used as carriers to drive genes into populations.

The kind of gene that we might wish to put into a population depends on the species. If an insect is a disease

vector, a gene which renders it incapable of harboring the disease may be ideal, both for man and for the insect. In cases where eradication of a pest is the ultimate aim, a conditional lethal gene may be best. Good candidates are genes which prevent an insect from going into diapause, at least in northern latitudes. Temperature sensitive lethals might also be useful.

There is a broad chasm between conceptualizing a genetic insect control program and implementing it. For many insect pests, a reasonable idea of the cost of rearing enough insects to suppress wild populations can be obtained. But we know very little about the time and effort required to produce the genetic stocks needed to implement any kind of sophisticated genetic insect control program. In fact, we can not, apriori, be sure that it is technically possible to engineer the right kind of stock at all.

Logically, the best way to fill this gap in our knowledge is to first obtain the relevant information by using, as a pilot organism, the genetically most tractable insect which has the genetic and life history features of important pest species. That insect is Drosophila melanogaster .

The main unresolved question that I have chosen to ask is: what proportion of homozygous viable translocations are fit enough to be useful in control programs. Many advocates of genetic insect control dogmatically assume that the proportion is high! However, practical experience suggests that the assumption is wrong. Robertson and Curtis (1973), in an experiment involving one Drosophila translocation, found that

their translocation could not compete successfully with wild flies. Foster (pers. Comm.) found that only one of thirty induced translocations in the sheep blowfly, Lucilia cuprina, was homozygous viable and fertile. It, however, had a low fecundity.

Another major objective of my research was to examine, in detail, the practicability of a simple procedure for isolating useful translocations. This method would be applicable in pest species where methods involving visible genetic markers, crossover-suppressors and polytene chromosome squashes can not be used.

LITERATURE REVIEW:

There are two categories of genetic insect control techniques. These will be termed genetic load techniques and the population replacement techniques.

The objective of genetic load techniques is to eradicate or lower to commercially tolerable levels pest populations through the introduction of translocated or otherwise genetically aberrant individuals: introduced individuals, by mating with members of a wild population, lower the average fitness of the population. Such techniques were first suggested by Serebrovskii (1940). The best known procedure of this sort is the sterile insect technique, which was used to eradicate, at least temporarly, the screw-worm fly from the South Western U.S.A.

Gene pool replacement of pest populations by specially taylored laboratory strains was suggested by Curtis (1968). The nature of an introduced genetic stock must be such that it will tend to replace the original wild-type stock. The key point is that the introduced genetic material must initially confer a fitness advantage so that individuals carrying it are favored in competition with wild stocks.

The more sophisticated variants of both categories of genetic control procedures, load and replacement, rely on a single principle of population genetics. This principle is termed negative heterosis. Negative heterosis implies that the heterozygote between two strains is less fit than either of the

two homozygotes. This results in an overall population fitness depression and in an unstable equilibrium between wild-type and translocated stocks. This in turn, results in frequency dependent selection in favor of the genotype which is present in excess of the unstable equilibrium level.

Genetic Load Population Suppression:

Moderately successful experimental genetic manipulations of pest populations have been reported. Wagoner et al. (1973), using sex-autosome translocations, produced a significant depression in a wild house fly population (field test). Laven (1969), also using sex-autosome translocations, eradicated structurally wild-type Culex pipiens (large cage test). These examples encourage further development of genetic insect control methods.

Gene Pool Replacement:

The development of replacement techniques was first suggested by Curtis (1968). His idea was to use single translocations to cause an unstable equilibrium condition which could be exploited to introduce closely linked desirable genes into insect populations. In such a case, the translocation is termed a carrier mechanism as it can be used to transport a desirable trait into wild populations. Desirable traits include high or low temperature lethality and insecticide susceptibility as well as the inability to be a disease vector.

The theory was later expanded by Curtis and Robinson(1972) who examined the use of double translocations for pest control. Whitten (1971a) further extended the theory by considering the use of multiply translocated strains which would carry desirable characters. He developed a simple discrete generation computer simulation model. The results of this model suggested to Whitten that for a translocated strain with a fitness as low as .7, replacement of the wild population would be virtually complete in seven generations.

Whitten (1971a) subscribed to two myths that pervade the literature of genetic insect control. The first is typified by the following quote:

> "Once a set of suitable strains has been developed
> ... and the system tested... its permanence is
> insured since natural selection cannot oppose it;
> rather natural selection is an essential
> ingredient of the program." (Whitten 1971a, page
> 684.)

This may be paraphrased as "God is in the laboratory and mother nature is no match for him".

The second myth is typified by the following quote:

> "Although this multiple translocation strain,
> (developed in the lab) can be expected to equal
> the base strain in fitness..." (Whitten 1971a,
> page 682)

This second myth may have arisen from a statement in Curtis (1968), "...But translocation homozygotes (T/T) are usually

fully fertile." Serebrovskii (1940) on the other hand had suggested that the greatest problem, even for genetic load techniques, would be development of highly fit laboratory strains.

A large portion of the theoretical and experimental literature is based on the second myth, that translocated strains with extremely high fitness can easily be constructed.

Foster et al. (1972) note, as did Curtis (1968), the problem of inseparably linking desired characters to the genetically rearranged strain. They pointed out that if a desirable allele is genetically separable from the transporting mechanism, then the desired allele (for temperature sensitivity etc.) could be accidently lost via genetic crossing-over in the heterozygote during gene-pool replacement. Thus the net effect of such a replacement program would be that one wild-type strain would be replaced by another at great expense.

Foster et al. (1972) also considered the use of compound chromosomes (a special type of translocated chromosome where the left and right arms are attached to the same centromere) as a transporting mechanism. Compound chromosomes seem very attractive as carriers since the desired alleles cannot be removed by crossing over in heterozygotes. This is so because compound by wild-type crosses produce no viable heterozygotes.

Foster et al. (1972) reported on discrete generation experiments showing density dependent selection in competition between a compound stock and a structurally wild-type stock.

The main problem with replacing pest species by releasing strains with compound chromosomes is that compound chromosomes are very difficult to construct in pest species which are not genetically well characterized.

Smith and von Borstel (1972) also noted the crossing over problem. They coined the term "Time Bomb Technique" for the replacement of insect populations by conditional lethals attached to a carrier. Wehrhahn and Klassen (1971) suggested the use of conditional lethals inseparably linked to genes for insecticide resistance. Among other things, they favor the use of insecticides to promote replacement of wild populations with conditional lethals. Tight linkage between resistance and conditional lethal genes is more critical to the usefulness of their method than any small decrement in fitness would be.

Wehrhahn (1973) noted that species with low powers of dispersal would be least amenable to control because "hot spots" of wild-type insects, not easily swamped by introduced genotypes, would develop after a few generations of a control program.

Fitz-Earle et al. (1974), using compound chromosomes of Drosophila melanogaster , achieved total replacement of the wild-type stock from which the compound was derived. This was the first time negative heterosis was used to achieve true wild-type (as opposed to structurally wild-type) population replacement.

Translocation Isolation Procedures:

All the work mentioned to this point has been done in insects which have previously been genetically very well characterized. The development of visible genetic markers and cross-over suppressors would cause a genetic insect control project in most pests to be delayed by several years.

Because of such time considerations, the work reported by Curtis (1971), with tsetse flies, and Wijnands-Stab and Van Heemert (1974), with the onion root-maggot fly, is of special interest. Curtis (1971) isolated translocation homozygotes in tsetse flies without resorting to the use of genetic markers. Wijnands-Stab and Van Heemert (1974) isolated translocations by irradiating sperm and checking for reduction in progeny numbers in the F2 generation. They were able to confirm their findings with the simplest of cytogenetic methods, somatic chromosome preparations.

MATERIALS AND METHODS:

Drosophila populations were kept in cages (see Appendix 1) similar to those first developed by Bennett (1956). These cages are described in Appendix 1. Each cage contained eight food vials. One food vial was replaced by a fresh one every three days.

During experiments, cages were kept operational for from one to two months. In all cases standard Oregon-r control populations were initiated at the same time as the experimental populations to aid in the detection of any change in conditions that, otherwise, might be construed to be an experimental effect.

My Drosophila melanogaster stocks were obtained from three sources. Translocated strains, glassy, smudge, and hairy, came from Dr. P. T. Ives' laboratory in Amherst Massachusetts. (The smudge stock is the same as that used by Robinson and Curtis (1974)). All other stocks except those referred to as TVR , were provided by Dr. D. Holm's laboratory at U.B.C. Those stocks labelled TVR1 through TVR57 are the translocated stocks which I derived from Dr. Holm's Oregon-r, wild-type, stock.

I isolated TVR1 through TVR57 by the standard Drosophila melanogaster marker system outlined in Figure 1a. These translocations were checked for homozygous viability by a sibling cross between males and females heterozygous for the translocation and a multiple-break, homozygous lethal, crossing-

Figure #1a.

A <u>Drosophila</u> <u>melanogaster</u> translocation identification procedure which utilizes
the two recessive eye colour markers, brown (bw), and scarlet (st). These interact to
produce the phenotype white eye. When the sperm contributes a 2-3-translocated haploid
genome, one half of the F_2 progeny never become adults.

Figure #1b.

A <u>Drosophila</u> <u>melanogaster</u> purification scheme which utilizes the "balancer" chromosome,
2L2R Cy bw (dominant phenotype Curly). When a homozygous viable translocation is
present, unmarked progeny will occur in the F_4 generation.

P_1	F_1	F_2	Phenotype	Alive (A) or Dead (D)	
				2-3-T	NON-2-3-T
(sperm) $+;+$ bw;st (ovum)	$\frac{+}{bw};\frac{+}{st}$ ♂ X $\frac{bw}{bw};\frac{st}{st}$ ♀	$\frac{+}{bw};\frac{+}{st}$	wild-type	A	A
		$\frac{+}{bw};\frac{st}{st}$	scarlet	D	A
		$\frac{bw}{bw};\frac{+}{st}$	brown	D	A
		$\frac{bw}{bw};\frac{st}{st}$	white	A	A

F_2	F_3	F_4	Phenotype	Alive (A) or Dead (D)	
				HOMOZYGOUS LETHAL	HOMOZYGOUS VIABLE
$\frac{+}{bw};\frac{+}{st}$ ♂ $\frac{Df(2R)\ bw}{In(2L2R)Cy\ bw'};\frac{+}{+}$ ♀	$\frac{+}{In(2L2R)\ Cy\ bw'};\frac{+}{+}$ $\frac{+}{In(2L2R)\ Cy\ bw'};\frac{+}{+}$	$\frac{+}{+};\frac{+}{+}$	wild-type	D	A
		$\frac{+}{In(2L2R)Cy\ bw'};\frac{+}{+}$	Curly wing	A	A
		$\frac{In(2L2R)Cy\ bw}{+};\frac{+}{+}$	Curly wing	A	A
		$\frac{In(2L2R)Cy\ bw}{In(2L2R)Cy\ bw'};\frac{+}{+}$	....	D	D

over suppressing, inversion, Curly-SM1. This is a "missing class system" in which homozygous viable translocated individuals are the only progeny produced. (see Figure 1b.)

