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THE GENETICS OF THE BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES OF DROSOPHILA MELANOGASTER TO THE INSECTICIDE MALATHION

By

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ABSTRACT

i) the more neglected of the two components of the adaptive response of insects to biocides, the behavioural (avoidance) response was investigated and found to be made up of a basic aversive and a secondary dispersive component.

ii) natural populations were found to posess a large amount of genetically determined variation in both physiological and behavioural responses to malathion. There is also evidence that these characteristics have been affected by selection in the local populations.

iii) the relationship between avoidance and resistance in natural populations indicates that both responses are independantly modifiable by selection.

iv) a class of X-linked mutations was induced which show a negative correlation between resistance and avoidance. These mutations may prove useful in further studies of resistance and avoidance mechanisms (eg. via genetic mosaic studies), and they may also represent a class of mutations which provide co-adapted changes in responses that may prove to be important in some evolutionary situations.

v) practical methods (already published) for assaying avoidance and resistance were developed, which may prove themselves to be useful in studies of economically important insects.

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In considering the Origin of Species, it is quite conceivable that a naturalist, reflecting on the mutual affinities of organic beings, on their embryological relations, their geographical distribution, geological succession, and other such facts, might come to the conclusion that each species had not been independantly created, but had descended, like varieties, from other species. Nevertheless, such a conclusion, even if well founded, would be unsatisfactory, until it could be shown how the innumerable species inhabiting this world have been modified, so as to acquire that perfection of structure and coadaptation which most justly excites our admiration.

- Charles Darwin
(1809 - 1882)

INTRODUCTION

The resistance of insects to toxins designed to control them has traditionally posed a major problem to the success of insecticide application programs (Margham 1975). A great deal of effort has been expended in attempts to elucidate the nature of this resistance, particularily its genetic basis (for reviews see Oppenoorth 1965, Georghiou 1969, Plapp 1976). This research has provided insights into both the prospects for human intervention in insect-related problems and into the dynamic processes of evolution which have been responsible for thwarting many of our best efforts to control insect populations.

The main thrust of research dealing with insect-insecticide interactions has focused upon the problem of physiological resistance, i.e. such processes as enzymatic detoxication of absorbed materials and changes in the structures of target enzymes of the insecticides, which reduce the deleterious effects of the insecticide in insects coming into contact with it. The possibility that behaviour might be an important component of insect resistance to toxins was noted shortly after the second world war by Kennedy (1947) who observed that it was possible for mosquitos to survive the first stages of DDT poisoning if the initial dose taken up altered their behaviour so that they tended to spend less time on treated surfaces. Until this fact was experimentally confirmed it had been assumed that DDT "irritability" in mosquitos, while commonly observed, was only manifested once a lethal dose had been absorbed (Buxton 1945, Rose 1944), and thus was of no importance with regards to control programs.

The phenomenon of insect irritability and avoidance of insecticides, not considered in itself to be as important as physiological resistance to control programs (Muirhead-Thomson 1960), was examined along with other types of insect behaviour in order to gauge the effects of behavioural factors on programs to eradicate mosquito-borne disease by Mattingly (1962), who concluded that a study of the nature and inheritance of irritability would be worthwhile, since irritability and other aspects of mosquito behaviour might determine what proportion of the local population was killed during spraying in domestic areas.

An investigation into the genetic basis of mosquito irritability and escape responses to DDT was done by Gerold and Laarman (1964, 1967), who reported success in enhancing these responses via directional selection in the species Anopheles atroparvus (Van Thiel). While they did not link irritability to physiological resistance, other reports indicated that there was a link, with strains with high levels of resistance to DDT being less irritable to it than normally resistant strains. This relationship between resistance and irritability was seen in several mosquito species (World Health Organization 1970, Brown 1964), and it is the type of relationship that would be expected if the insecticide was detoxified before penetrating to the afferent nerves of the legs, which is presumably the first step in triggering the irritability response (Hooper and Brown 1965).

Instances where behaviour had an effect on the control of other insect species have been noted, for example Keiding (1965) noted that knowledge of the resting surface preferences of houseflies (Musca domestica L.) was useful in increasing the effectiveness of spraying procedures, an important consideration with the advent of the organophosphate insecticides which, being much more expensive than DDT, make discriminate use profitable.

In the housefly, DDT-resistant flies were observed to avoid sprayed surfaces (Silverman and Mer 1952), and although no detailed study of DDT-induced irritability was done in that study, the results indicate the opposite relationship between physiological resistance and irritability (or avoidance) to the one described above for mosquitos.

Similar instances of a resistance-behaviour relationship have been reported in studies with the organophosphate insecticides. Using malathion, Hooper and Brown (1965) reported a case of the development of increased irritability to insecticide due to the decreased ability to detoxify it in a strain of the spotted root maggot, Euxesta notata (Wiehemann). In the housefly, several different studies with the same insecticide (malathion) gave the reverse relationship as a result: the strains with high resistance (physiological) also showed the greatest tendancy to avoid malathion-sugar baits. The relationship held whether, as in Hooper and Brown's study, the resistant lines were obtained via artificial selection (Schmidt and La Brecque 1959) or were wild-caught (Kilpatrick and Schoof 1958, Fay et al. 1958).

Little has been done to investigate the reasons for the reported relationships between resistance and avoidance. Since it is not considered to be important in determining the effectiveness of control programs in general, insect avoidance of insecticides has not received the research attention given to the various modes of physiological resistance, which is understandable since most research in this area is done with practical applications in mind. However, insect-insecticide interactions are also interesting because they provide examples of newly-evolving adaptive responses, which arise in populations subjected to controlling chemicals. The rise of resistance in populations subjected to selection pressures from insecticides provides modern examples of the processes involved in Darwinian evolution, since genetically preadapted individuals,(Bennett 1960, Kerr et al. 1957) gaining a selective advantage once the insecticides are applied, quickly become the dominant type in the local population.

It is from the evolutionary viewpoint that the responses of the fruit fly Drosophila melanogaster to the insecticide malathion will be looked at in this study. While some techniques were developed over its course which may prove to have practical applications, the main concern was to investigate the ranges of the behavioural and physiological responses to malathion and the relationships between them in both natural populations and in laboratory lines whose responses were altered by the induction of single mutations.

While the fruit fly is not currently the target of any specific control programmes, malathion is ubiquitously employed throughout southern Ontario, and hence there has been ample opportunity for it to act as a selective agent upon local Drosophila populations. The ease with which this insect can be cultured make it an ideal organism for laboratory study; and, most importantly, the methods of genetic manipulation which have been developed for this species (eg. mutant induction, mapping) make it ideal for a study of the precice effects of genetic changes upon the responses under investigation.

Thus the use of D. melanogaster permits a comparison of the behavioural and physiological responses to malathion that have arisen in two different situations. The wild-caught lines provide a sample of the range of expression and interactions between these responses in natural populations; and because of the likely differences in selection pressure from insecticide that exists between some of the locales sampled (i.e. urban vs. rural), the effects of selection on local populations can also be examined to a modest degree. The laboratory lines allow us to look at the responses to malathion present in a population which has never been exposed to any insecticide, and via the induction of mutations it is possible to look at the effects of the type of genetic differences which serve as the basis of preadaptations in populations subjected to insecticide pressure.

Before this study could be carried out, methods were required to quantify the responses under observation, and this was accomplished via the development of two novel techniques, details of which have been published, and are also included in the Methods section:

i) the assay used to determine the strength of the behavioural response of flies to malathion involves the measurement of the degree to which they avoid an insecticide-treated surface when given a choice between it and an untreated surface. (Pluthero and Threlkeld 1981)

ii) the assay used to determine the levels of resistance to malathion involves its application via vacuum-injection, thus eliminating the effects of fly behaviour on the results - i.e. avoidance of malathion is not a factor as it might be in a feeding test. (Pluthero and Threlkeld 1980).

Employing these techniques, the study was conducted on the basis of the following questions, with the results of the investigation given in the sections to come:

a) in the natural population: i) what is the range of genetic variability in the responses to malathion, and is this variation due mainly to quantitative or qualitative genetic effects?

ii) is there any relationship between behaviour and resistance of the type reported in the studies mentioned earlier in the Introduction?

iii) are there differences among wild lines that can be attributed to different selection pressures in the area from which they were sampled?

b) in the laboratory stocks: i) is it possible to isolate single induced mutations on the X-chromosome which alter the resistance of flies to malathion?

ii) what effects does a change in the level of resistance have upon the behavioural response?

iii) what could be the possible roles of such mutations in producing adaptive responses in evolution?

MATERIALS AND METHODS

I - Lines Used: i) Wild population lines - these were raised from the progeny of single females caught in the area of Hamilton, Ontario by Dr. R. A. Morton in the summer of 1977. They are designated in the text by a letter followed by a number (eg. A-16), and those named with the letters A or B were caught in an urban location (Dr. Morton's backyard in the Westdale area of Hamilton) while the other lines were caught in orchards in either Jordon, Ontario (J lines) or Vineland, Ontario (V lines).

The line designated as A-15-R was isolated as a malathion-resistant sub-population of line A-15 via selection with the vacuum-injection resistance assay described later in the Methods. Other than for this line, no selection was applied to the wild population lines.

An analysis of variance experiment conducted by R.A. Morton and R.S. Singh (unpublished data) using a malathion feeding resistance assay showed that while some genetic polymorphism for resistance exists within the isofemale lines, the largest part of the variance in resistance can be attributed to differences between lines, and hence these differnces can be reliably determined.

ii) Mutant lines - these were derived from a parent stock, Canton Special (designated in the text as Canton S or CS), a laboratory wild-type strain in common use for genetic studies of <u>D. melanogaster</u>. Mutations were induced in this line by feeding males with the mutagen ethyl mehtane sulfonate (EMS) via the procedure of Lewis and Bacher (1968). These males were then mated to females carrying the attached-X chromosome C(1)Dx, establishing lines in which the X-chromosome is passed from the male parent to the male offspring, while the female parents (karyotypically XXY) supply the Y chromosome; thus if the original male parent posessed a mutation on the X-chromosome as a result of EMS treatment, all of his male offspring will carry the mutated chromosome. (For a description of the chromosome stocks used see Lindsley and Grell, 1968) The attached-X lines thus established were subjected to the screening process (to be described), and once the lines had been selected that were to be used for further study, the females were made homozygous for the mutant X-chromosome via crossing to the balancer line FM6. Balanced lines are indicated in the text by the suffix cs (eg. 59cs) added to the original numerical designation of the line.

II - Handling Techniques: i) Culturing - stocks were maintained at a constant temperature of 25° Celsius in an incubator. They were grown on yeast-agar medium (Carpenter's) in individual 8 X 3.5 cm. diameter cylindrical clear plastic shell vials fitted with sponge plugs at the top, to allow air in. Conditions within the cultures (i.e. numbers of flies, age of medium) were carefully watched to ensure that flies were of a constant size and did not suffer from the deleterious effects of overcrowding. The recipe for the medium is in Appendix 0.

ii) Experimental preparation - flies were commonly collected at ages of 1 - 2 days, sexed at 2 - 4 days and used for experimentation at 4 - 7 days. After sexing, some small groups (approx. 100) were kept in 13 x 100 mm plastic tubes with medium, but typically they were returned to the larger vials to allow continuation of the stocks. Unless it is otherwise noted in the text, all experimental groups were of a single sex.

When necessary during culture transfers, anaesthesia was applied in the form of gaseous carbon dioxide, released under pressure from a tank via a stopcock valve. During sexing and mutant scoring, when longer-term anaesthesia was required, CO_2-treated flies were placed on a cooled surface (a plastic petri dish over ice) which kept the flies immobile for several minutes.

All the experiments in this study were conducted between the hours of 10 a.m. and 4 p.m., and while the incubator was kept in darkness most of the time, the time of first light exposure for the flies was roughly the same (between 8 and 10 a.m.) each day, hence the effects of activity cycles on the results is likely to be small, and since all stocks were kept in the same incubator, cycle differnences between lines should not exist.

III - Resistance testing: details of the vacuum-injection assay employed in this study have been published in Pluthero and Threlkeld (1980), and the following description of the method is largely derived from that publication.

Malathion was applied to the test subjects as follows: i) flies were lightly anaesthetized with CO_2 and counted into 20 ml serum bottles (Wheaton Scientific) in groups of twenty flies per bottle. The bottles were stoppered with plastic sponge and the flies were allowed one hour to recover from anaesthesia (this time was more than sufficient, since the effects of CO_2 wear off after a few minutes for the flies used in this study).

ii) each serum bottle was then plugged with a rubber serum cap and evacuated via aspiration to 52 ± 2 cm of Hg vacuum. Previous testing showed that the vacuum itself had no delterious effects upon the flies over the length of time they were subjected to it during resistance testing.

iii) one microliter of malathion solution was injected into the syringe end of a hypodermic needle (Yale 23G1 disposable - from Becton Dickinson) which was then carefully fitted to a syringe (Plastipak - Becton Dickinson) which had the plunger previously withdrawn to 0.1 ml. The ends of the syringes used were trimmed to allow them to fit into the loaded needles without ejecting the sample of solution.

iv) held by its top, the needle was pushed through the serum cap and the plunger of the syringe was depressed before the needle was withdrawn. The actual injection of the malathion sample was done by the effect of the negative pressure inside the bottle, the plunger depression was merely done to insure that no air leaked in around the syringe-needle junction.

v) after 120 seconds, the vacuum in the bottle was released via the insertion of a no. 18 hypodermic needle through the cap which allowed air to enter the bottle. The serum cap was then replaced by a sponge to allow air in for the length of time over which the knockdown of the flies was observed.