FITNESS OF TRANSLOCATIONS: CAGE EXPERIMENTS, MEASUREMENT, AND SIMULATION

As I have mentioned previously, (Literature review page 7) many workers in the field of genetic insect control have assumed that most translocations will be fit enough to be useful. There is reason to suspect that this assumption may be false. The main objective of my research is to determine what proportion of laboratory isolated translocations are sufficiently fit to be useful in genetic insect control.

In order to achieve this objective, three things must be done. The first is to devise fitness measures and to incorporate them into a simulation model. Such a model can be used to predict the acceptability or unacceptability of any translocation. The second thing that must be done is to test the reliability of the model and of the fitness measures upon which it is based. Finally it is necessary to generate a sample of new unmarked translocations and then, using the fitness measures and the model, determine the proportion of translocations which might be useful in genetic insect control.

Previous Work:

Robinson and Curtis (1973) performed fitness tests on a single _Drosophila melanogaster_ homozygous viable translocated strain. They obtained this strain, smudge, from Dr. P. T. Ives of Amherst College, Amherst Massachusetts. Robinson and Curtis devised two measures of fitness, egg-production and egg-

hatchability. Utilizing these measures of fitness they constructed a simulation model of competition between the translocated strain and wild-type flies. Because of discrepancies between the predictions from their discrete-generation computer simulation model and results from caged populations, their measures of fitness must be regarded as inadequate. The apparent reason for the discrepencies is that their measures give a gross over-estimate of the true fitness of the strain under study.

Fitness: Measures And Models.

I chose to use more complete measures of fitness. These are (1) carrying capacity in a population cage and (2) maximum production of adult offspring by single females.

My basic simulation model (Appendix 3) has four main features. The first is that it iterates from one three-day, simulation period to the next. At the end of each iteration all adult individuals mate panmictically. These two unrealistic assumptions, 3-day mating period and panmixis, affect the model only in that they increase the speed of allele frequency changes occuring in the populations. It is unlikely that these assumptions will alter the direction of selection, and it is the direction rather than the speed with which I am concerned.

The second feature is the simulation of replacement activity over many three-day iterations. The third feature determines the carrying capacity which will produce equilibrium

gene frequencies at a given translocation's maximum production. Finally, by performing many binary searches the model can describe an equilibrium line on the fitness space above which the translocation will replace the wild-type, and below which it will be eliminated.(Fig. 2.)

Methodology:

Determination Of Fitness Measures:

To estimate the carrying capacity of a strain involves cage experiments lasting about two months. Hence, replication is time consuming and I limited myself to three replicates per strain. Individual replicates are shown in Figure 2 as single small dots. Smudge is unreplicated. Experiments to measure maximum production are, however, easy to perform and consequently were replicated 9 or more times. The mean values shown in figure 2 are thus based on 9 or more replicates.

Experiments Involving Marked Translocations

Using the four available marked translocations , replicated fitness measures were determined. Cage populations were initiated with various initial frequencies of Oregon-r and translocated flies. The experimental cages were incubated for one months at 25 c. At the end of this period the cage experiments were terminated and samples of adults taken to determine translocation frequencies.

FIGURE #2.

Mean progeny production from single females of five genotypes: SM-smudge,

H-hairy, GL-glassey, TVR5-brilliant, and ORR-oregon-r, versus their cage

carrying populations. Both axes are expressed as a proportion of wild-type,

ORR, performance. 3 replicates of the cage populations are separate dots

except in SM where 2 cages were terminated because of mold.

The line expresses the modelled prediction above which replacement of a

wild population will occur via negative heterosis.
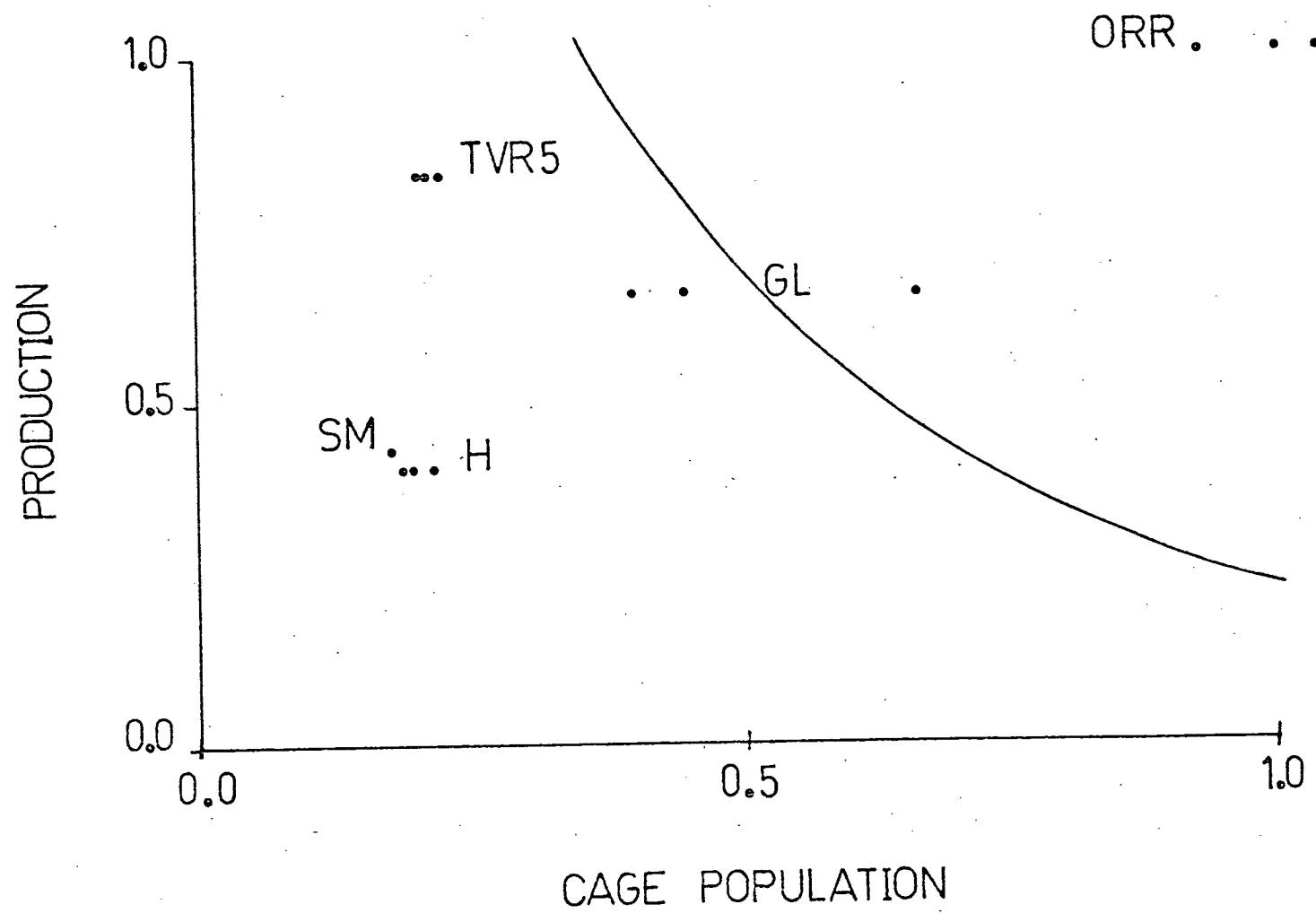
Experiments Involving Unmarked Translocations:

To create new translocations, I treated males with gamma radiation (1000 rd.) and collected progeny from the fifth and sixth days of exhaustive mating. I detected new translocations by the procedure outlined in Figure 1a. Those translocations which were homozygous viable, (as detected by using the system out-lined in Figure 2b.) were sorted on the basis of two preliminary measures of fitness. (See Figure 3.)

The first measure of fitness is the number of progeny produced when a wild-type female is mated to the translocation heterozygote. The number of progeny produced should be inversely related to the usefulness of the translocation.

The second measure of fitness is the ratio of translocation homozygote to wild-type progeny from a sib-cross of translocation heterozygotes (Fig. 3). The heterozygote contains a homozygous lethal multiple-break-inversion and thus the expected proportions of translocation homozygotes to heterozygotes is 1:2. So the expected position for a fully viable translocation is .5 on the "y" axis of Figure 3. I consider this to be an indirect measure of translocation homozygote viability.

I took a sample of five suitable homozygous viable translocations, as judged by two preliminary fitness criteria: (1) proportion of translocation homozygotes recovered from translocation heterozygote sib crosses, and (2) the number of progeny obtained from crosses of wild females with male

Figure #3.

Locations of the homozygous viable translocations on the plane formed by two preliminary

fitness measures. The two measures are: (X-axis) number of offspring produced by a

single wild karyotype female when mated to a T/+ male (no replicates), (Y-axis) ratio

of T/T to T/+ progeny from a T/+ by T/+ cross, where + carries a recessive lethal

(0 - 5 replicates).

PRODUCTION OF T/T FROM T/+ X T/+ MATINGS

0.5

0.0

0

T/+ X +/+ PRODUCTION

30

60

•14

•33

•26

9 •19

•37

12• •45

•18

•10

•41

•34

•32

•15

•38

•39
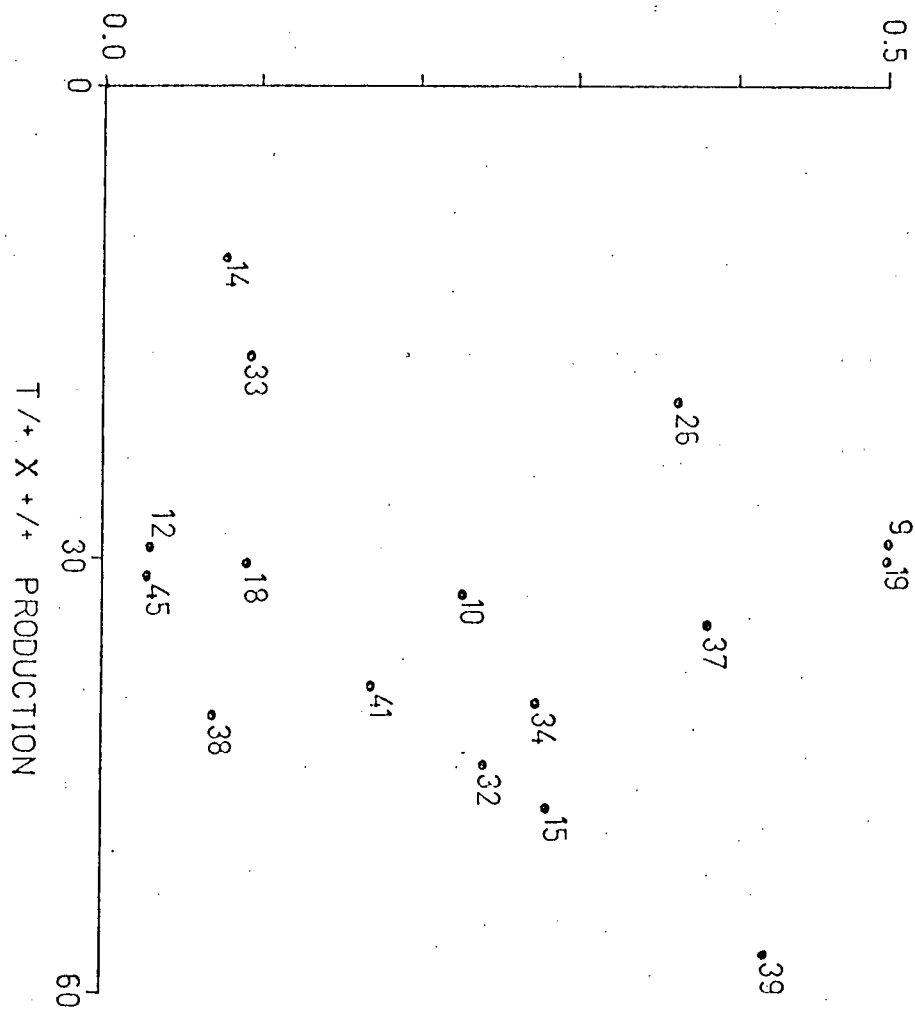
translocation heterozygotes. These homozygous viable translocations were chosen in such a way that they would cover a range of the two primary fitness measures. The five translocations, TVR's 10, 19, 26, 34, and 39 were tested using the more comprehensive fitness measures outlined in Fitness: Measures and Models. I compared these results with results from wild-type controls and checked for significant differences.

All five homozygous translocations chosen, were tested for their ability to compete with the wild-type Oregon-r stock from which they had been derived. To this end, cages were initiated with 18 pairs of translocation homozygotes and two pairs of normals. Cages initiated with the reverse ratio were also maintained. At the end of a one month incubation period, males from all experimental cages were sampled to determine the translocation frequencies.

The sampling procedure was identical to the procedure for the recovery of translocations (Fig. 1), except that five progeny from each male were collected and checked for karyotype. In this way the original male's genotype could be inferred (Table 1).

TABLE 1. Number of sampled progeny of various genotypes and resulting conclusions as to male parent's genotype.

Original Male's Karyotype	Karyotype-	
	2-3-T	Non-2-3-T
T/T	5	0
+/+	0	5
+/T	1 to 4	1 to 4

A preliminary estimate of the frequency of the two karyotypes in the original population can be obtained before the above procedure has been done. Because of the reduced number of progeny produced when one parent is a translocation heterozygote (Fig. 5), production of progeny from the sample-male X wild-karyotype-female cross together with cytogenetic subsamples (Fig. 4) of larvae from vials with various numbers of progeny, can give us such an estimate (Data of this type is presented in Table 4.). (The sampling procedure is outlined in Appendix 4.)

Figure # 4.

    An example polytene chromosome preparation. This shows clearly that both break-
points are in euchromatin, though one is near to the heterochromatin.
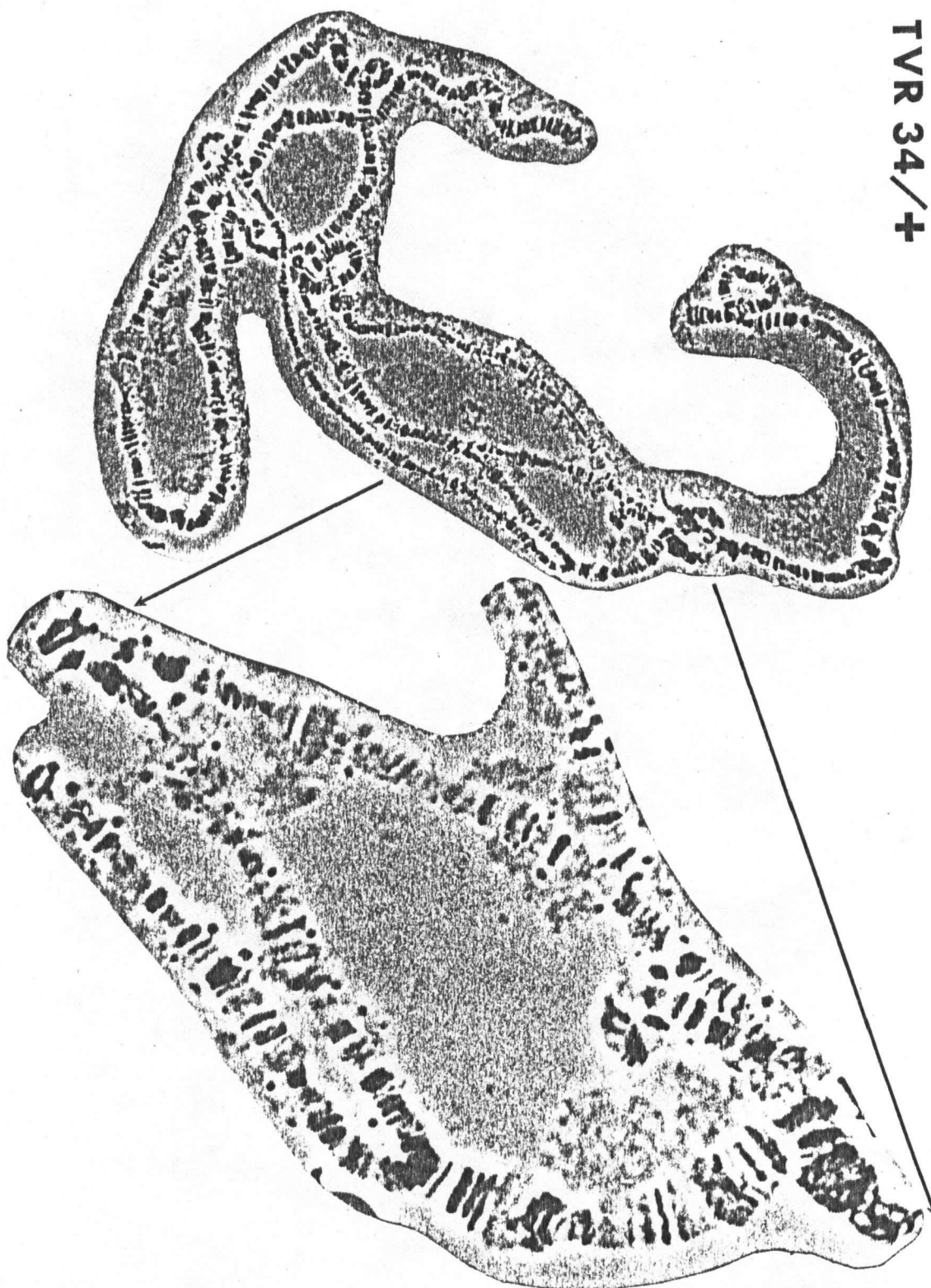
Results:

Experiments Involving Marked Translocations:

Figure 2 is a scatter diagram of mean progeny production versus carrying capacity for each of four marked translocation strains and Oregon-r. My simulation model predicts that translocations which fall below the curve in Figure 2, are incapable of replacing wild-type populations. Thus the prediction is that smudge, hairy, and TVR5(brilliant) will be unable to out-compete the wild-type. Glassy falls close to the border line but is also predicted to be unable to out-compete Oregon-r. The results of my competition cage experiments clearly validate the model, since at the end of the competition cage experiments in all cases not one translocation homozygote appeared in cage samples of between 50 and 100 flies. These results were found even in cages where the translocation was introduced at a frequency of 48 pairs to 2 wild-type pairs.

Experiments Involving Unmarked Translocations:

Figure 3 shows that there is no high correlation between the two preliminary measures of fitness. Two (TVR19 and TVR9) of the sixteen translocations for which I have data seem to be at the upper theoretical limit (.5) of the viability measure of fitness.

Using my two comprehensive measures of fitness, production

(Table 2) and carrying capacity (Table 3), there are two significant deviations between the fitness of the unmarked translocations and the Oregon-r wild-type stock. TVR10 shows a negative deviation in carrying capacity, while TVR19 shows a positive deviation in progeny production.

The results of the cage experiments with TVR26 indicated that only about one in twenty translocated chromosomes remained at the end of a one and one-half month experiment started with an initial population that was 90% translocated. Preliminary results of cage experiments involving the other four translocations are presented in table 4.

These preliminary results indicate that all translocations except for TVR19, are poor competitors with the Oregon-r stock from which they were derived.

TVR19, TVR34, TVR39, TVR10, and TVR26 have both of their breakpoints in euchromatin. An example of such a break is shown in Figure 4. The first three of these translocations are simple two-breakpoint translocations, and the last two are multiple breakpoint translocations.

Table 2. Number of progeny produced by single females of
Oregon-r and five translocated stocks.

Oregon-r	TVR10	TVR19	TVR32	TVR34	TVR39
42	62	52	37	37	20
32	43	48	16	38	39
37	54	51	35	56	46
48	55	48	38	38	41
45	44	36	37	63	36
38	45	36	30	38	47
35	41	54	29	46	44
38	37	52	26	37	57
28	27	54	17	38	46
21		62			36
45					
44					
53					
20					
MEAN 37.6	45.4	49.3*	29.5*	42.8	41.2

*Significantly different from Oregon-r.

Table 3. Number per population cage for Oregon-r and four
translocated stocks. Twenty pairs of flies were used to
initiate each population. The populations were censused
after incubation for one month at 25^0 C.

	Oregon-r	TVR10	TVR19	TVR34	TVR39
	1406	1086	900	993	1089
	1315	757	1860	1082	1257
	1046	707		1389	1012
	1203				
MEAN	1242.5	850	1380	1155	1119

*Significantly different from Oregon-r

Table 4. Number of individuals of each of three possible
karyotypes in samples from population cages initiated
with 18 pairs of the translocation and 2 pairs wild-type.
Populations were incubated for one month at 25^0C.