The apparatus used to evacuate the serum bottles is illustrated in Figure 2.1, and it consisted of a 1 L. flask with tubulation attached via vacuum tubing to an aspirator and a pressure guage, which was in turn fitted with a no. 18 needle through which air was drawn when water was run through the aspirator. The serum bottles

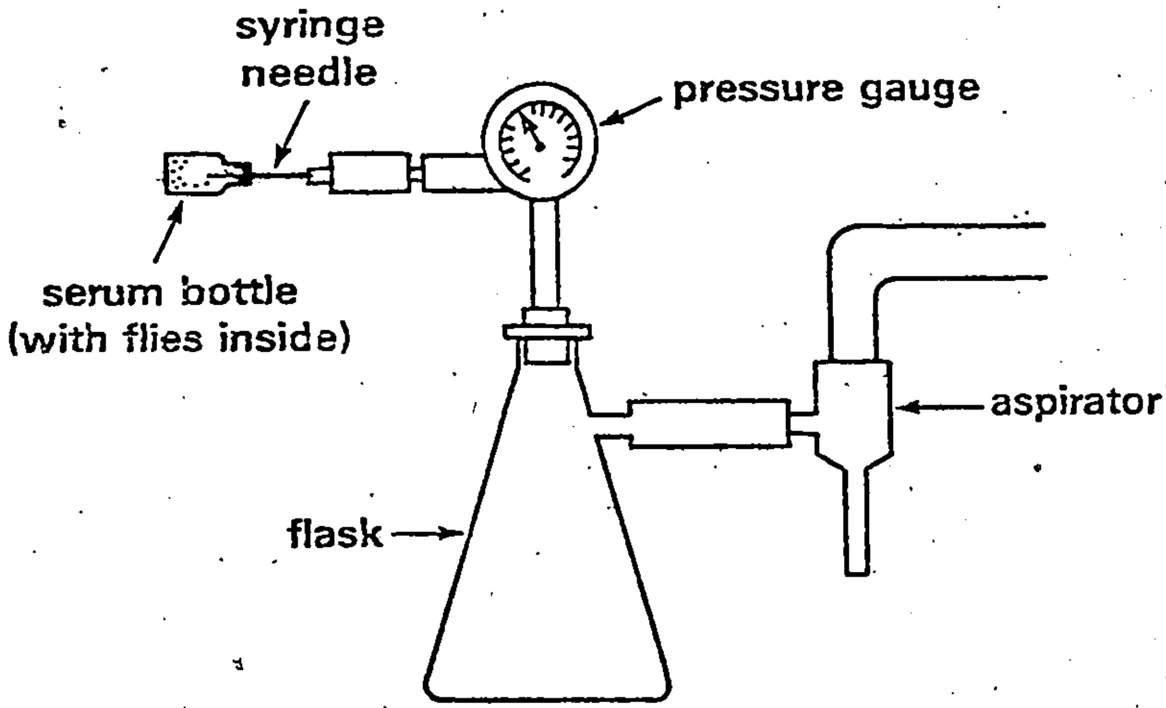


Fig. 2.1 - apparatus used to evacuate serum bottles for the vacuum-injection assay.

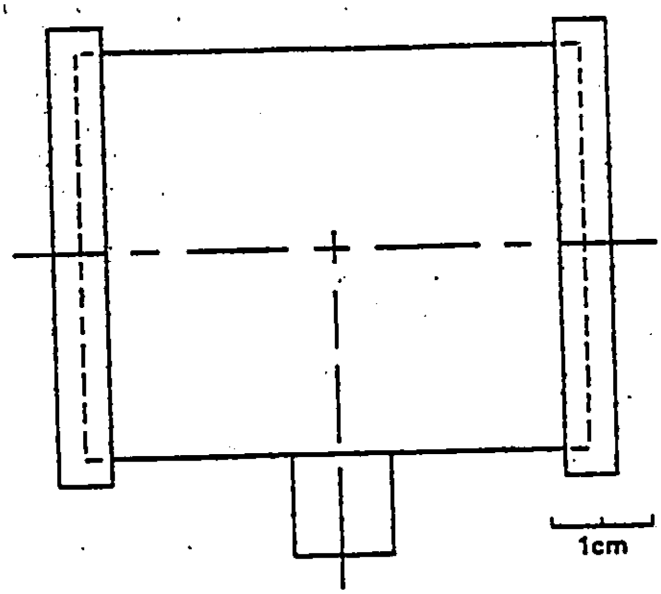


Fig. 2.2 - chamber used for avoidance testing.

were evacuated by pushing the serum cap over the needle and running the aspirator to withdraw the air inside the bottle, with the internal pressure being monitored by the gauge. None of the lines tested in this study showed any mortality due to this treatment, although one laboratory strain, an ebony mutant, was seen to be killed by the effects of the vacuum alone.

Testing was done with various concentrations of a commercial preparation of malathion (95% technical grade malathion in a solvent, SC100, with added emulsifyers - Wilson Laboratories, Dundas, Ontario) mixed with a carrier solution of 2-propanol and stored at 12°C. This carrier was chosen after extensive testing with other solutions because: a) malathion is miscible with it and retains constant potency in solution for at least 24 hours. b) 2-propanol alone does not kill flies when vacuum-injected, unlike other alcohols such as methanol, and ketones such as acetone. c) Ethanol, while not in itself lethal to the flies, gives a "false knockdown" when vacuum injected, i.e. up to 50% of the flies in a test group are knocked down for up to 30 minutes and then subsequently recover. Since most of the recording of knockdown due to malathion was done in the 10 to 60 minutes post-injection period, elimination of this false knockdown is essential, and it is not seen with 2-propanol.

Once the insecticide had been applied, the flies in each test bottle were scored for number knocked down versus time after the sample of malathion had been injected. These results were treated in the manner described in a following section.

The vacuum-injection method of applying malathion was employed in this study because it allows resistance of the flies to be assayed in a manner which ensures that the uptake of malathion is independant of the flies' behaviour, which would not be the case if the insecticide was adminstered by feeding. This is an important consideration when the connections between behaviour and resistance are being investigated. While most topical application methods also meet the behaviour-independance requirement, the small size of D. melanogaster individuals and the large numbers that were planned to be tested simultaneously made methods such as direct injection unsuitable.

The vacuum-injection procedure described above is basically a modification of that employed by Sega and Lee (1970) to inject uniform doses of mutagen into D. melanogaster males. According to their report, the vacuum apparatus gave superior consistency of results and uniformity of dose over other topical application methods, a characteristic exploited by Howard et al. (1975) in testing the effects of various neurotropic drugs on this species.

In their description of the technique, Sega and Lee (1970) hypothesized that the injected sample was drawn out of the needle, vapourized, and then subsequently diffused into the tracheal systems of the insects. Once the vacuum was ruptured, the increase in air pressure forced the material into the tissues of the flies. While no attempts have been made here to verify this proposition, there is evidence that in this technique the insecticide and carrier gain entrance to the fly tissue directly, since, although the flies were commonly left in the same serum vial for injection and scoring, there was no difference seen in the knockdown vs. time relationship if they were transferred to fresh bottles after the vacuum was broken. This indicates that the insecticide is not simply being picked up as a condensate from the sides of the bottle (an unlikely possibility given the small size of the injected sample). Although Sega and Lee used the term "vaporized" to describe what happens to the sample on injection into the evacuated bottle, in fact it is not possible to say whether the sample is in the form of a vapour or a very fine aerosol unless further investigation is carried out.

The advantages of the vacuum-injection method of applying insecticide are: a) uptake is independant of behaviour, b) it is a fast and relatively simple procedure (several hundred flies can be treated in an hour), c) the results indicate good dose uniformity within and between test groups, d) it allows measurements of the responses of groups of test subjects which are injected simultaneously, e) by itself, or with minor modifications (e.g. increasing the size of groups treated by using larger holding vessels) it is useful as a technique for screening out resistant sub-populations.

Some problems had to be overcome, however, in order to obtain consistent results. Aside from the finding of a suitable carrier solution, it was most important to establish a sequence in which the times between different steps in the procedure remained the same from run to run, since some of the intervals (i.e. the length of time between sample injection and the breaking of the vacuum) have a large effect on subsequent results. There is also a significant effect due to temperature, which was carefully monitored within each set of runs (actual testing temperatures are given with the results). On the average, less than 25% of the runs were failures, which were easily detected since little or no knockdown was seen due to errors in injecting the malathion in the affected groups.

With respect to general applicability, the vacuum-injection technique appears to be adaptable to many test situations, although for other test substances different carriers may be necessary, and other insects may require further changes in technique, such as the use of larger testing bottles or different timing schedules.

IV - Avoidance testing: the assay for avoidance of malathion by D. melanogaster used in this study has also been described in Pluthero and Threlkeld (1981).

Since the behavioural responses of insects to insecticides typically involve the effects of physical contact, an apparatus was needed which allowed the test subjects to come in contact with insecticide-treated surfaces, such as occurs in the standard World Health Organization test kit for measuring DDT-irritability in mosquitos (Brown 1964). It was also desired to have an internal control for each test run, hence a choice was offered to the flies between an insecticide-treated surface and an untreated surface, in a manner similar to that used in the repellency studies of Bar-Zeev (1962) and Bovingdon (1958).

Figure 2.2 shows a diagram of the type of test chamber used for avoidance testing, as seen from above. The main body of the chamber is a clear plastic cylinder, each end of which accepts a translucent plastic cap containing a piece of 4.5 cm diameter filter paper, which serve as the test surfaces. Flies are introduced into the chamber via the entrance tube which points to the bottom of the diagram. This chamber is simple in design, easy to fabricate and simple to clean (with 70% ethanol), and offers two large surfaces (the discs of filter paper) in close proximity to each other for potential choice by the flies.

During the experimental runs, the chambers were oriented such that in Figure 2.2 the page represents the horizontal plane, hence neither side was favoured by geotaxis. All tests were run inside a wooden light box, with the chambers held by three-finger clamps between two flourescent tube lights. In order to prevent any phototaxis shown by the flies from affecting their choices, the chambers were positioned so that the amount of light coming in either end was equal, as measured with a photometer (LI-COR Model LI-185A, Lambda Instruments Corp.). Temperature during testing was 27-28°C in the box, with a room humidity of 30-35%, although the humidity within the chambers was higher due to the moistening of the test surfaces.

Flies were run in groups of 50, counted out 2 hours prior to testing under light CO_2 anaesthesia, and kept in 10 ml blacked-out holding tubes without food. They were admitted into the test chamber by lightly tapping them from the holding tube through the entrance tube, and scoring commenced 5 minutes after they had entered the chamber, this time being allowed for the flies to recover from the transferring process.

Immediately prior to each test run, the filter papers in each chamber cap were wet with uniform amounts of either of two test solutions: a) a solution of 5% sucrose by weight in water, or b) the same solution plus 1% malathion by volume, which was kept constantly stirring while sampled to maintain an even suspension. For the remainder of the text these solutions will be referred to as 0% and 1% respectively. They were kept refrigerated at 10 - 12°C between applications, and the 1% solution was made up fresh each day from a stock of 0% solution. The tests were run with wet papers because they were found to be more attractive to the flies than dry ones, and also this eliminated death by dessication during testing.

At the end of the recovery period, the flies were scored at intervals of thirty seconds for the numbers standing on each end paper. The number knocked down was recorded approximately every 3 minutes (longer intervals were used when the rate of knockdown was low), and recording ceased once 60% of the flies were down, or 30 minutes had passed, whichever came first.

For avoidance tests, the chambers were set up with one end paper treated with the 1% solution and the other with 0% solution, hence the raw data consisted of pairs of numbers, each recording the number of flies on each surface at each observation time. For further analysis of the data, these number pairs were converted to index values. The index used is similar to the ones employed by Fuyama (1976, 1978) and Shreck (1977), and is called the avoidance index, A. The value of A for each observation was determined according to the following formula:

$$\text{Avoidance index, A} = \frac{\text{\#flies on 0\% surface}}{\text{\#flies on either surface}}$$

This value can range from 0 (all flies on 1% surface for one observation) to 1 (all on 0% surface). Note that the value of A involves only those flies "responding" at any one time, which is usually a subset of the total number of flies within the chamber.

Controls: in order to determine whether differences noted in avoidance index values between sexes and strains were due to specific responses to the malathion or the conditions of testing, control runs were done in which no choice was offered - both end papers during a run were treated with the same solution (0% or 1%). Instead of the avoidance index, results for these runs were turned into control index values as follows:

$$\text{control index value} = \frac{\text{\#flies on left side}}{\text{\#flies on both sides}}$$

V - Data analysis: i) Resistance testing - the raw data from the resistance assay consists of numbers of flies seen to be knocked down recorded versus the time post application of malathion. For further analysis and visualization, the individual run results were converted into cumulative distributions of percentages of total flies down versus time (eg. since there were 20 flies in each test group, the number knocked down was multiplied by five in each instance). These cumulative distributions were subjected to further analysis as follows:

a) knockdown curves were obtained by plotting the percentage knocked down on a probit scale versus the time post application on a logarithmic scale (ld-p plot - see Figure 2.3). When all of the observations at one dose for a particular class of flies had been plotted, a curve was fitted to them to give the knockdown curve, which, since the knockdown distributions were normal, is in the form of a straight line on the log-probit plot through most of the percentage range.