		Translocation			
		TVR10	TVR19	TVR34	TVR39
Karyotype	T/T	16	28	30	25
	T/+	4	3	6	8
	+/+	6	2	5	15
Number of wild allels		16	7	16	38
Total allels sampled		52	66	80	96
Frequency of wild allels		.31	.11	.2	.26

Discussion:

Since the four marked translocations and TVR10 were shown to be significantly less fit than Oregon-r and since these five translocations have been shown to be poor competitors, I conclude that my fitness measures are an improvement over the previously used measures; egg-production and hatchability (Robinson and Curtis 1974). (TVR26 was not subjected to these two fitness measures because scarlet markers were inadvertantly incorporated into it during the initial isolation phase, likely by male somatic crossing-over).

However TVR34 and TVR39 also appear to be at a competitive disadvantage in competition with Oregon-r even though my fitness tests indicated that neither one is of significantly lower fitness than oregon-r. In these two cases the preliminary fitness measures appear to be more reliable.

It is interesting that, of the 57 translocations isolated, only 21 were viable in the homozygous state. Of these, only one was shown to be a good competitor with the Oregon-r type. It may be that TVR9 would have done as well as TVR19 had TVR9 been as intensively tested. Thus the results of my cage experiments suggest that only about two in twenty homozygous viable translocations are worth testing in a field situation.

It is not surprising that the fitness measures used by Robinson and Curtis (1974) gave over-estimates of the true fitness of strains. One would expect egg production and hatchability to overestimate fitness since these measures are

affected by only a small portion of the insect's life-cycle.

It should be mentioned at this juncture that, in cage experiments lasting one month one cannot expect negative heterosis to have a strong effect, since only two full generations of incubation elapse before termination of the experiments and heterozygotes are not produced until the end of the second generation. Thus heterozygotes, upon which negative heterosis acts, are not produced until very near the end of each experiment.

TRANSLOCATION ISOLATION PROCEDURES:

The use of translocation procedures has a tremendous advantage over other genetic insect control methods in that the needed genetic rearrangements can be isolated without the use of visible markers, cross-over-suppressors or extensive cytogenetics (Wijnands-Stab and Van Heemert (1974) and Curtis (1971)).

The technique used is to irradiate males and mate them to untreated virgin females. The progeny are then crossed to untreated flies and crosses in which there is low progeny production are suspected of carrying translocations. The suspected lines are then examined cytologically, (Wijnands-Stab and Van Heemert (1974) or test crossed, (Curtis 1971), to determine if a translocation is indeed present.

Materials And Methods

I treated Oregon-r Drosophila melanogaster males with 1000 rd. of gamma radiation and then mated them to karyotypically wild-type females homozygous for the recessive markers brown(chromosome 2) and scarlet(chromosome 3). Then I back-crossed the progeny to the maternal strain. Any 2-3 translocated stocks can be identified by an inseparable linkage of the brown and scarlet markers. By this procedure all 2-3 translocations produced are picked up in the heterozygous state. This should account for about four-fifths of all translocations produced.

Progeny production of the translocations was estimated at the time they were picked up and thus we can test the usefulness of a progeny reduction scheme of translocation identification.

Results:

There is a marked reduction in progeny numbers when translocation heterozygote male parents are mated to wild karyotype females (Fig. 5). The mean production per mating for non-translocated males mated to wild females was 55 while the mean production of adults for 2-3 translocated males was 35.

Two questions remain. What proportion of the translocations produced could be detected by noting a reduction in progeny numbers? Secondly how can we maximize the number or quality of translocations produced per unit effort? Figure 5 demonstrates that there is a considerable difference in the number of progeny produced, between translocation heterozygote males and normal males. Unfortunately translocations made up only a maximum of 4 percent of the genomes treated. (It is wise not to attempt to increase the proportion of translocations by increasing the gamma ray dose because large numbers of recessive lethals are produced.) Thus if Figure 5 is modified to account for this and the translocated progeny are added to the normalized version of the histogram, those crosses indicating the presence of a translocation become much harder to separate, Fig. 6.

Figure 7, derived from Figure 6, gives the relationship

FIGURE #5.

Histograms of frequency versus the progeny produced per karyotypically wild female mated to males either, non-2-3-translocated (control, top graph), or 2-3-translocation heterozygotes (experimental, bottom graph).

Data represents 47 translocations and their controls.

NUMBER OBSERVED

0

7

14

PERCENT ANESTHESIA

FEMALE SEE FEMALE

0   55   100

SOLID HETEROZYGOTE
2-3-TRANSLOCATION
+ FEMALES MATED TO HETEROZYGOTE MALES

NUMBER OBSERVED

0

7

14

0   55   100

SOLID WILD MALES
+ FEMALES MATED TO WILD MALES
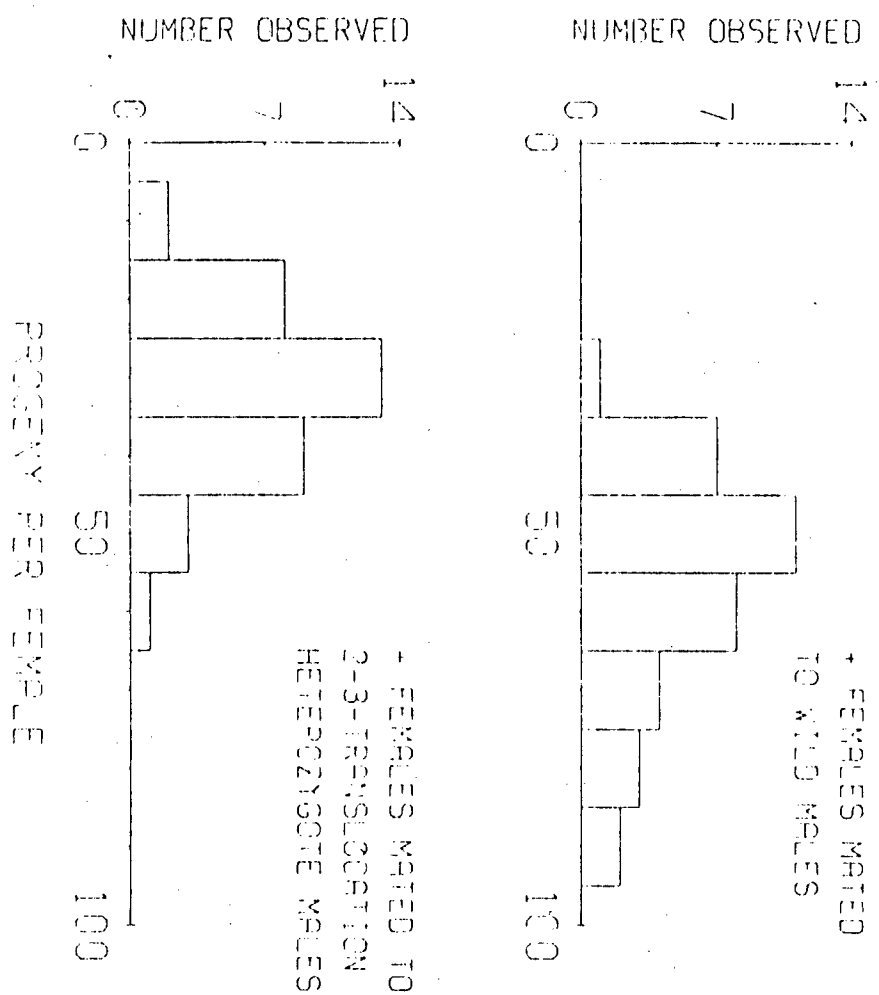
FIGURE #6.

Histogram of frequency versus number of progeny produced per karyotypically
wild female mated to non-2-3-translocated males (data expanded from figure 5,
to account for sample size), and mated to 2-3-translocation heterozygote
males (shaded area).

NUMBER OBSERVED

PROGENY PER FEMALE

MALES 2-3 TRANSLOCATED
MALES 2-3 WILD TYPE

FIGURE #7.

Graph of proportion of translocations that will be identified versus the proportion of treated genomes that the investigator examines.

PROPORTION OF TRANSLOCATIONS IDENTIFIED

PROPORTION OF TREATED GENOMES EXAMINED

between the cumulative proportion of all translocations which would be detected and the cummulative proportion of the treated genomes examined. From this relationship it is apparent that most translocations can be isolated from those matings yielding fewest progeny.