From these knockdown curves, the values for the time to 50% knockdown (KT_{50}) can be determined. This was typically done via a computer program, which also calculated the standard error of the KT_{50} value (see Appendix A).

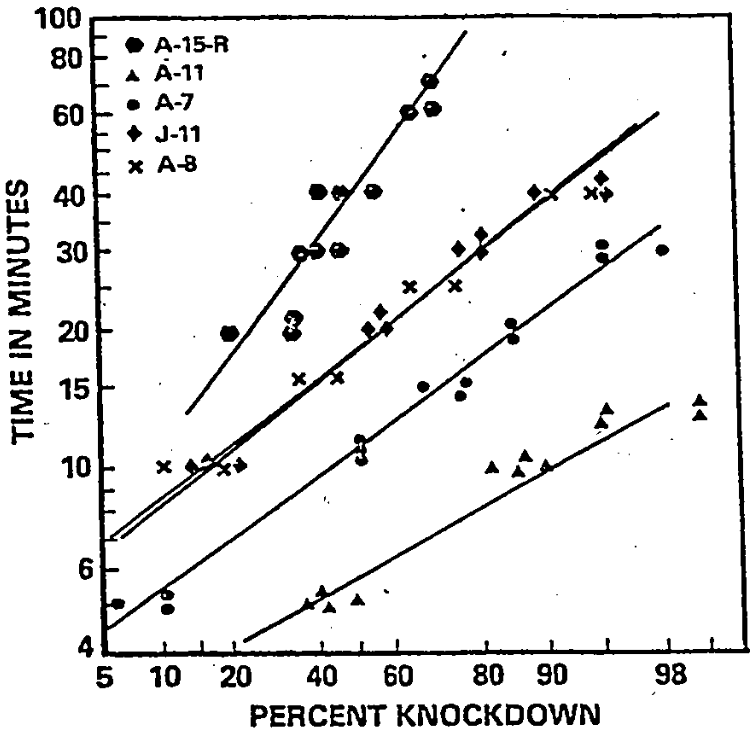


Figure 2.3 - plot of the percentage of males knocked down versus time post malathion application (2% dose) for five wild population lines. (# replicates = 2 for A-8, 4 for A-11, 3 for rest)

b) dose-response curves were obtained from the knockdown curves in two ways: for the wild populations, the percentage of flies knocked down at a given time (eg. 15 minutes post-injection, for Figure 2.4, given here as an example) was plotted on a probability scale versus the applied dose to give ld-p plots (Hoskins 1960). Uncertainty is indicated by bars showing the standard error for each point, which was calculated from the knockdown data. The cut-off time selected for the ld-p plots was chosen to be one which best illustrates graphically the range of resistances among the lines plotted.

For the mutant lines, the dose-response curves are in the form of plots of log dose versus log KT_{50} (cf. Singh and Morton 1981). In each case, the method used to plot the dose-response relationship was chosen to best illustrate the results (these plots were not used to determine any significant differences in resistance between lines), and to conform to the requirements of the publications to which the results were submitted.

c) the cumulative distributions were averaged for each class to give mean distributions of knockdown for each dose, which were used in comparisons between groups to determine significant differences in resistance. The test used to compare these mean distributions was the Kolmogorov-Smirnov two sample test (Beyer 1966), in which a critical difference value, D is calculated as follows:

$$D = k\sqrt{\frac{n_1+n_2}{n_1 n_2}}$$

where n_1, n_2 = sizes of samples

k is a constant whose value depends upon the level of significance being tested for (eg. k=1.36 for α=0.05)

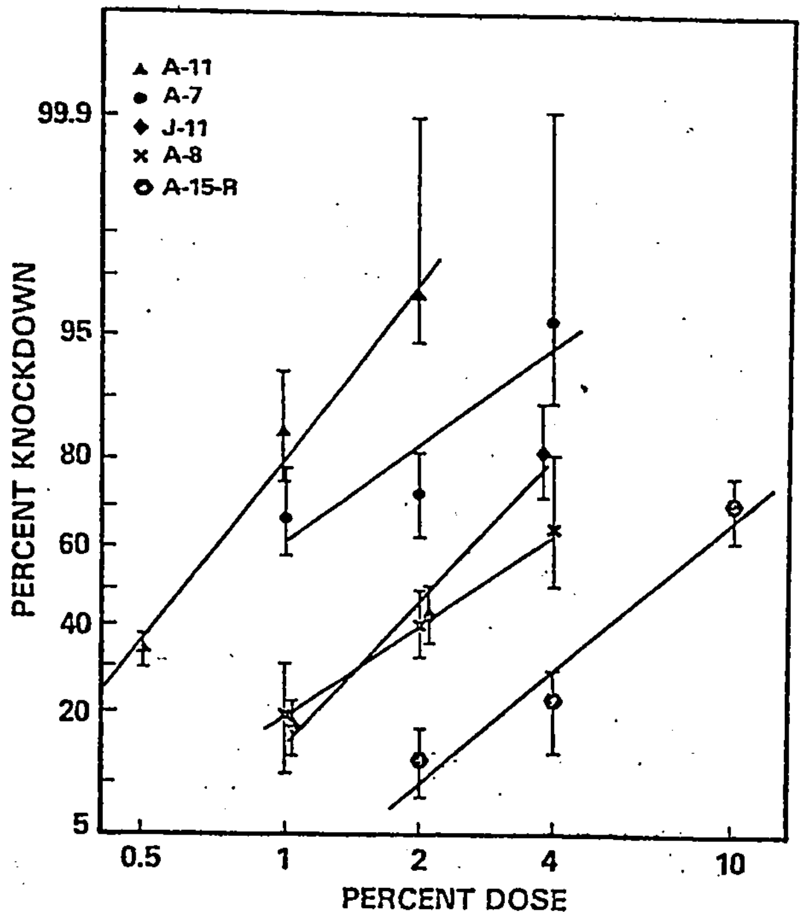


Fig. 2.4 ld-p plot showing susceptibility of male flies to malathion.

Expressed as a percentage, D was compared to the values of the maximum differences between cumulative distributions (Dmax), and cases where Dmax exceeded D at a level of significance of p= .05 or less were taken to be indicative of significant differences in resistance between the groups compared.

ii) Avoidance testing - the raw data from the avoidance test runs, as has already been described, was converted into values of the avoidance index, A. The mean A values for each run of a group (i.e. all the runs for one sex of one line) were compared for homogeneity via analysis of variance, after the homogeneity of their variances had been confirmed via Bartlett's test. Both types of analysis were done by computer using methods described in Sokal and Rohlf (1969). Data from runs showing no significant differences within the group were pooled to give single sets of A values for the group.

These pooled sets of indices for each group were used for intergroup comparisons via a means test (Sokal and Rohlf 1969), since in general there was no homogeneity of variances in the A values among groups. (For further explanation see Part C of the Results). These data sets were used to calculate values of the mean avoidance index, $\overline{A}$, and to make computer-generated histograms of the index distributions, which are used to illustrate differnces in avoidance behaviour seen among the lines tested throughout the text (see Figure 2.5 for an example of these distributions).

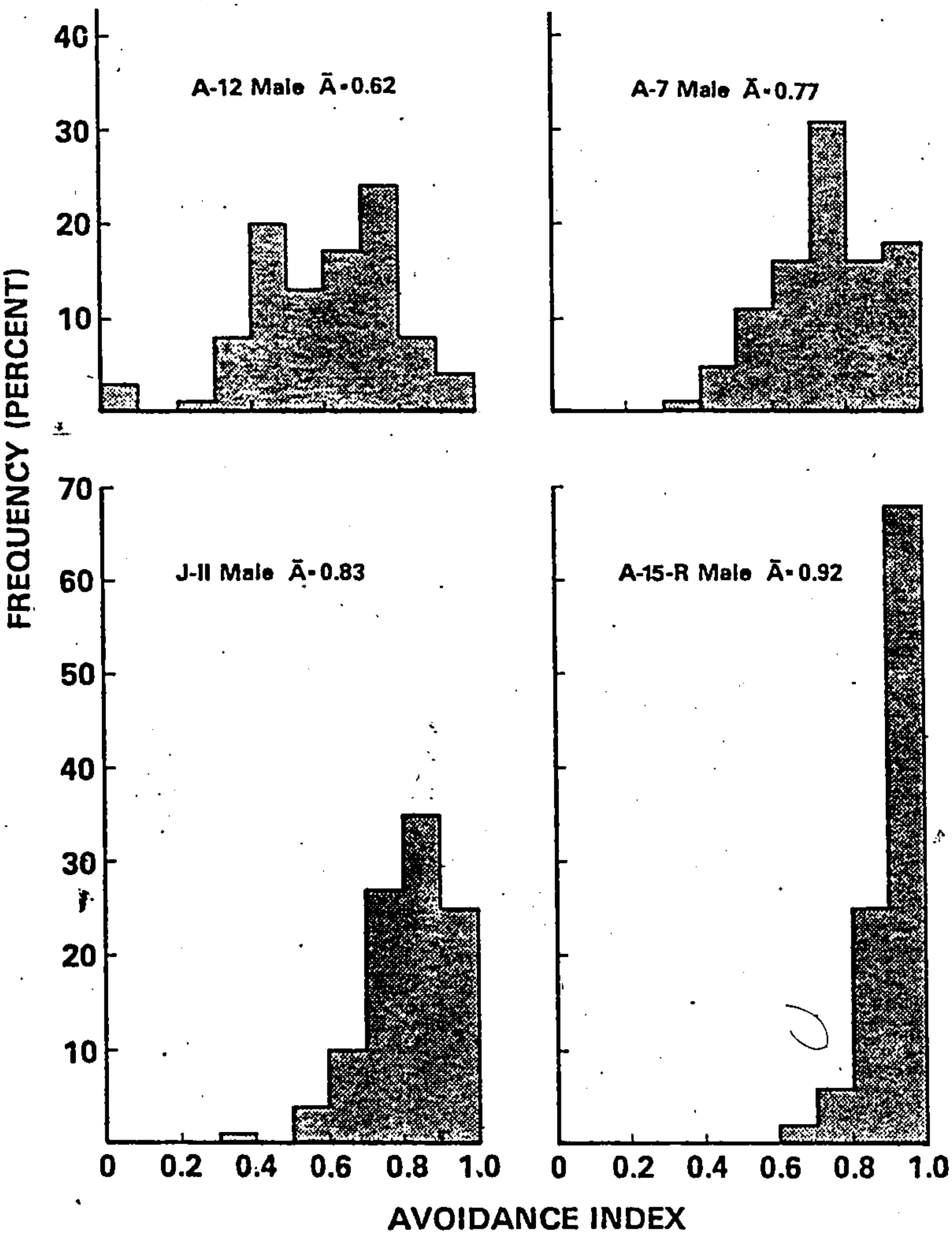


Fig. 2.5 - avoidance index distributions of male flies for four different lines.

VI - Isolation of resistant sub-population A-15-R: during the course of resistance testing of the wild population lines, some of them showed evidence of split-populations for malathion resistance. Figure 2.6 shows the knockdown curve for one of these lines, A-15, obtained via a medium-feeding resistance assay in which a dose of 1% malathion was administered by feeding (Appendix O). The plateau for knockdown reached at approximately 70 minutes after the start of the test indicates the presence of a sub-population within the group which posesses a level of resistance much higher than the rest of the flies (app. 75% were knocked down in the first 70 minutes).

Similar results were obtained for this line when it was tested with the vacuum-injection assay (1% malathion dose), which showed that about 10% of the flies survived testing at that dose. Since the existance of a resistant sub-class so distinct from the others in an iso-female line is a good indication of a major gene for resistance segregating within the line, the resistant sub-population was isolated in order to test for the existance of this major gene.

Line A-15-R was selected from line A-15 by subjecting 3 successive generations to a 1% dose of malathion via vacuum-injection and culturing those flies which survived (i.e. were alive 120 minutes post-treatment - most of the sensitive flies were dead in less than an hour). After 3 generations of selection, over 95% of the A-15-R flies were seen to be able to tolerate a vacuum-injected dose of 1% malathion, and this level of resistance has remained constant within the line, indicating that a major gene for resistance has been fixed within it, a possibility discussed further in the Results.