Discussion

The best strategy for isolating translocations when suitable markers are not available is dependent on two features of the pest in questio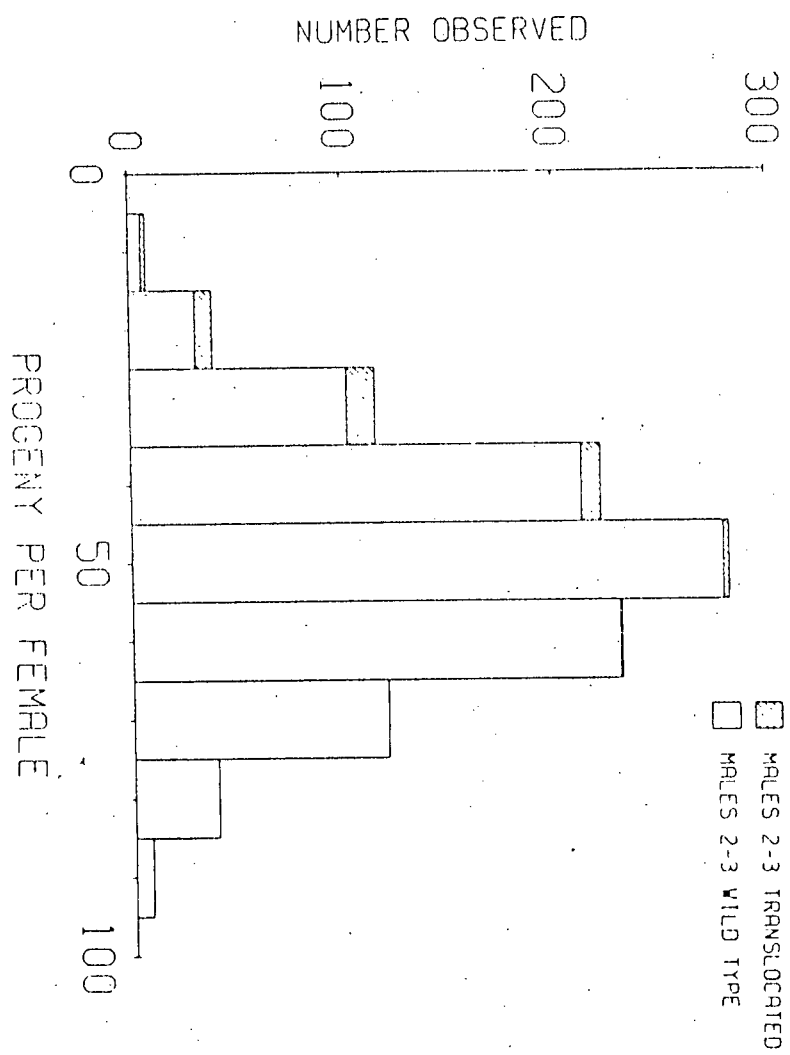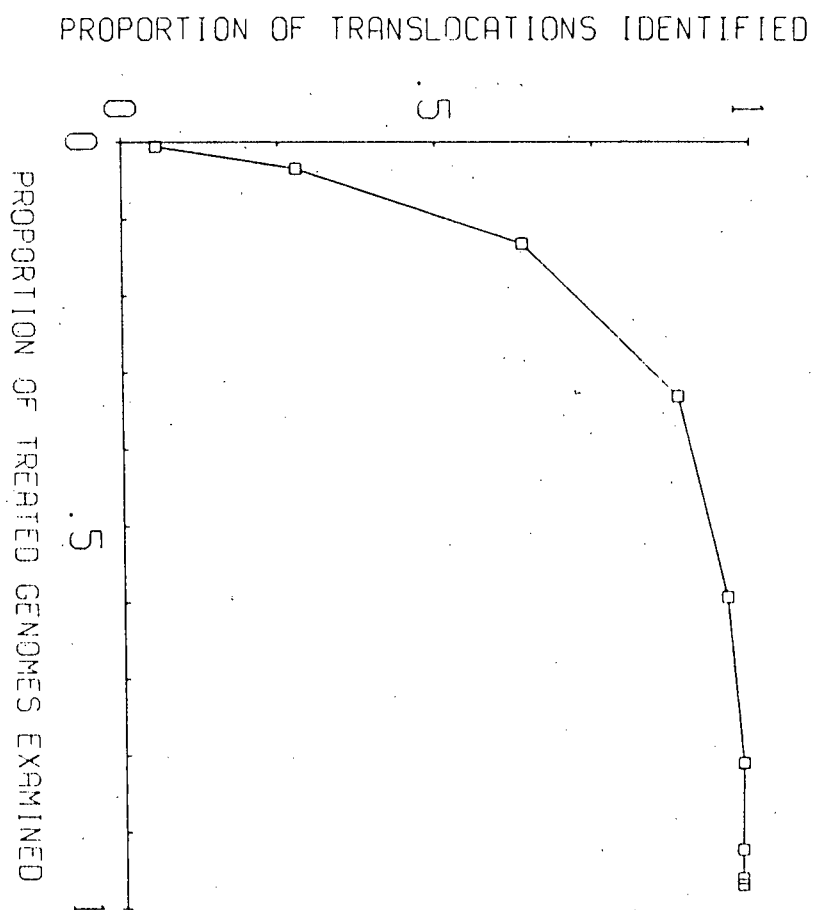n. These are the effort required to raise single pair off-spring and the effort to examine the off-spring, either cytologically or with single pair mating experiments. If much effort is required to make single pair matings then the best strategy is to cytologically examine a large proportion of those matings yielding fewest progeny. Alternatively, if the single pair mating is easy relative to the effort of karyotyping the progeny, then only genomes from the most greatly depressed progenies should be examined. In these respects I agree with the approach taken by Wijnands-Stab and Van Heemert (1974) and Curtis (1971).

Since there appears to be no correlation between my two preliminary measures of fitness, it is quite possible that some very fit translocations may come from the extreme left tail of the distribution Figure 6. Wijnands-Stab and Van Heemert (1974) and Curtis (1971) chose to ignore the extreme deviants and probably missed promising translocations.

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APPENDIX 1: A CAGE FOR DROSOPHILA POPULATION STUDIES.

Bennett (1956) introduced an inexpensive Drosophila population cage which consisted of a small square plastic freezer container with a removable lid. This was modified by inserting four holes on each of two opposite sides along with two air holes. This cage, though a great advance over previous wooden models, has two drawbacks. Firstly, removal of adult flies is an inefficient and laborious task; and secondly, in my experience, the cage walls invariably develop cracks around the food vial holes, allowing flies to escape.

I have developed a new cage (Fig. 8) which solves both of these problems, and is cheaper than the plexiglass cages used by Fitz-Earl et al. (1974). Each cage consists of a 2 qt. Dairyland plastic milk bottle. Eight holes are placed in two rows on one side and at least two air holes covered in fine mesh are bored in the adjacent sides.

As with Bennett cages, one food vial is removed once every two to four days. My cages support similar adult populations to those observed by Frydenberg (1962), eg. 1000 to 2000 adults. The adults can be as easily removed by banging the open cage into an etherizer. Twenty-five of these cages have been in use for the past two years, but only one has cracked around the vial openings.

Figure #8.

A small, economical <u>Drosophila</u> population cage. It consists of a 2qt. plastic milk-jug with eight holes, suitably sized for food vials, bored in one side. At least two air holes are bored in the adjacent sides and covered with fine fly-proof mesh.

DROSOPHILA POPULATION CAGE
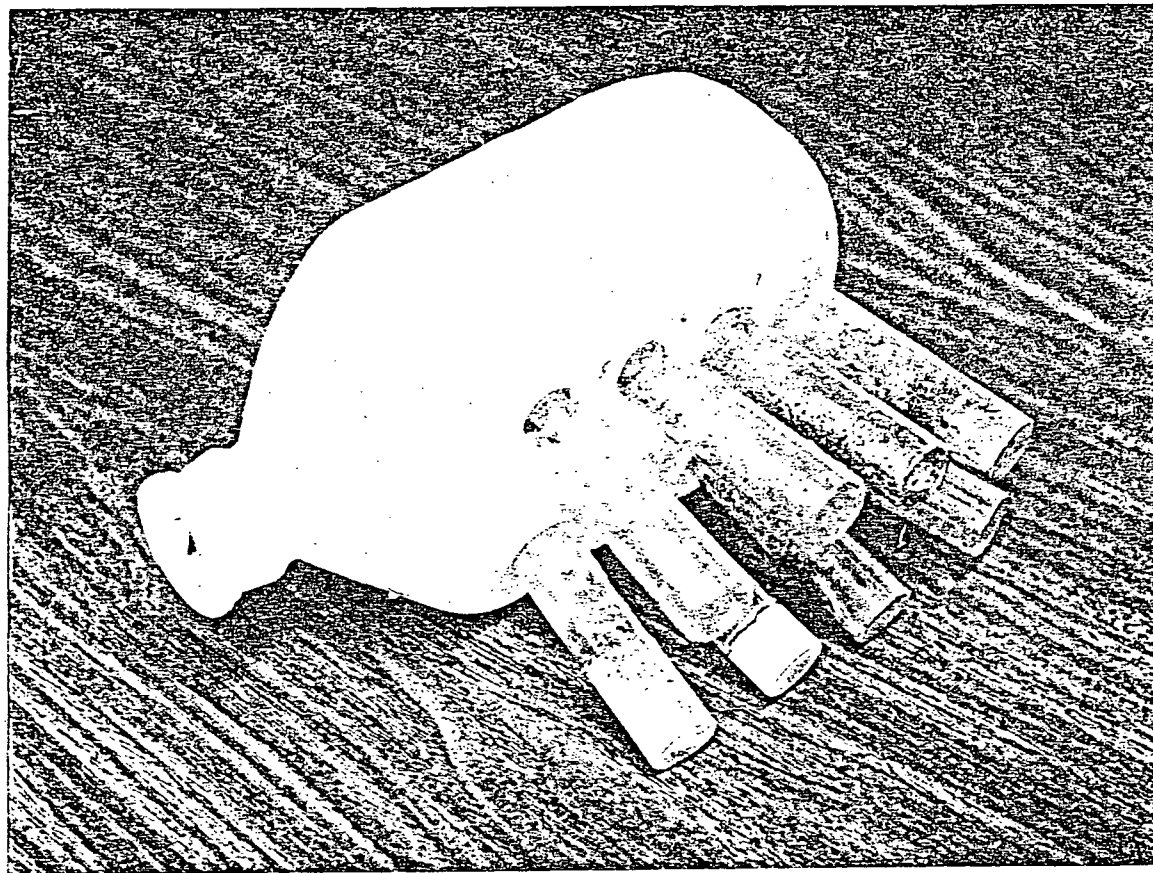
APPENDIX 2: A FAST, ACCURATE, ELECTRONIC FLY COUNTER:

This counter was developed to solve the problem of counting large numbers of live etherised Drosophila where counting by hand requires such a long time period that heavy mortality almost always results. The apparatus was constructed and perfected (with the great assistance of A. Reid) over the course of the two year study at a cost of approximately two hundred dollars.

It consists of three basic parts (Fig. 9): (1) a photo-transistor and light beam through which the flies are passed via a vacuum apparatus, (2) a pre-amplifier which increases the difference in resistance caused by fluctuations in beam intensity detected by the photo transistor, and (3) an electronic beam interruption counter.

The accuracy achieved by the counter is dependent on the uniformity in size of the insects being counted, as well as the likelihood of their sticking together. A test of accuracy is reported in Table 5. As can be seen from these results, the counter never over-estimates the number of insects passed through the light beam but often will slightly under-estimate the correct count. The extent of this effect varies with the size variability and stickyness of the insects being counted. The effect can largely be removed by doing a small sample hand count and machine count to establish a conversion factor. (See Table 2.)

TABLE 5. COMPARISON OF EXACT HAND COUNTS OF FLIES AND COUNTS INDICATED BY MY ELECTRONIC BEAM-INTERUPTION COUNTER.

EXACT HAND COUNT	ELECTRONIC COUNT	D**2	CORRECTED ELECTRONIC COUNT	D2**2
37	35	4.00	36.16	0.70
16	16	0.00	16.53	0.28
35	35	0.00	36.16	1.35
38	36	4.00	37.19	0.65
37	37	0.00	38.23	1.51
30	30	0.00	31.00	0.99
29	27	4.00	27.90	1.22
26	25	1.00	25.83	0.03
17	17	0.00	17.56	0.32
62	61	1.00	63.02	1.05
43	40	9.00	41.33	2.80
54	51	9.00	52.69	1.71
55	51	16.00	52.69	5.33
44	44	0.00	45.46	2.13
45	42	9.00	43.39	2.58
41	39	4.00	40.29	0.50
27	26	1.00	26.86	0.02
34	34	0.00	35.13	1.27
38	36	4.00	37.19	0.65
56	54	4.00	55.79	0.04
38	38	0.00	39.26	1.59
63	62	1.00	64.06	1.12
38	38	0.00	39.26	1.59
TOTALS 903	874	71.00	903.00	29.42

58.6% of squared deviations accounted for by corrected count.