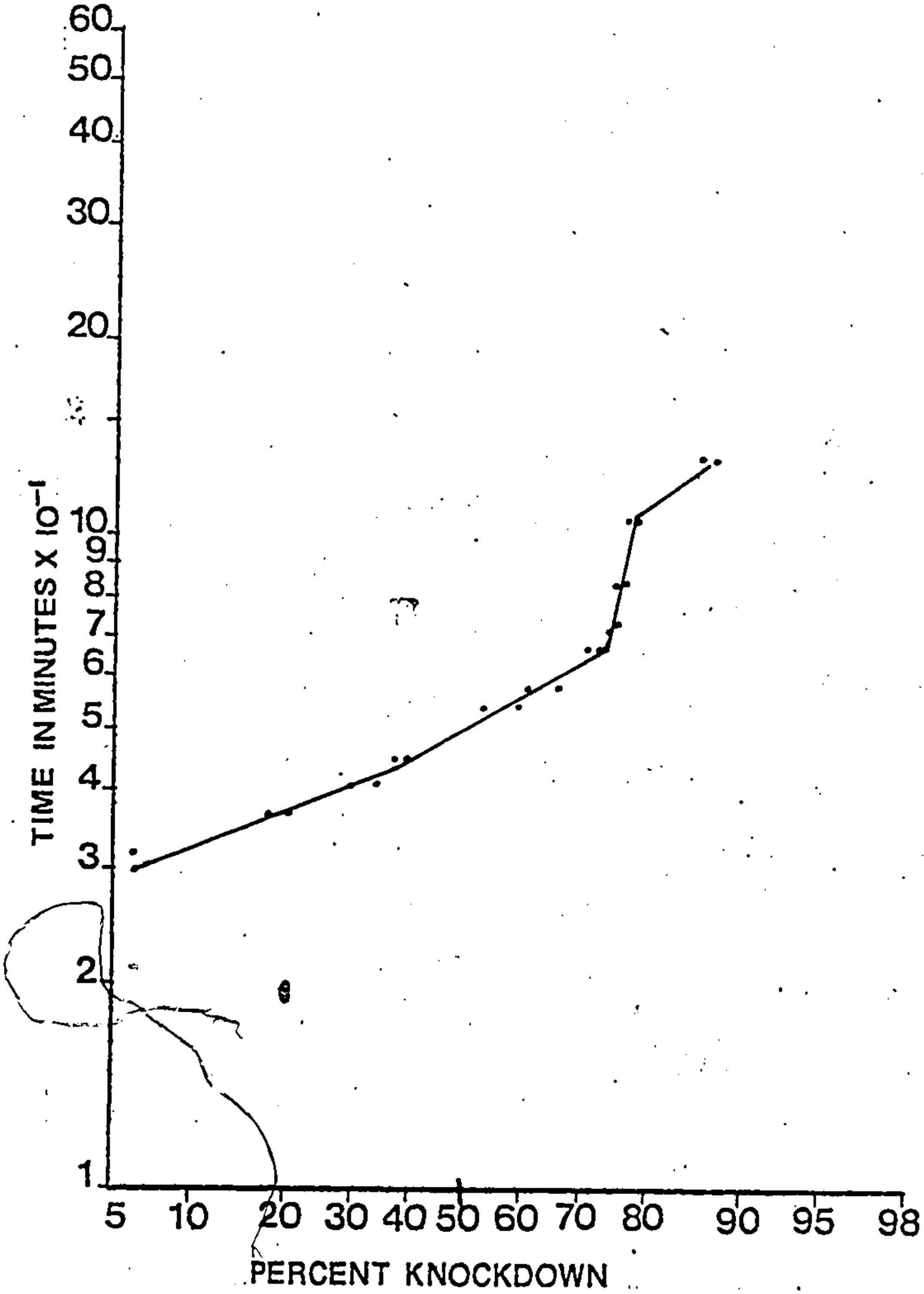


Fig. 2.6 - knockdown curve for line A-15 indicating the presence of a resistant sub-population (2 replicates).

VII - Mutant screening: males from the attached-X lines were rough-screened for differences in resistance to malathion in an apparatus consisting of a plastic shell vial with the closed end cut off and plugged with a foam stopper to let air in. The top of the vial was fitted with a translucent plastic cap into which was placed a circular piece of filter paper soaked with a solution of 5% sucrose by weight and 1% malathion by volume in distilled water. The testing chamber was placed in a light box so that the cap with the filter paper was pointing up, and was illuminated from above by a tube fluorescent light, hence the negative geotaxis and positive phototaxis of the flies would lead them to the malathion treated surface. Each line was scored for the number of males knocked down versus time after they were introduced into the chamber, and those showing a different knockdown profile from Canton S males were kept for further testing.

After the mutant lines had been rough-screened, they were put through a secondary screen in which they were tested for malathion resistance via the vacuum-injection assay. They were tested at a dose of 1% malathion, and those lines which still showed significant differences from Canton S males were kept for the next phase of the study, provided that their knockdown curves indicated that the line was a uniform population with respect to resistance (i.e. gave a straight line on the knockdown versus time plot). This was an important consideration, since the lack of female controls did not eliminate autosomal mutations from being screened out as well (the knockdown curve of attached-X females was grossly divergent from those for Canton S flies presumably because of their unusual genetic constitution, hence they were useless as a resistance control). In fact, several lines were rejected because they showed multiple resistance classes within them, indicating that their differences in resistance from Canton S were not likely to be due to X-linked mutations, which would have been carried by all of the males in a line, but rather arose from the effects of autosomal mutations segregating within the line, giving results similar to those seen in the wild population line A-15.

VIII - Mutant mapping: the mutations were mapped via their phenotypic effects upon resistance, since differences in this characteristic can be detected within a population from knockdown curves. Populations containing two resistance classes give stepped bimodal curves on these graphs, such as those shown on Figure 2.7, which illustrates the knockdown curves obtained for mixed mutant populations (the lines used were 59cs and 151cs, whose resistance lines are shown on the graph for comparison). As the curves illustrate, it is not difficult to detect a mixed population via this method, but the curves themselves are not reliable indicators of the relative proportions of the two resistance classes present in the sample.

Mapping was done in two steps: i) first the mutations were located between marker genes on the X chromosome by crossing mutant males to females homozygous for an X chromosome containing the marker loci yellow (y), crossveinless (cv), vermillion (v), and forked (f) at map locations 0, 13.7, 33 and 56.7 respectively. The F_1 progeny from each cross were allowed to cross and the F_2 males were sorted into the different

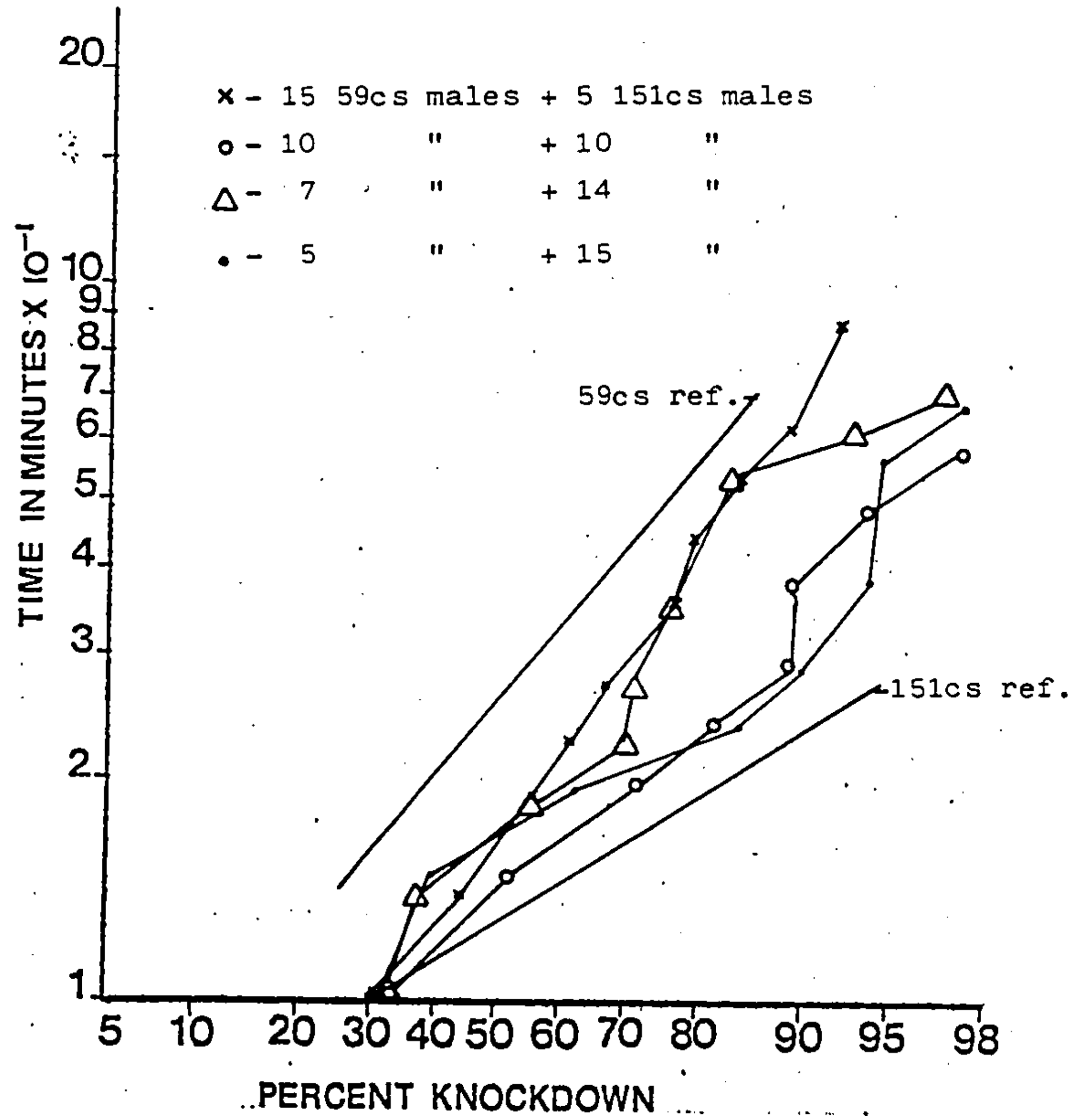
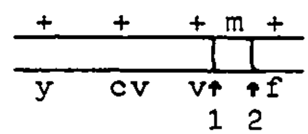


Fig. 2.7 - knockdown curves (1% malathion dose) for mixed groups of males from two mutant lines (reference lines shown).

recombinant classes: ++++, +++f, ++vf, +cvvf, ycvvf, ycvv+, and y+++. These different classes of recombinant males were tested for resistance to a dose of 1% malathion via the vacuum-injection assay. If the mutant genes retained their expression in the presence of the marker genes, it was expected that the recombinant classes resulting from crossovers in the region of the mutation would show split populations with respect to resistance. For example, for a mutation between v and f, in the heterozygous female there are two possibilities for a crossover between these loci :



Thus within a class of recombinants arising from a crossover between these loci, eg. +++f males, some will carry the mutant gene (c/o at position 2) while some will not (c/o at position 1), and hence when these males are tested for their malathion resistance, their knockdown curves should indicate a split population like those on Figure 2.7. In this manner the mutations were located between marker loci.

Once the flanking markers for a mutation had been determined, an approximate map location was arrived at by crossing single males of the split population recombinant class with attached-X females and examining the male progeny of the lines thus established. The ratio of the frequencies of the two resistance types within the set of lines was used to calculate the frequencies of crossovers between the marker loci and the mutation, and hance the map location of the mutation could be estimated. For example, line 59cs gave a split population for the ++vf F_2 male recombinat class, and a sample of the attached-X lines derived from males of this class showed that of

36 lines, the males of 15 carried the 59cs line mutation, as indicated by their higher level of resistance than lines of the same genotype (++vf) known not to carry the mutation. Thus the map location of the 59cs mutation was calculated as 15/36 x (distance between cv and v loci) map units to the left of the vermillion locus.

RESULTS

PART 1 - Wild Population Lines

I - Resistance testing: the raw data from individual vacuum injection resistance assay runs are given in Appendix B, along with the knockdown curves for each group at each testing dose. In order to get some idea of the range of malathion resistance existing in the wild population lines, dose-response curves were determined for 8 of them: A-6, A-7, A-8, A-10, A-11, A-12, J-11 and A-15-R. (as mentioned in the methods, line A-15-R is a resistant sub-population isolated from line A-15).

The dose-response curves for these lines are shown in Figures 3.1.1 (males) and 3.1.2 (females). The curves were obtained by plotting the logarithm of applied malathion dose versus the percent knockdown for that dose at t=15 minutes post application on a probability scale.

The wide range in resistances existing within the wild population is illustrated by a comparison of lines A-11 and A-15-R. A dose of 1% malathion administered to both lines gives, for A-11, greater than 80% knockdown at 15 minutes post-injection, and total knockdown at 25 minutes (see Figure 3.1.3), while the same dose given to A-15-R flies gives a total mortality of less than 10%.

Tests for significant differences in resistance were done by comparing the cumulative knockdown distributions obtained for a dose of 2% malathion (the lowest dose for which all lines tested showed mortality). The complete analysis is given in Appendix C, and the KT_{50} values for

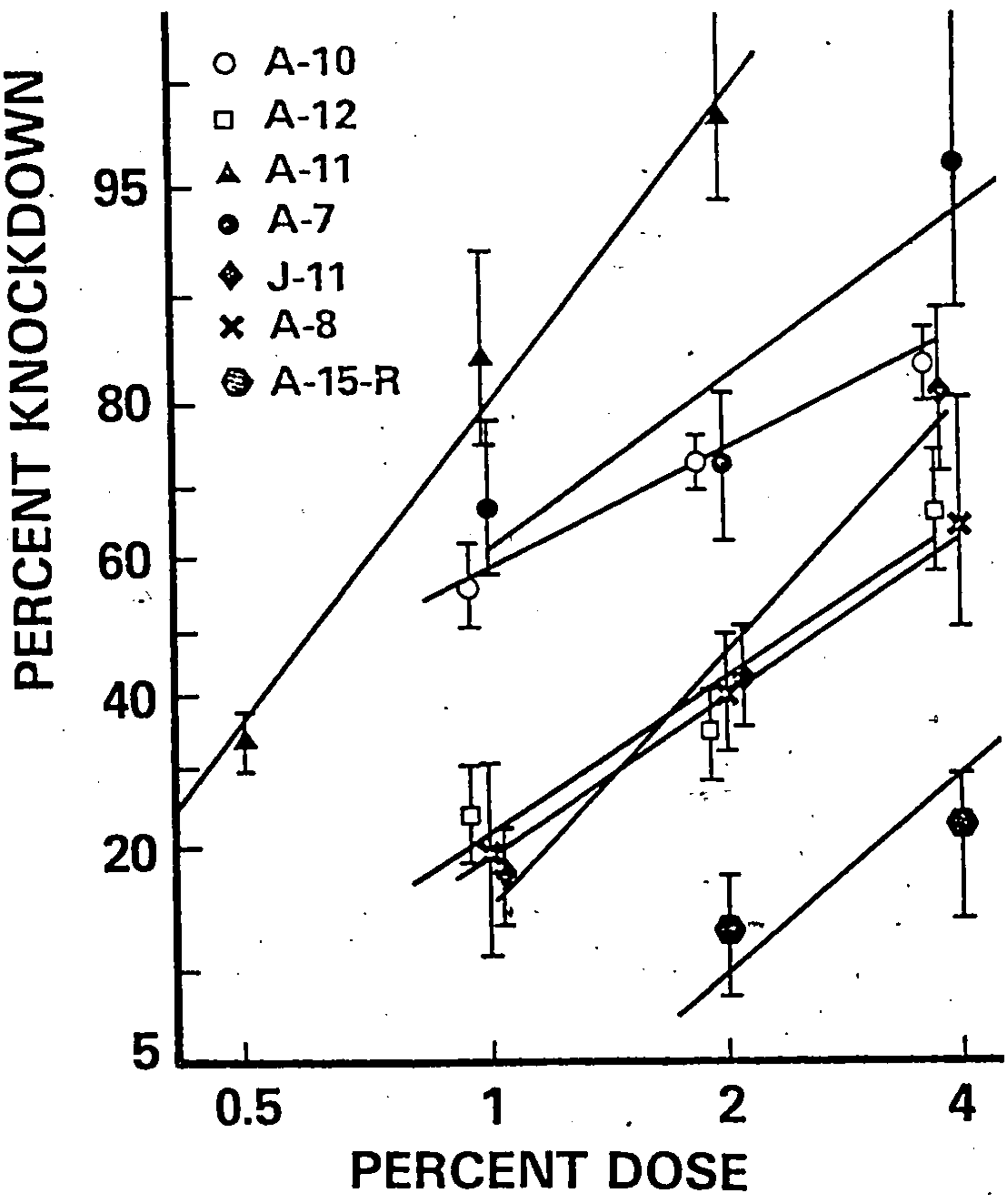


Fig. 3.1.1 dose-response curves for males of wild-population lines (see Appendix B for numbers of replicates).

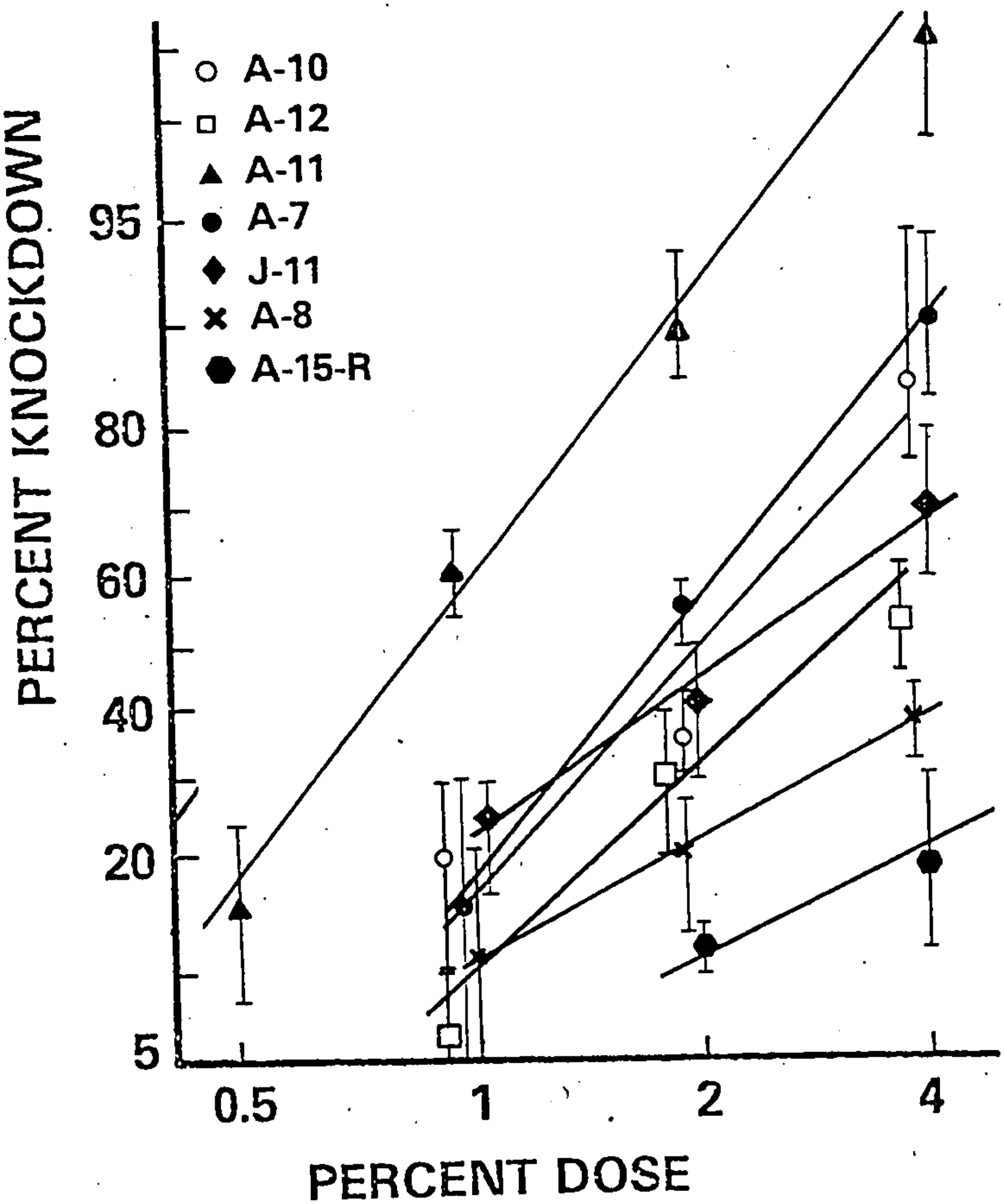


Fig. 3.1.2 dose-response curves for females of wild-population lines

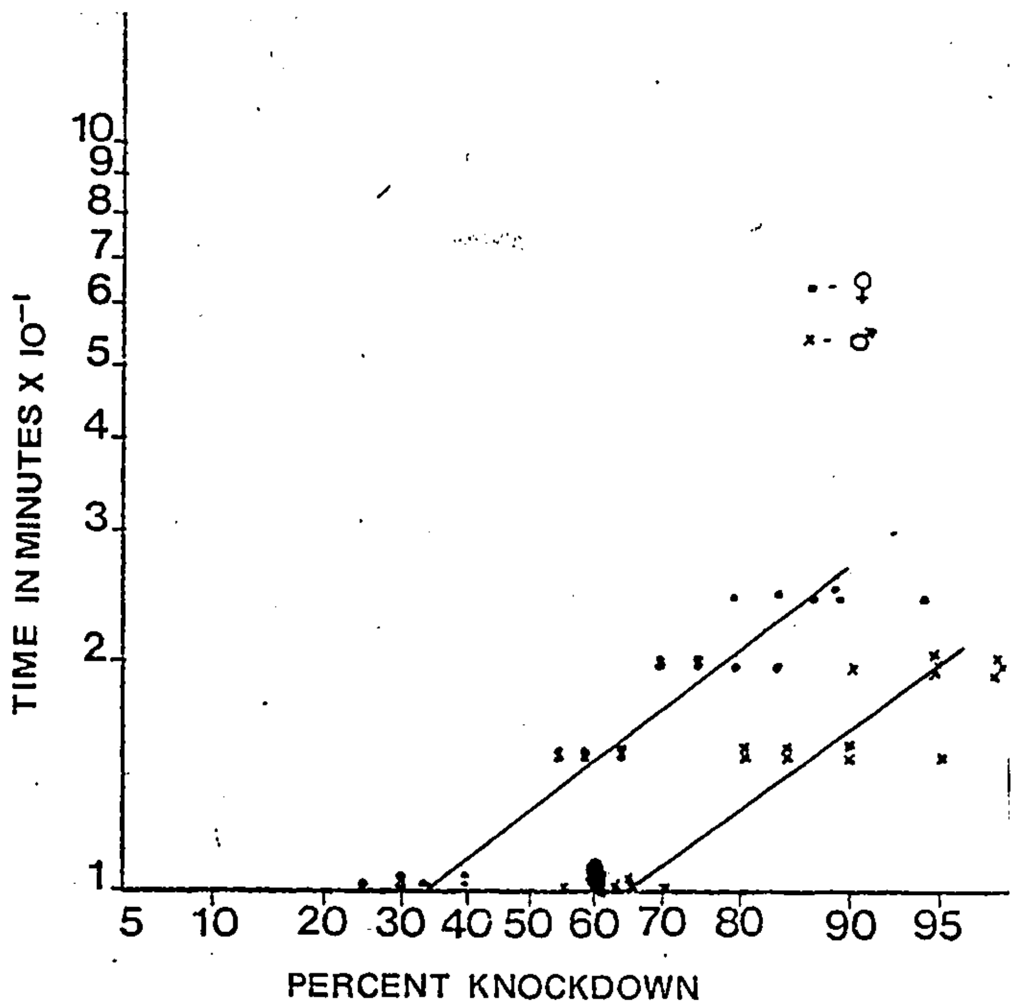


Fig. 3.1.3 knockdown curves for flies of line A-11 (1%malathion dose).

this dose are listed in Table 1.

a) Inter-strain comparisons - within each sex, the most resistant line is A-15-R, and the most sensitive is A-11. The rest of the lines can be put into groups within which there are no significant differences in resistance. For males, the groupings are: (A-6, A-7, A-10) and (A-8, J-11, A-12); while for females the groupings are (A-7), (A-6, A-10, A-12), and (A-8, J-11). In both cases the groups are listed in ascending order of resistance, and these groupings can be clearly seen on the dose-response curves (Figs. 3.1.1 and 3.1.2).

b) Inter-sex comparisons - the lines indicated by asterisks on Table 1 show significant differences between the sexes for resistance (Kolmogorov-Smirnov test).

In addition to the lines for which complete resistance profiles were determined, knockdown curves for a dose of 1% malathion were also determined for males of lines V-3, B-2, V-11 and V-31. These results will be given later, and are also recorded in Appendix B.

II - Avoidance testing: in addition to the lines which were tested for malathion resistance, several other wild population lines were tested for the strength of their avoidance response towards malathion. In all, 26 lines were tested (both sexes) via the avoidance assay described in the Methods, and the raw data and homogeneity analyses are given in Appendix D.

Table 2 lists the mean avoidance indices and standard deviations for each sex and line tested. These results show a wide range in the strength of the tendency to avoid malathion among these lines, and therefore within the wild populations from which they were sampled. The strongest avoidance is shown by males of line V-11, with a

TABLE 1. Fifty % knockdown times (KT_{50}) for all strains of flies treated with a 2% malathion dose in the vacuum apparatus.

	KT_{50} (min)			
Strain	# runs	Male	# runs	Female
A-6	3	11*	4	25
A-7	5	12	5	15
A-8	3	24	5	26
A-10	2	9*	3	33
A-11	4	6	5	9
A-12	3	18*	3	23
J-11	4	16	3	18
A-15-R	3	48*	2	100

*Significant difference between sexes, $P < 0.01$.

TABLE 2. Results of the wild population avoidance survey.

Strain	Female		Male	
	$\bar{A}$	St. dev.	$\bar{A}$	St. dev.
*A-4	0.61	0.17	0.81	0.13
A-5	0.69	0.18	0.70	0.17
A-6	0.74	0.18	0.71	0.17
*A-7	0.69	0.20	0.75	0.15
A-8	0.64	0.18	0.63	0.23
A-9	0.60	0.16	0.64	0.16
*A-10	0.80	0.11	0.63	0.26
A-11	0.66	0.21	0.71	0.20
*A-12	0.67	0.15	0.59	0.23
*A-15-R	0.71	0.16	0.92	0.07
A-16	0.70	0.20	0.68	0.17
A-17	0.70	0.20	0.69	0.15
B-2	0.91	0.07	0.92	0.08
*B-4	0.87	0.13	0.81	0.13
B-7	0.87	0.10	0.84	0.12
B-8	0.86	0.11	0.89	0.10
B-10	0.85	0.08	0.86	0.12
B-13	0.63	0.17	0.63	0.17
B-16	0.74	0.15	0.73	0.21
*B-17	0.78	0.12	0.71	0.16
J-8	0.69	0.26	0.62	0.17
*J-11	0.70	0.27	0.84	0.11
J-19	0.67	0.14	0.71	0.13
V-3	0.89	0.12	0.88	0.15
*V-11	0.89	0.08	0.95	0.04
*V-31	0.76	0.13	0.81	0.10

*Significant difference between sexes, $p < 0.05$

mean avoidance index value of 0.95, while at the low end of the range, A-4 females have a $\bar{A}$ value of 0.61. These results can be visualized in the avoidance index histograms shown in Figure 3.1.4 (distributions of the A values for all lines are given in Appendix D).