EXACT HAND COUNT	ELECTRONIC COUNT	D**2	CORRECTED ELECTRONIC COUNT	D2**2
53	51	4.00	52.69	0.09
73	71	4.00	73.36	0.13
67	67	0.00	69.22	4.94
55	52	9.00	53.73	1.62
79	78	1.00	80.59	2.52
97	91	36.00	94.02	8.88
99	95	16.00	98.15	0.72
86	81	25.00	83.69	5.35
61	60	1.00	61.99	0.98
94	90	16.00	92.99	1.03
101	100	1.00	103.32	5.37
38	38	0.00	39.26	1.59
TOTALS 903	874	113.00	903.00	33.23

70% of squared deviations accounted for by corrected count.

Figure #9.

A block diagram of a light beam-interruption insect counter. Insects move through

the beam from the light source in an air stream, causing varriation in the light intensity

reaching the photo sensor. This variarion produces a varriation in the resistance of

the photo sensor which is amplified and finally counted as a beam interruption.
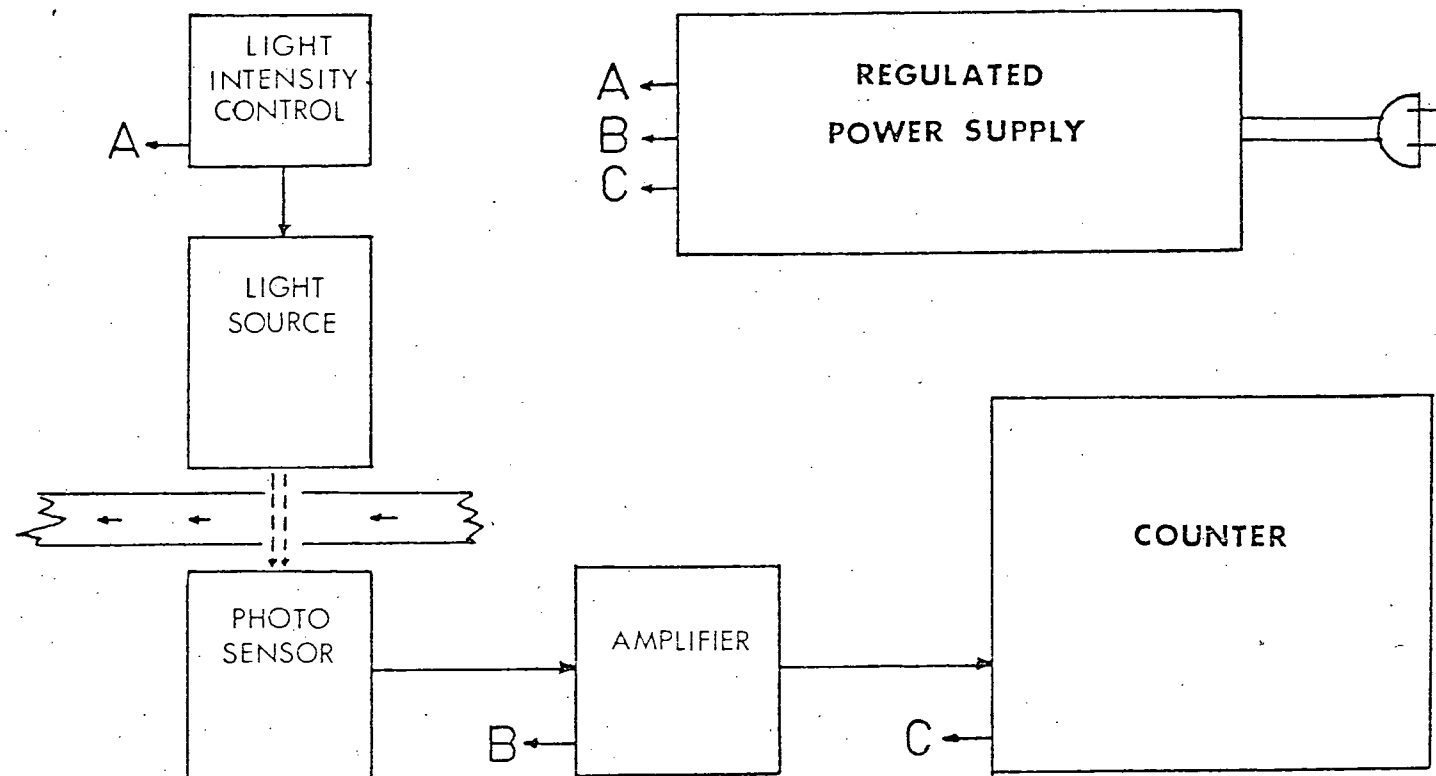
BEAM INTERRUPTION INSECT COUNTER

Figure #10.

· Photo of light beam–interruption insect counter. Insects move through the system from right to left interrupting the light beam between the light source and the photo sensor. the insects then pass into the collecting bottle at left.

BEAM INTERRUPTION INSECT COUNTER

COUNTER

LIGHT INTENSITY

PHOTO SENSOR

VACUUM

LIGHT SOURCE

53
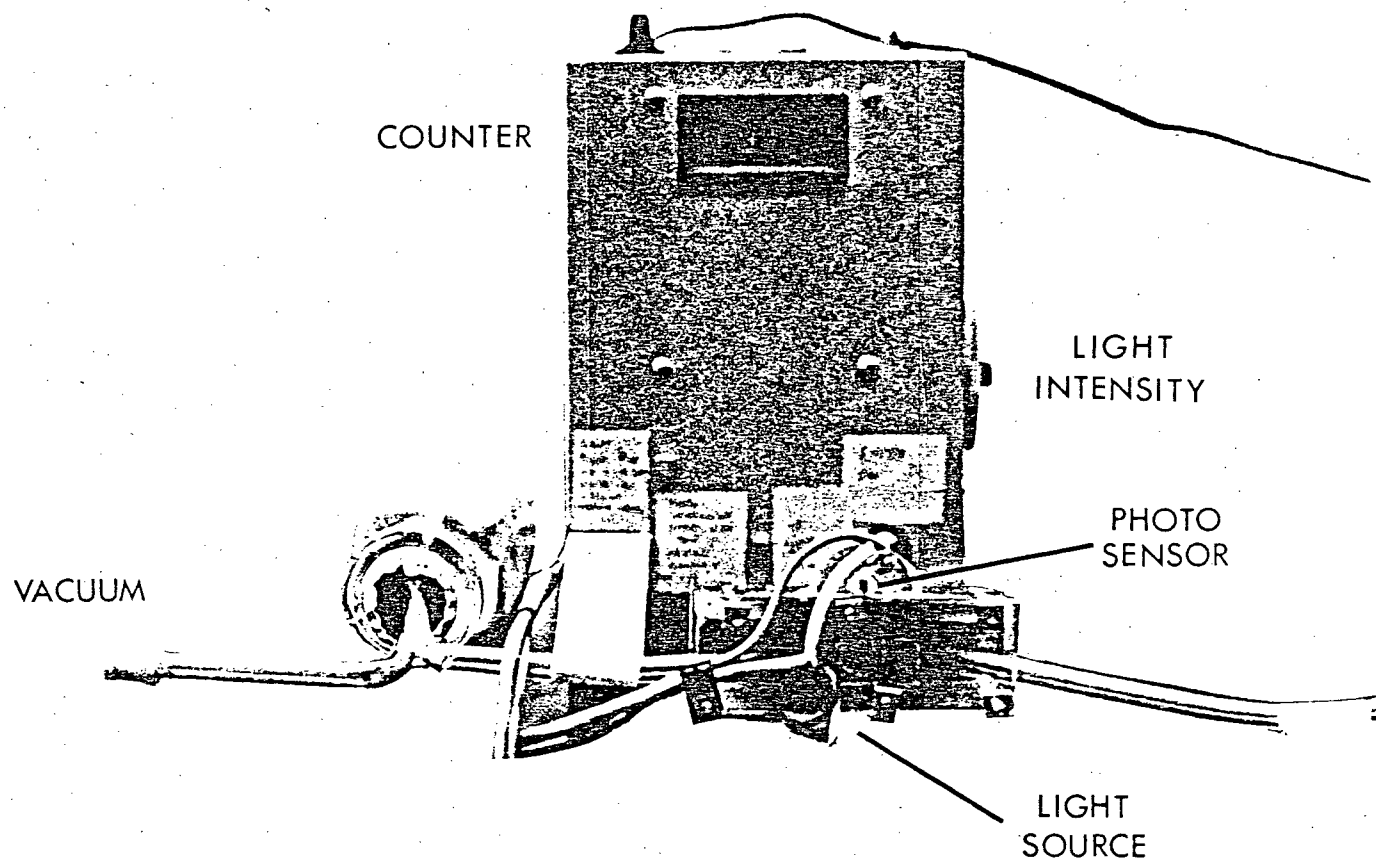
APPENDIX 3. SIMULATION OF SEMI-STERILE GENETIC SYSTEMS.

Basic Algorithm

Realistic simulation models of semi-incompatible genetic systems, such as wild populations under a translocation release program, quickly become completely intractable. Therefore I have developed a new modelling system specifically designed to handle such problems.

The basis of this system is a Markovian time-iteration procedure which utilizes an INCOMPATIBILITY MATRIX, a type of transition matrix formed by the serial addition of Incompatibility Vectors. Also required is simple, fortran defined function to convert the results into new animals or nonsense (incomplete) animals.

As an example, the Incompatibility Matrix for the simple case of one wild-type genome and one homozygous viable translocation is constructed as follows:

Incompatibility Vectors for one homozygous viable translocation.

```
+/+ [ 0, 0, 0, 0]
T/+ [ 0,-3, 4, 1]
T/T [ 1, 1, 1, 1]
```

The identity numbers of the +/+, T/+, and T/T genotypes are

0, 1, and 2 respectively. The fortran function is simply defined and has the following action.

FUNCTION= F(IR)= F(1 to 2= same, -infinity to -1 = 3, and 3 to infinity = 3)

Thus a T/+ by T/+ mating gives the following INCOMPATIBILITY MATRIX.

```
 0,-3, 4, 1                          0, 3, 3, 1
-3,-6, 1,-2                          3, 3, 1, 3
 4, 1, 8, 5     WHICH THE FUNCTION   3, 1, 3, 3
 1,-2, 5, 2       CONVERTS INTO      1, 3, 3, 2
```

All 3's appearing in the matrix indicate nonsense or incomplete animals. We can easily calculate the proportions of the three possible genotypes produced by a translocation heterozygote by translocation heterozygote mating. These are T/T= 1/16, T/+= 4/16, +/+= 1/16, and lethal= 10/16.

This modelling system was used extensively to assist in choosing those translocations most likely to compete favorably with the wild-type genome.

NAME: CPU TRANS.SIM

AUTHOR: Keith Reid

Date: JAN 1, 1974

CPU TRANS.SIM - Iterative Homozygous Translocation Fitness
Phase-space Construction Procedure.