Significance testing - the full results of significance testing are given in Appendix D. Lines which showed significant differences in avoidance are indicated on Table 2, while the results of interstrain comparisons (via the means test mentioned in the Methods) are summarized in Table 3, which shows how the lines can be grouped according to avoidance. There are eight such groups, within which there are no significant differences in avoidance, and the average values of the $\bar{A}$ values for lines within each group given in the table show a fairly continuous range from a value of 0.63 to 0.92.

The distribution of $\bar{A}$ values among all of the lines tested is shown in Figure 3.1.5, which shows that the values of $\bar{A}$ between 0.6 and 0.7 have the highest frequency of occurrance in the population at large, and over 90% of the lines tested have $\bar{A}$ values between 0.6 and 0.9. The overall average value of $\bar{A}$ for the population is 0.75, and it should be noted that for each line tested, a definite aversion to malathion was seen, as shown by the fact that all values of $\bar{A}$ are above 0.5, hence none of the lines were ~~indifferent~~ ($\bar{A}$ = 0.5) or attracted to ($\bar{A}$ less than 0.5) malathion. All differences between groups represent differences in the strength of this common avoidance response.

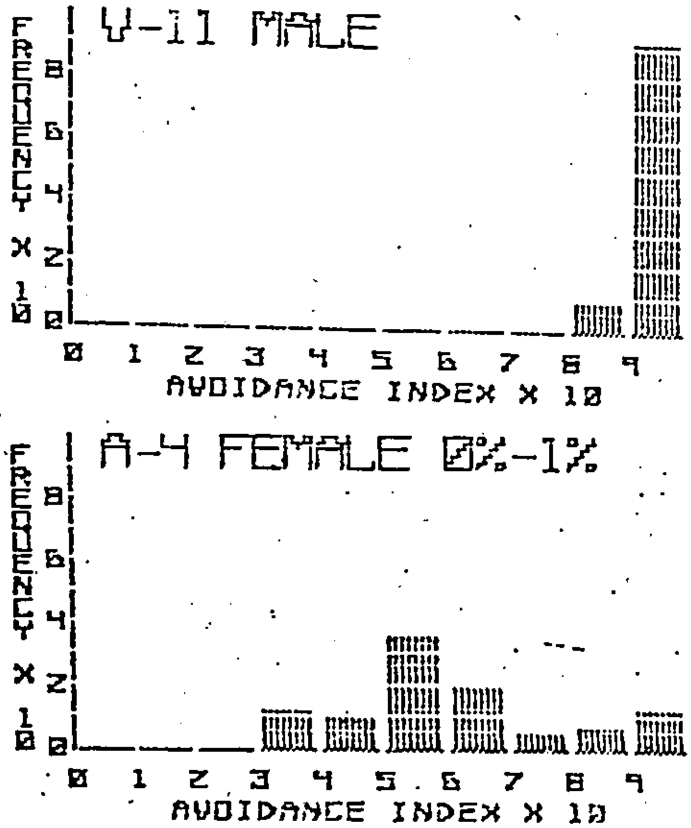


Fig. 3.1.4 avoidance index distributions for lines of high and low avoidance strengths.

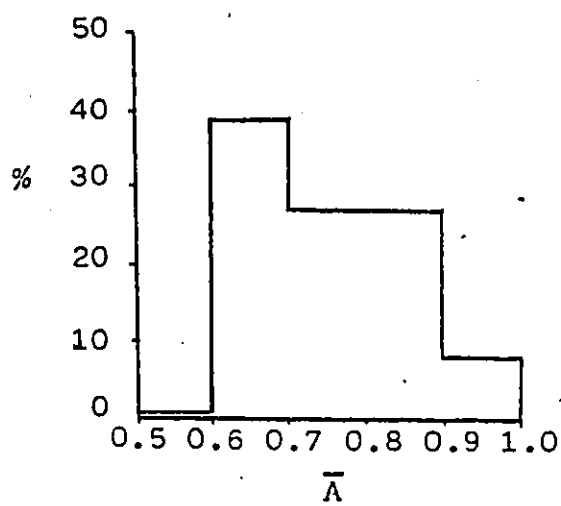


Fig. 3.1.5 distribution of $\bar{A}$ values within the wild population lines.

TABLE 3. Groupings by avoidance of wild population lines (compared via means test).

Compared	d.f.	F	Average $\bar{A}$
V-11, A-15-Rm, B-2	4,10.1	0.81	0.92
B-4f, B-7, B-8, V-3, B-10	8,29.6	0.62	0.87
A-4m, B-4m, J-11m, V-31m, A-10f	4,10.2	0.66	0.81
A-7m, B-17f, J-11f, V-31f, A-10f	4,10.2	3.01	0.77
A-15-Rf, A-6, B-16	4,10.2	0.36	0.73
A-5, A-11, A-16, A-17, J-8, J-19, A-7f, A-12f, B-17 m	14,79.4	1.78	0.68
A-8, A-9, A-12, B-13, J-8, A-10m, A-4f	10,51	1.26	0.63
		Overall	0.75

m = male, f=female

Controls - in order to ensure that the differences in avoidance index values seen between the various groups were due to specific responses to the insecticide and not the effects of testing itself, control runs were done in which no choices were offered to the flies. Both of the end papers in the chambers were treated with either the 0% or 1% solution, with these runs being referred to as (0%-0%) and (1%-1%) runs respectively. (Runs in which a choice was offered are sometimes referred to as 0%-1% runs in the text.) As mentioned in the methods, the results for such runs were converted into values of a control index in which the numerator is the number of flies seen on the paper on the left side of the chamber.

The raw data for the control runs is given in Appendix E, and using these results, analysis of variance testing gave a $F_{(15,844)}$ value of 1.19, (p greater than 0.1) for the (1%-1%) runs, and $F_{(7,503)} = 1.1$, (p greater than 0.1) for the (0%-0%) runs comparing all sexes and strains together. No significant inter-sex or -strain differences were found, and the average control index value over all runs was 0.50 (s.d. = 0.03) indicating that the differences in the avoidance index values seen are specific to the behaviour of the flies in the choice runs (0%-1%) and cannot be attributed to biases inherent in the testing apparatus, since such biases would show up through deviations in the mean control index values from the value of 0.5 expected by random chance.

Intersex correlations - comparisons between the resistances (KT_{50} for a dose of 2% malathion) and $\overline{A}$ values for males and females were made (see graphs in Figure 3.1.6). Both cases gave a significant correlation; for resistance r=0.91, df=6; for avoidance r=0.68, df=24, and in both cases p is less than 0.001 that r=0.

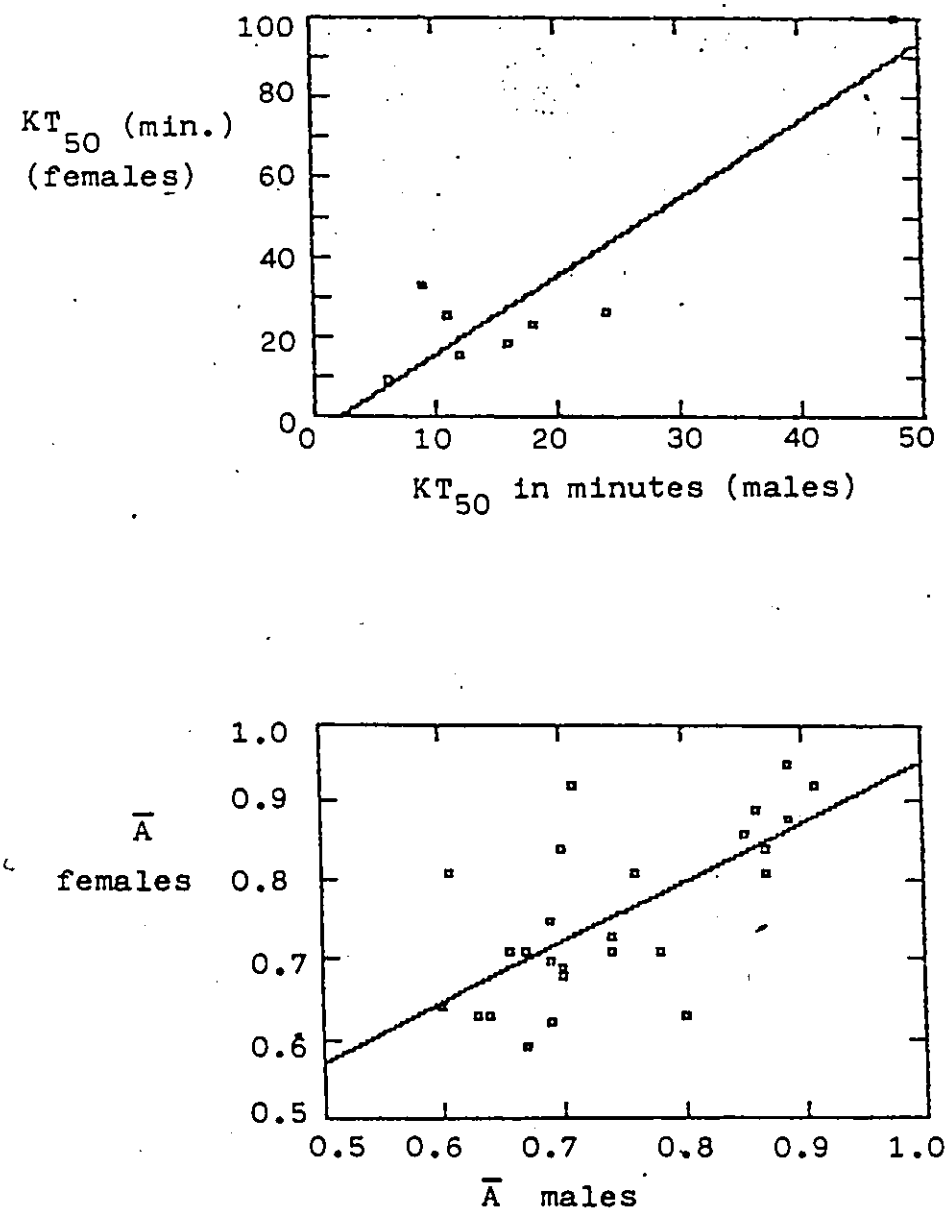


Fig. 3.1.6 plots used for intersex resistance and avoidance comparisons.

III - Results of crosses: to check for major gene effects upon responses to malathion in the wild population lines, a series of crosses was done as follows:

Cross 1: A-11 male X J-11 female; Cross 2: reciprocal
Cross 3: A-11 male X A-10 female; Cross 4: reciprocal
Cross 5:A-15R male X A-11 female; Cross 6: reciprocal

10 flies from each parental line were used in the crosses.

Line A-11 was chosen as a common parent because it was found to be the least resistant of the wild lines, while the other lines were chosen because their resistance or avoidance results showed a marked difference to A-11. Line A-15-R was also used because it was suspected of carrying a major gene for resistance, since it was isolated from its parental line as a resistant sub-population.

i) Resistance: the raw data from resistance testing of the progeny of the crosses is given in Appendix F, and these results are summarized in Table 4, which gives results for the parents, F_1 and F_2 generations of each cross. The KT_{50} values (1% malathion dose) for the F_1 and F_2 from crosses 1 to 4 are all similar to those for the original parents from line A-11, and neither the F_2 KT_{50} values nor their knockdown curves indicate split-populations for resistance, thus there do not appear to be any major genes affecting resistance segregating within lines A-11, A-10 and J-11.

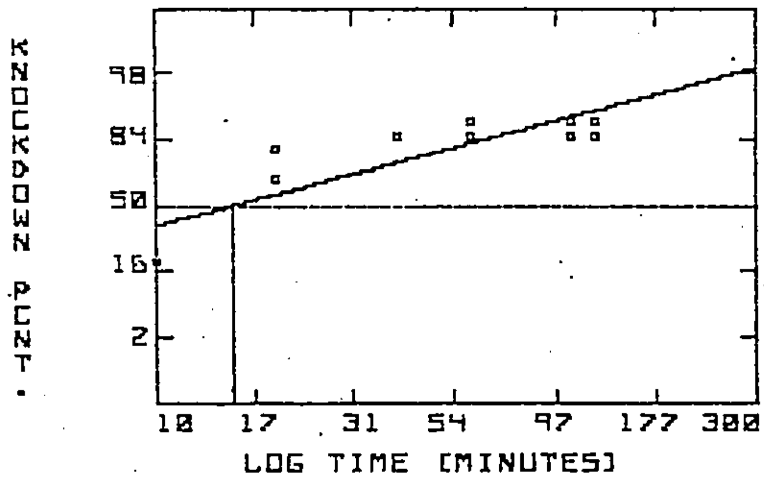
The knockdown results for the progeny from cross 5 and its reciprocal cross 6, however, do indicate the presence of a major gene for resistance. In the F_2 generation, the KT_{50} values are greatly elevated over the values for the F_1, and the knockdown curves (Figures 3.1.7 and 3.1.8) show that the F_2 populations are split with respect to resistance. If fact some of the F_2 flies from these crosses survived resistance testing at the 1% malathion

TABLE 4. Results of resistance and avoidance testing of cross progeny.