I.   TYPE OF ROUTINE:

       Main-line program written in Fortran

II.   PURPOSE:

       To construct a phase-space of two fitness measures,
       carrying capacity and maximum progeny production
       (which can be obtained from various genetic insect
       strains9. The phase space gives translocation fitness
       levels which will produce unstable equilibria between
       a translocated strain and the wild-type.

III.   RESTRICTIONS:

       Must be concatinated with link:plot or the PDP 11
       system routines, KGA, KLINE, and KLABL (see listings
       included). Also should be run on syn-6 (see "outputs
       section").

IV.   VIRTUAL MEMORY:

       Fits on the current PDP 11

V.   METHOD:

       Iterative search over the phase-space to locate the
       areas of unstable equilibria. At each Y-fitness level
       a binary search is used to locate the relevant X-axis
       level for equilibrium.

VI.   HOW TO USE:

       $RUN *FTN PAR=S=SIM.S L=-L

       $RUN -L+LINK:PLOT 5=DATA 6=OUTPUT

"5=data"
,   the data is to be stored in DATA

"6=output"
this is where the printed (as opposed to plotted) out-put goes.

1) Inputs:

Birth rates (min and max --treated density-dependently), death rates starting populations , carrying capacities, and maximum births per iteration.

2) Outputs:

All output, plotted goes to the syn-6 screen, but if paper plots are required, then use "HCOPY" o or concatinate the run command with webb:lib instead of link:plot and run plot:q par=-plot# after the first run is complete .

VII.   ACKNOWLEDGEMENT:

No thanks would be too much for my good friends and buddies who like to dragg me off to the "pit". Also I would like to thank Bill Webb and R. Yourk for their help.

```
$RUN  *FTN PAR=S=MOD(4)    L=MOD.O
$RUN  WEBB:TRAP+MOD.O+PLOT.O+LINK:PLOT 6=-SP 5=MOD(200)
$END
      DIMENSION T(3,4)
      DIMENSION T1(3,4)
      DIMENSION XY(2,1),XYB(2,15),XYT(2,15)
C THE ABOVE IS FOR PLOT PURPOSES ONLY
      COMMON D(3),B(3)
C THE ABOVE ARE DEATH AND BIRTH RATES FOR TT TP AND PP
      COMMON DM(3)
      COMMON POP
CC THE ABOVE ARE THE STATE MATRIX (TENETIC STATE, AGE), AND PT
      COMMON TK(3),TMAX
C ABOVE ARE GENETIC SPECIFIC CARRYING CAPACITIES,AND MAX GROW
      COMMON FINT
C ABOVE IS NUMBER OF FLIES INTRODUCED PER STEM
C THE ABOVE IS THE NUMBER OF T HOMOS INTRODUCED PER TIME PERIOD
      COMMON Y,Y1,YSY1
      NYSK=1
   12 READ(5,100) B,D,DM,((T(I,J),I=1,3),J=1,4),TK,TMAX,FINT
      WRITE(6,100)B,D,DM,((T(I,J),I=1,3),J=1,4),TK,TMAX,FINT
  100 FORMAT(3F10.2)
      IF(TMAX.EQ.0) GO TO 999
      YM=B(1)
      XM=TK(1)
      CALL KGA(XM,YM)
      XY(1,1)=0.
      XY(2,1)=YM*1.05
      CALL KLINE(XY,1,-1,1)
      WRITE(7,104)D,TMAX,FINT
      CALL PFLAG
  104 FORMAT(' DEATH RTS. DTT=',F5.3,'  DTP=',F5.3,' DPP='
     2 ,F5,3,
     1 '   MAX YOUNG/ITT.=',F6.0,'  TT INTRODUCTION=',F6.0)
      CALL  KLABL(XM,YM,2)
      WRITE(7,107)
      CALL PFLAG
  107 FORMAT('            BIRTH RATE OF TT')
      CALL KLABL(XM,YM,1)
      WRITE(7,108)
      CALL PFLAG
  108 FORMAT('           CARRYING CAP.OF TT')
      CALL FCHAR(0.,YM,.10,.15,0.0)
C OUTERMOST LOOP OVER THE INITAL STARTING VALUES
      DO 8 IR=1,4
C ************** SET/RESET B(1)
      B(1)=B(3)
C *** ****** ZERO OUT PLOT FILES
      DO 11 J=1,15
      DO 11 I=1,2
      XYB(I,J)=0.
```

```
     11 XYT(I,J)=0.
C BINARY   SEARCH OF REAGON BETWEEN TK(3) AND
C AND BETWEEN B(3) AND 10.
      K=0
    7 CONTINUE
      K=K+1
      WRITE(6,100)B,TK
C ************* SET RESET TK(1)=TK(3)
      TT=TK(3)
       TB=0.
      TK(1)=TT
C SET UP FLIP-FLOP
      FF=1
      DO 5  I=1,10
C WEE WILL MAKE THE ABOVE NO OF CUTS ON THE BINARY SEARCH
      DO 1 II=1,3
      DO 14 JJ=1,3
   14 T1(II,JJ)=0.
    1 T1(II,4)=T(II,IR)
C THE SIMULATE DO
      DO 2 III=1,200
      TEMP=T1(3,4)
      CALL KOD(T1)
      IF(III.LT.40) GO TO 9
      IF(T1(3,4).GE.10.AND.T1(3,4).GT.TEMP) GO TO 4
      IF(T1(3,4).LT.1.AND.T1(3,4).LE.TEMP) GO TO 3
    9 CONTINUE
    2 CONTINUE
      WRITE(6,102)((T1(II,J),II=1,3),J=1,4),TEMP,TT,TB,TX
  102 FORMAT(' FELL OUT OF THE BOTTOM OF THE SIM LOOP T IS='
     2,/12F6.0
     1 ,'TEMP,TT,TB,TX',6F10.5)
      TX=2.
      WRITE(6,103)(T1(IIII,4),IIII=1,3),TEMP,B(1),TK(1),TT,TB,TX
  103 FORMAT(11F12.3)
      XY(2,1)=B(1)
      XY(1,1)=TK(1)
      CALL KLINE(XY,IR,1,1)
      IF(TT-TB.LT.10) GO TO 6
C ********* IF THIS HAS BEEN DONE HERE BEFORE --BAILOUT
      IF(FF+TX.EQ.3) GO TO 6
C ********SO WE GO TO THE UP FF=1 OR DOWN FF=0.
      TK(1)=(TK(1)-(FF*TT-(FF-1)*TB))/1.2+FF*TT-(FF-1)*TB
      WRITE(6,100)FF,TK(1),TB, TT
C ********* FLIPPER IS SET-RESET
      FF=(FF-1)*(-1)
      GO TO 5
    3 TX=0.
C TRANSLOCATION DID MAKE IT
C      WRITE(6,103)(T1(IIII,4),IIII=1,3),TEMP,B(1),TK(1),TT,TB,TX
```

```
      XYT(2,K)=B(1)
      XYT(1,K)=TK(1)
      TT=TK(1)
      IF(TT-TB.LT.10) GO TO 6
      TK(1)=(TK(1)-TB)/2.+TB
      GO TO 5
    4 TX=1
C THE TRANSLOCATION DIDNT MAKE IT
C     WRITE(6,103)(T1(IIII,4),IIII=1,3),TEMP,B(1),TK(1),TT,TB,TX
      XYB(1,K)=TK(1)
      XYB(2,K)=B(1)
      TB=TK(1)
      IF(TT-TB.LT.10) GO TO 6
      TK(1)=(TK(1)-TT)/2.+TT
    5 CONTINUE
    6 B(1)=B(1)/1.3
      IF(B(1).GT.9.) GO TO 7
      KK=K
      DO 10 K=2,KK
      IF(XYB(1,K)+XYB(2,K).NE.0) GO TO 13
      XYB(1,K)=XYB(1,K-1)
      XYB(2,K)=XYB(2,K-1)
   13 CONTINUE
      IF(XYT(1,K)+XYT(2,K).NE.0) GO TO 10
      XYT(1,K)=XYT(1,K-1)
      XYT(2,K)=XYT(2,K-1)
   10  CONTINUE
      CALL KLINE(XYB,KK,-1,0)
      CALL KLINE(XYT,KK,-1,0)
      WRITE(7,106)T(1,IR),T(3,IR)
      CALL PFLAG
  106 FORMAT(' TT/PP=',F4.0,'/',F4.0,')')
    8 CONTINUE
      XY(1,1)=XM*1.2
      XY(2,1)=0.
      CALL KLINE(XY,1,-1,1)
      GO TO 12
  999 CONTINUE
      WRITE(6,105)
  105 FORMAT('   JOB DONE ******************'////)
      CALL EXIT
      RETURN
      END
      SUBROUTINE KOD(T)
      DIMENSION T(3,4)
      COMMON D(3),B(3)
C THE ABOVE ARE DEATH AND BIRTH RATES FOR TT TP AND PP
      COMMON DM(3)
      COMMON POP
CC THE ABOVE ARE THE STATE MATRIX (TENETIC STATE, AGE), AND POP
```

```
      COMMON TK(3),TMAX
C ABOVE ARE GENETIC SPECIFIC CARRYING CAPACITIES,AND MAX GROWTH
      COMMON FINT
C ABOVE IS NUMBER OF FLIES INTRODUCED PER STEM
C THE ABOVE IS THE NUMBER OF T HOMOS INTRODUCED PER TIME PERIOD
      COMMON Y,Y1,YSY1
C DEMINISH ADULTS BY DRT.
      POP=T(2,4)+T(3,4)+T(1,4)
      DO 4 I=1,3
    4 T(I,4)=T(I,4)-(D(I)-DM(I)*(1.-POP/TK(I))  )*T(I,4)
C MOVE UP LARVAE
      DO 1 I=1,3
      J=5-I
      II=J-1
      T(2,J)=T(2,J)+T(2,II)
      T(3,J)=T(3,J)+T(3,II)
      T(1,J)=T(1,J)+T(1,II)
      T(2,II)=0.
      T(3,II)=0.
    1 T(1,II)=0.
C HERE WE INTRODUCE THE INTRODUCTION FUDGE FACTOR
      T(1,4)=T(1,4)+FINT
C ADD ON NEW BABIES
      T(2,1)=B(2)*T(2,4)*(.25*(T(2,4)/POP)+.125*(T(3,4)/POP)+
    2 .125*(T(1,4)/POP))+
    1 B(1)*T(1,4)*(.5*(T(3,4)/POP))+B(3)*T(3,4)*(.5*T(1,4)/POP)
      T(1,1)=B(1)*T(1,4)*((T(1,4)/POP)+.123*(T(2,4)/POP))
    1 +B(2)*T(2,4)*(.0625*T(2,4)/POP+.125*T(1,4)/POP)
      T(3,1)=B(3)*T(3,4)*((T(3,4)/POP)+.125*(T(2,4)/POP))
    1 +B(2)*T(2,4)*((T(2,4)/POP)*.0625+(T(3,4)/POP)*.125)
C WE CALCULATE THE EFFECTOF POPULATION LIMITATION ON BIRTHS
      Y1=T(2,1)+T(1,1)+T(3,1)
      DO 3 I=1,3
      Y=TMAX*(1-POP/TK(I))
      IF(Y.GT.Y1) GO TO 3
      IF(Y.LE.0.)  Y=.0001
CANT LET THE NUMBER OF LARVAE GO NEGATIVE
      YSY1=Y/Y1
      T(I,1)=T(I,1)*YSY1
    3 CONTINUE
      RETURN
      END
$ENDFILE
```