Cross	Parents		KT_{50} (minutes)				$\bar{A}$		
	Male	Female	F_1	F_2	Male Parent	Female Parent	F_1	Male Parent	Female Parent
1 Male	A-11	J-11	13	9	8	33	0.74	0.71	0.77
Female			11	14			0.78		
2 Male	J-11	A-11	16	8	35	12	0.65	0.83	0.66
Female			14	9			0.69		
3 Male	A-11	A-10	8	10	8	33	0.59	0.71	0.80
Female			8	11			0.64		
4 Male	A-10	A-11	10	10	14	12	0.59	0.63	0.66
Female			9	10			0.58		
5 Male	A-15R	A-11	14	16	-	12	0.59	0.92	0.66
Female			13	30			0.55		
6 Male	A-11	A-15R	12	44	8	-	0.55	0.71	0.71
Female			25	31			0.57		

CROSS 5 F2 MALE 1%

KT-50 = 15.6213209 +/- 3.17107214 (SE)



CROSS 5 F2 FEMALE 1% VAC

KT-50 = 29.8342967 +/- 2.85829924 (SE)

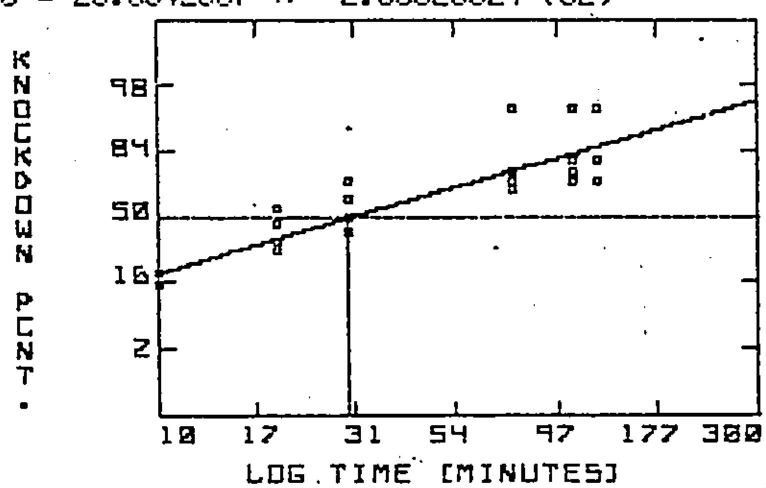
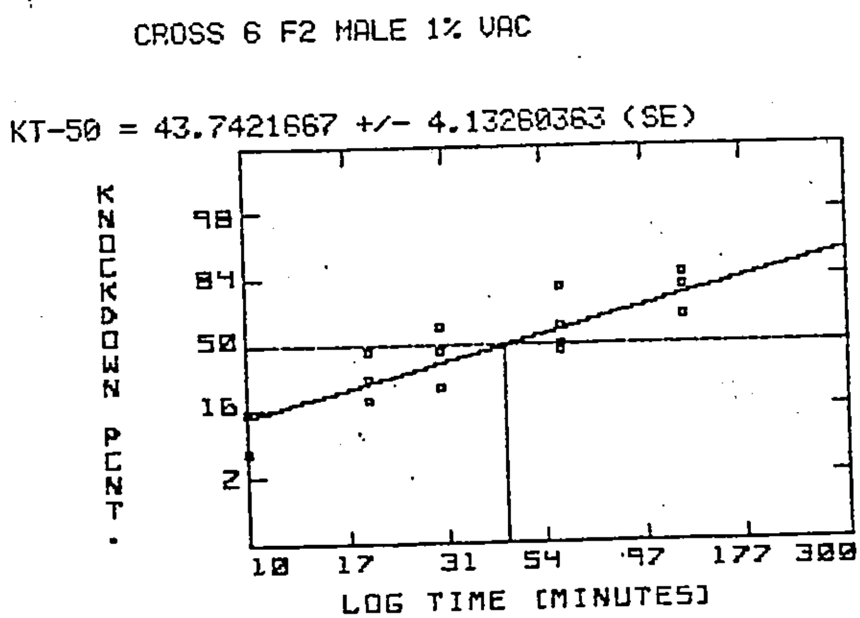


Fig. 3.1.7 knockdown curves for F_2 generation flies from cross of A-15-R male with A-11 female.



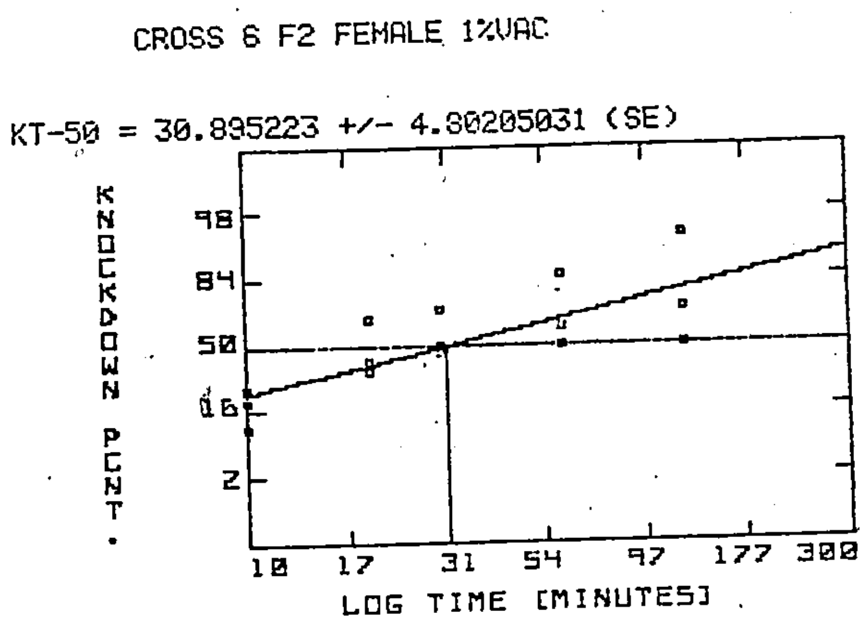


Fig. 3.1.8 knockdown curves for flies from F_2 generation of cross between A-11 male and A-15-R female.

dose, as do the A-15-R flies, and these survivors were used to raise an F_3 generation, which when tested with the 1% malathion dose gave approximately 10% total knock-down, indicating a level of resistance comparable to that posessed by A-15-R line flies.

Of a total of 260 F_2 generation flies from crosses 5 and 6 tested for resistance with a dose of 1% malathion, 58 were alive 120 minutes post-injection and survived to be cultured. The ratio of survivors to total flies tested is 0.22, which is not significantly different from the ratio of 0.25 (chi-square = 0.52, p greater than 0.5) which is expected if the survivors were homozygous for an autosomal recessive gene carried by the parental A-15-R flies.

ii) Avoidance: since the avoidance assay used in this study is not appropriate for detecting split populations with respect to behaviour, only the F_1 generations from the crosses were tested for avoidance, along with the F_3 population obtained from crosses 5 and 6.

The raw data from this testing is given in Appendix F, and the results are summarized in Table 4. The results for males and females of crosses 3, 4, 5 and 6 taken as a group do not differ significantly (compared via anova, $F_{(7,851)}=2.05$, p is greater than 0.5), and in each case the F_1 avoidance is significantly less than that of either parent. No F_1 group showed a significant male versus female difference in avoidance (see Appendix F for results of full comparisons). For cross 1, the F_1 avoidance results do not differ significantly from either parent (F_1 vs. A-11 male: $F_{(2,253)}=2.5$; F_1 vs. J-11 female: $F_{(2,309)}=1.42$; for both p is greater than 0.1), while in cross 2 the F_1 flies do differ from the male parent ($F_{(2,387)}=44$, p less than 0.01) but not the female parent.

Taken as a group, the F_1 avoidance results show that in those cases where a strongly avoiding tendency is seen in the parents (eg. J-1d and A-15-R males), it does not show up in the cross progeny, who, in several cases (crosses 3, 4, 5, and 6) show less avoidance than either parent.

As described in the preceding section, the F_3 generation from crosses 5 and 6 was obtained from the survivors of the resistance testing of the F_2 flies at a dose of 1% malathion. These F_3 flies were tested for avoidance with the following results being obtained: for males $\bar{A}$=0.48, s.d.=0.20; for females $\bar{A}$=0.58, s.d.= 0.17. Since the F_3 population is presumably made up of individuals all of whom are homozygous for the major resistance gene carried by line A-15-R, if this gene is also responsible for the high $\bar{A}$ value seen in the males of this line, this behaviour should also be seen in the F_3 flies. It was not in fact seen, and the low $\bar{A}$ values of the F_3 flies show that the resistance gene is not responsible for the strong avoidance seen in A-15-R flies.

PART 2 - Mutant Lines

I- Resistance Testing: the results of the mutant screening procedure are given in Appendix G, and the raw data from resistance testing of the lines chosen for further study can be found in Appendix H. Of the more than 100 lines tested, 3 were found to carry X-linked mutations which caused them to differ significantly from Canton S with respect to their malathion resistance.

I have chosen to designate these lines as trm mutants, for toxin response - malathion, and there are, as already mentioned, three of them: trm-59cs, trm-12cs, and trm-151cs. The numbers denote the original attached-X line, while the letters "cs" stand for the parent strain, Canton S. For simplicity, these lines will usually be referred to as 59cs, 12cs, and 151cs respectively in the text.

Dose-response curves for both sexes of the trm lines and Canton S are shown in Figures 3.2.1 and 3.2.2. These curves show the log % applied dose of malathion plotted versus the log of the KT_{50} values obtained via the vacuum injection assay (the sizes of the symbols indicate the standard error). Significant differences between lines were determined by comparing the cumulative distributions of knockdown versus time for each sex and strain with each dose (see Appendix H).

With respect to malathion resistance, all of the trm line flies differ significantly from Canton S. Flies of lines 59cs and 12cs are more resistant, while 151cs flies are more sensitive to malathion. Flies of the mutant lines 59cs and 12cs did not show any significant differences between each other at any dose for either sex, however significant differences between sexes were seen in

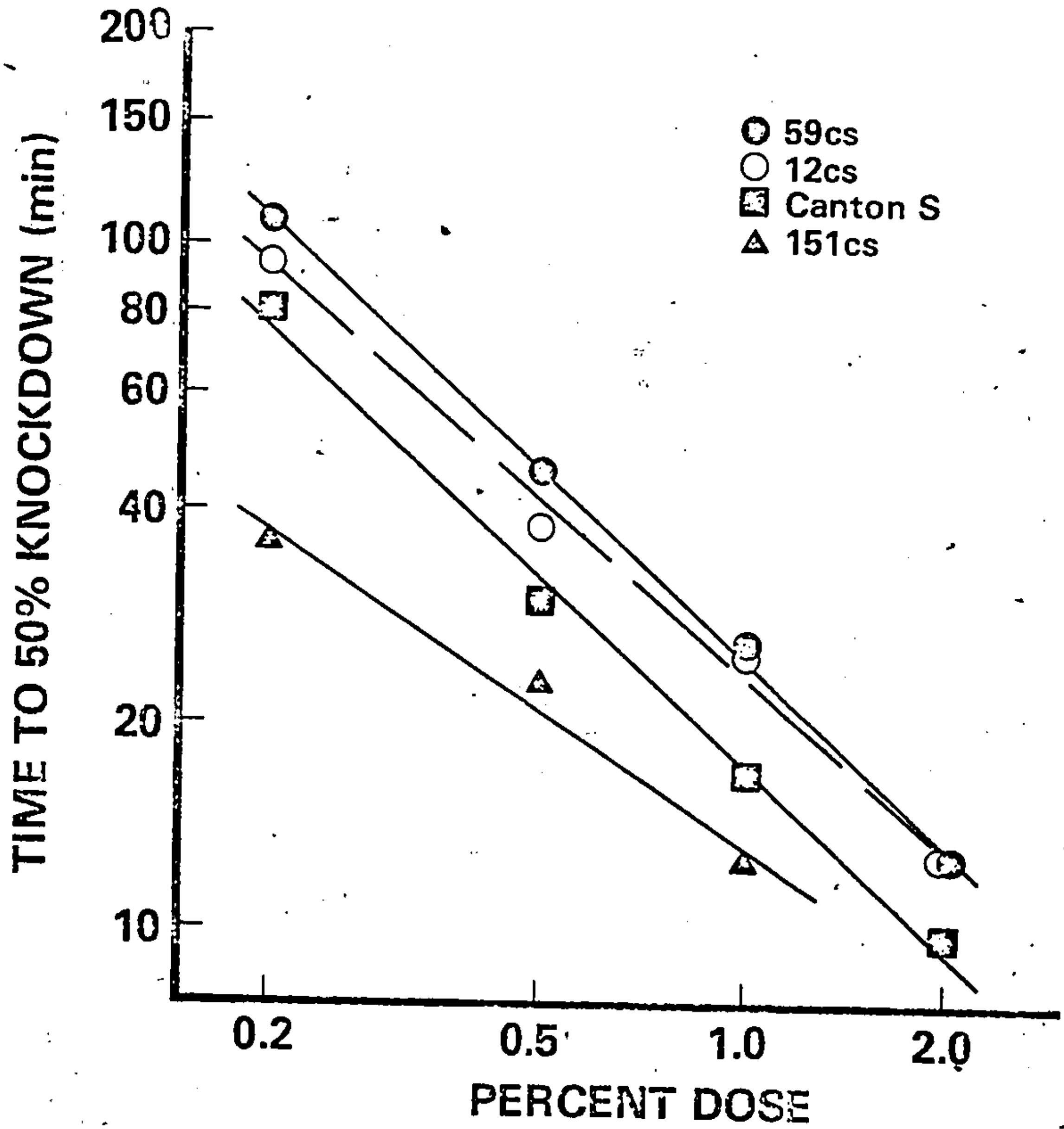


Fig. 3.2.1 dose-response curves for mutant males.