80.	80.	80.	BIRTH RTS
.4	.4	.4	DEATH RTS
.3	.3	.3	ABOV - THIS=MIN DEATH RT
98.	0.	2.	ADULT STARTING POPS OF TT TP PP
94.	0.	6	ADULT STARTING POPS OF TT TP PP
86.	0.	1	ADULT STARTING POPS OF TT TP PP
70.	0.		ADULT STARTING POPS OF TT TP PP

```
2000.      2000.      20          CARRYING CAPACITIES OF TT TP PP
300.         0.                              TMAX FINTRO
          SUBROUTINE KGA(MAXX,MAXY)
C** * * * * * * * * * * * * * * * * * * * * * * * * * * * **
C**   THE FOLLOWING IS A ROUTINE TO DRAW THE GRAPH AXIES TO A**
C** POINTS  BETWEEN 0.0 AND THE STATED MAXIMA   , MAXX AND M**
C** AXIES ARE LABELED WITH ROUNDED OFF VALUES IN F5.2 WHERE **
C** ELSE WHERE  'E' FORMAT IS USED                         **
C** * * * * * * * * * * * * * * * * * * * * * * * * * * * **
          REAL MAXX,MAXY
          XIN=10.
          YIN=7.
          XIN=8.
          YIN=6.
101       FORMAT(2F10.3)
          CALL P1130
          X=XIN/MAXX
          Y=YIN/MAXY
          YP=-MAXY/7.0
          XP=-MAXX/5.
          CALL SCALF(X,Y,XP,YP)
C**   DRAW X AXIS
          U=MAXX/5.0
          CALL FGRID(0,0.0,0.0,U,5)
C**   PUT NUMBERS ON X AXIS
          YN=-MAXY/23.
          XN=MAXX
          DO 2 I=1,6
          IF(I-6) 3,4,4
4         XN=0.0
3         CALL FCHAR(XN,YN,0.10,0.15,0.0)
          IF(MAXX-99.99)10,10,11
10        WRITE(7,102) XN
          CALL PFLAG
102       FORMAT(F5.2)
          GO TO 12
11        WRITE(7,100)XN
          CALL PFLAG
12        CONTINUE
C**   PUT NUMBERS ON Y AXIS
2         XN=XN-MAXX/5.
          XN=-MAXX/9.
          YN=0.000
          DO 1 I=1,6
          IF(I-1) 5,6,5
6         YN=0.0
5         CALL FCHAR(XN,YN,.10,.15,0.0)
          YN=YN*.00005+YN
          IF(MAXY-99.99)7,7,8
7         WRITE(7,102)YN
          CALL PFLAG
```

```
        GO TO 9
8       WRITE(7,100) YN
        CALL PFLAG
100     FORMAT(E8.2)
9       CONTINUE
        YN=YN+MAXY/5.
1       CONTINUE
C**  DRAW Y AXIS
        U=MAXY/5
        CALL FGRID(3,0.0,MAXY,U,5)
        RETURN
        END
        SUBROUTINE KLINE(XY,NP,J,K)
C** * * * * * * * * * * * * * * * * * * * * * * * * * * **
C**     K        CONTROLES WHETHER A LINE WILL BE PLOTTED BETWEE**
C**              ON ENTRANCE 0=LINE 1=NOLINE                   **
C**     XY       2 BY NP ARRAY OF DATA TO BE PLOTTED            *
C**     J        INDICATES WHAT TYPE OF MARKER TO BE  ON THE LIN *
C** * * * * * * * * * * * * * * * * * * * * * * * * * * **
        DIMENSION XY(2,1)
        X=XY(1,1)
        Y=XY(2,1)
        CALL FPLOT(K,X,Y)
        DO 1 I=1,NP
        X=XY(1,I)
        Y=XY(2,I)
        CALL FPLOT(0,X,Y)
        CALL FPLOT(2,X,Y)
        CALL POINT$(J)
1       CALL FPLOT(K,X,Y)
        CALL FPLOT(1,X,Y)
        RETURN
        END
        SUBROUTINE KLABL(XM,YM,J)
        YIN=6.
        XIN=8.
        GO TO (1,2),J
1       XN=0.0
        YN=(-YM/(1.2*YIN))*.9
        D=0.0
        GO TO 3
2       YN=0.0
        D=1.5708
        XN=(-XM/(.80*XIN))*1.4
3       CALL FCHAR(XN,YN,.2,.2,D)
        RETURN
        END


$SIG
```

APPENDIX 4: ESTIMATION OF KARYOTYPE WITHOUT MARKERS.

The purpose of this appendix is to describe a method to estimate the karyotype, i.e. T/T, T/+, +/+, of individual male insects.

Male flies of unknown genotype were singly mated to females of known wild karyotype, and the progeny production recorded. This data was arranged in the tabular graphic form of Figure 11, (top four tables). For purposes of normality these top four tables were summed to produce the bottom table, (entitled TOTAL) figure 11, bottom shows three normal distributions, overlapping slightly in their tails. The procedure for estimating genotype is then to consider as I have that the left-most normal curve is the result of of T/+ X +/+ matings, the center curve is the result of T/T X +/+ matings, and that the right-most curve is the result of +/+ X +/+ matings.

The assumptions were confirmed by cytological methods as follows. I examined a high producing TVR10, three medium producers, TVR34, 39, and 19, and examples from all four translocation in low producers. In all instances the cytological methods agree with my assumptions.
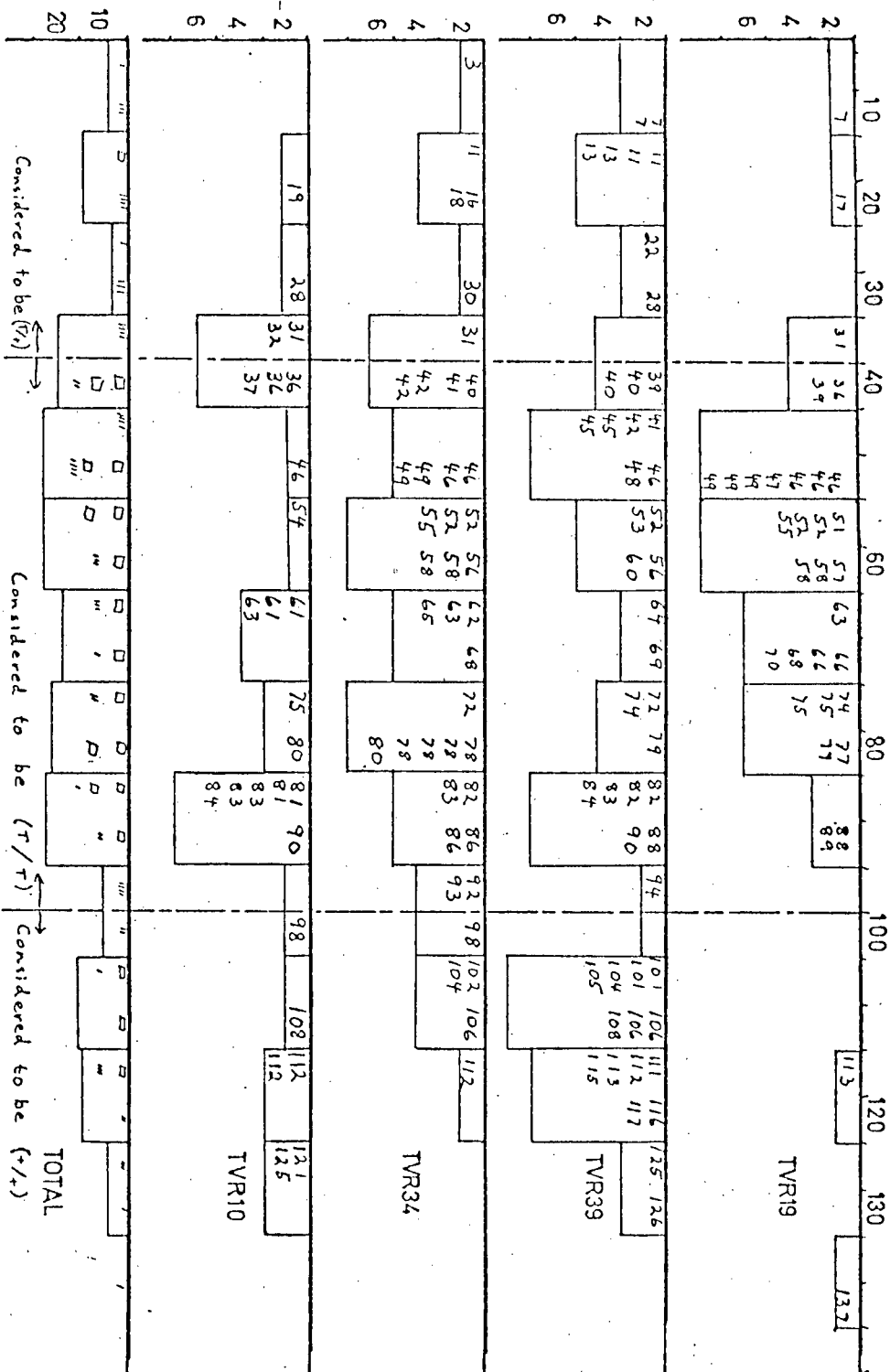
Using these tested assumptions and making the further assumption that there will be no "slop" about the dividing lines, we can construct the final table, table 4.

Figure 11. Procedure for estimation of karyotype without the use of visible
  marker genes. The 3 mormal distributions on the bottom axis are assumed
  to be the result of T/+, T/T, and +/+ male parents, respectively. Results
  must be confirmed cytologically.

FREQUENCY/DIVSION

PROGENY PRODUCTION

TVR19

TVR39

TVR34

TVR10

TOTAL

Considered to be (r/r)

Considered to be (r/+)

Considered to be (+/+)

These results indicate that TVR19 is a far superior competitor with the Oregon-r stock than are the other translocations.