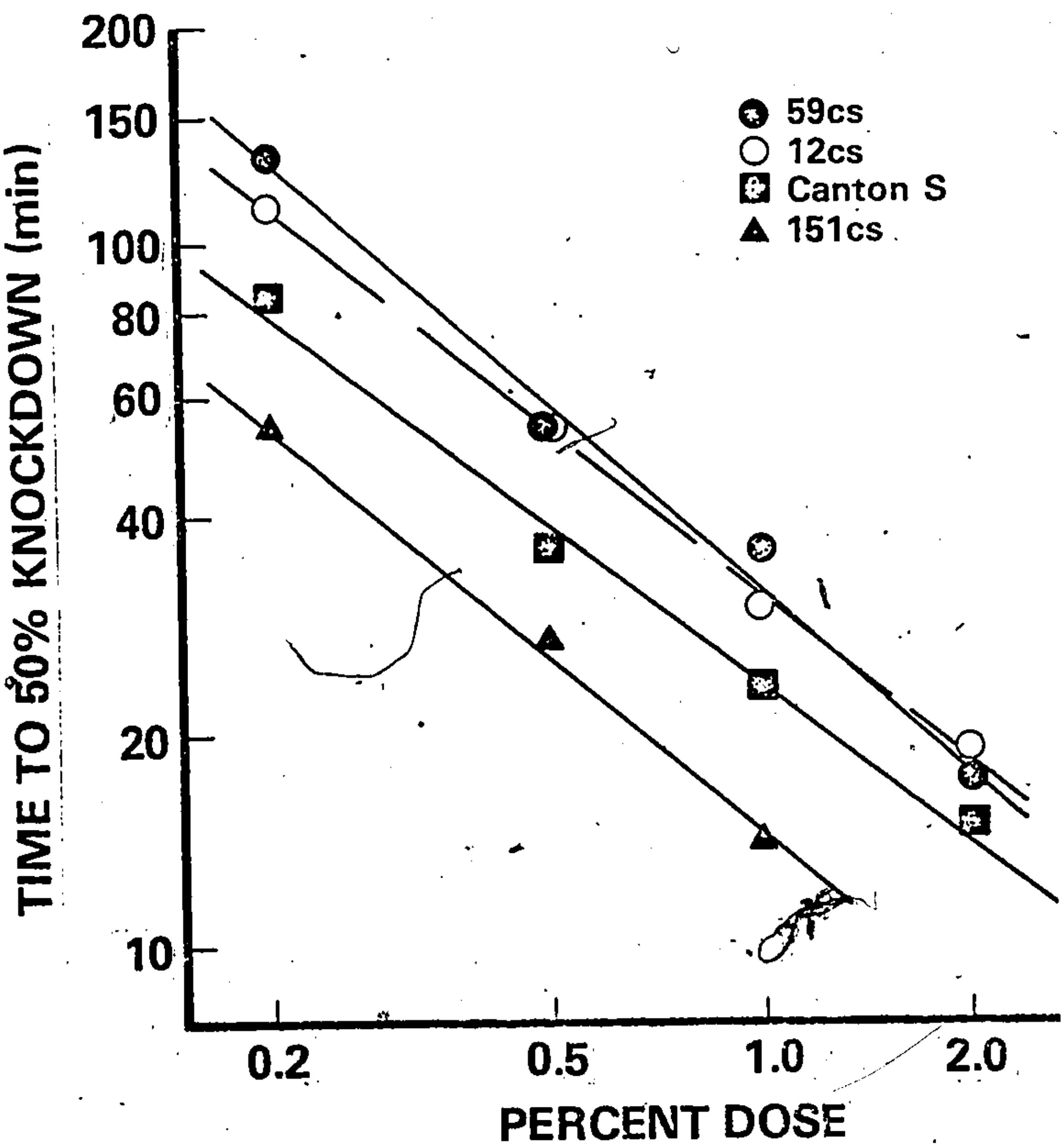


Fig. 3.2.2 dose-response curves for mutant females.

each line (Appendix H). In each the case the female is the more resistant sex. The results of the resistance testing are summarized in Table 5, which lists the KT_{50} values for a dose of 0.5% malathion.

II - Avoidance testing: the raw data from avoidance testing of the mutant lines and Canton S are given in Appendix I. The distributions of the avoidance index for each strain are shown in Figures 3.2.3 (males) and 3.2.4 (females), while the values of the mean avoidance index ($\bar{A}$) are listed in Table 6, along with the results of the control runs, given as mean values of the control index ($\bar{C}$).

The individual run data within each sex and strain were compared for homogeneity via analysis of variance (see Appendix I), and in all cases the data were found to be homogeneous, hence the individual run results were pooled for further comparisons. Since the variances between sexes and among lines were not homogeneous in most instances, further significance tests were done via the means test used in Part A of this section. (The results of Bartlett's test for homogeneity of variances were as follows: for males $\chi^2 = 26.8$, d.f.=3; for females $\chi^2 = 35.3$, d.f.=3; for both p is less than 0.001).

Intersex comparisons gave significant differences for avoidance in each line (all comparisons can be found in Appendix I). Comparisons between lines show that they can be ranked from lowest to greatest avoidance response strength as follows: 59cs, 12cs, Canton S, and 151cs, (see Table 6). The results of significance testing between lines are summarized in Table 7, and these results show that all of the mutant lines differ significantly

TABLE 5. Results of resistance testing of mutant lines and Canton S with 0.5% malathion dose.

Line		KT_{50}(min.)	S.E.
Canton S	male	31	1.8
	female	38	1.4
59cs	male	47	1.9
	female	56	1.8
12cs	male	39	1.5
	female	56	2.4
151cs	male	24	0.8
	female	28	1.2

TABLE 6. Results of avoidance testing.

Line	Female $\bar{A}$	Female s.d.	Male $\bar{A}$	Male s.d.	1%-1% $\bar{C}$	1%-1% s.d.	0%-0% $\bar{C}$	0%-0% s.d.
Canton S	0.78	0.15	0.73	0.18	0.56	0.34	0.48	0.17
59cs	0.65	0.15	0.55	0.18	0.55	0.27	0.47	0.11
12cs	0.69	0.20	0.58	0.22	0.51	0.30	0.52	0.22
151cs	0.81	0.21	0.93	0.08	0.55	0.32	0.48	0.23

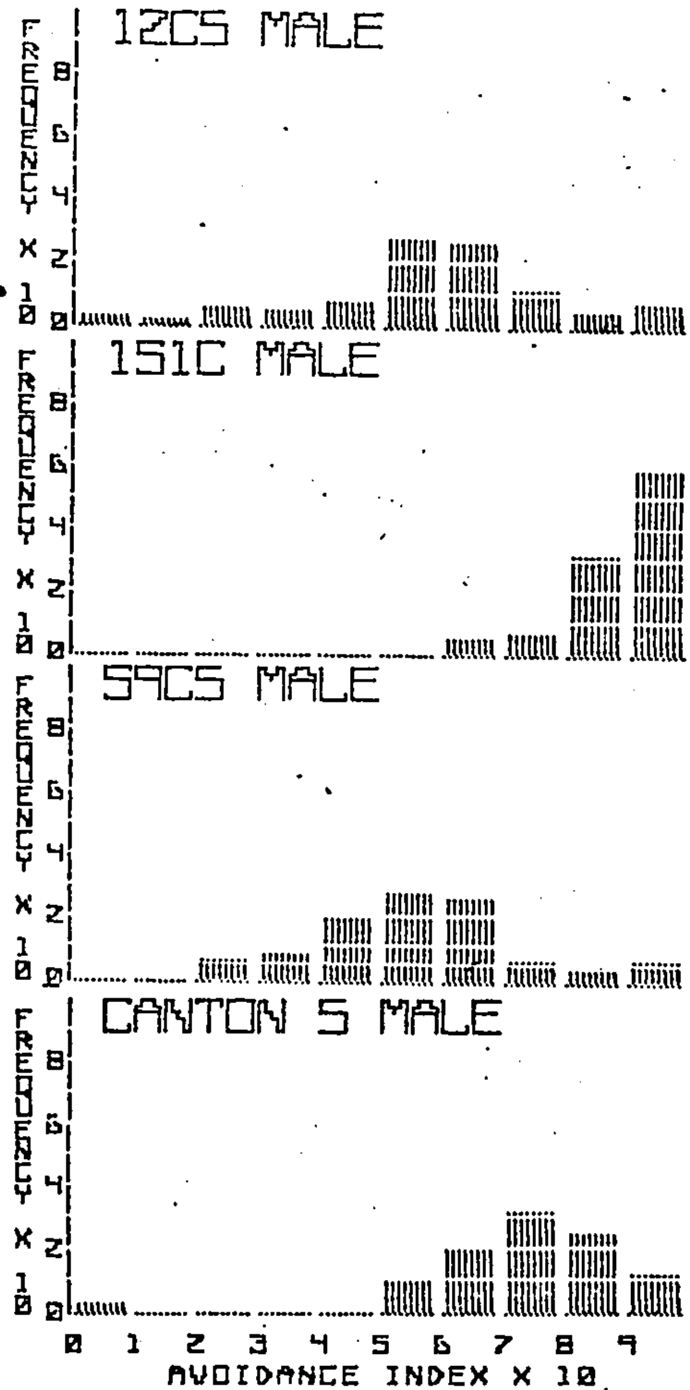


Fig. 3.2.3 avoidance index distributions for males of the mutant lines.

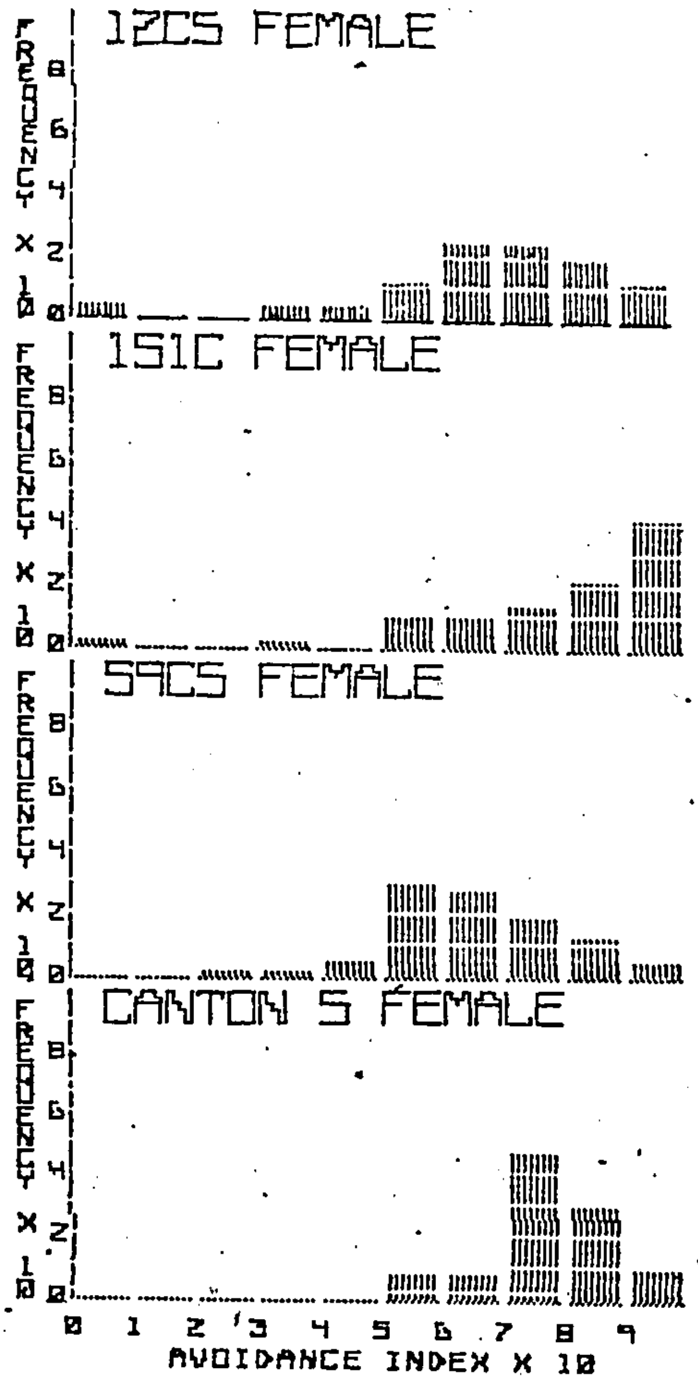


Fig. 3.2.4 avoidance index distributions for females of the mutant lines.

from Canton S for avoidance, except for 151cs females, which do not differ significantly from Canton S females. As was seen with their resistance testing results, flies of lines 59cs and 12cs do not differ significantly for avoidance in either sex.

TABLE 7. Results of significance testing of avoidance results via the means test.

Compared	Males		Females	
	d.f.	F	d.f.	F
59cs, 12cs	1,5.04	0.74	1,5.05	1.6
59cs, CS	1,5.04	30.2*	1,5.05	16.8*
12cs, CS	1,5.05	21.5*	1,5.04	8.0*
151cs, CS	1,5.04	34.1*	1,5.05	0.88

*$p<0.01$

Control runs: the results of analysis of variance comparisons were: (1%-1%) runs from all lines, $F_{(3,120)}=0.548$; (0%-0%) runs from all lines, $F_{(3,244)}=0.49$; all control runs together, $F_{(7,364)}=1.02$. None of the F values are significant, and the mean control index value averaged over all runs is 0.51, which does not differ significantly from the value of 0.5 expected if the flies were showing no surface preference when no choice was given. Thus for these lines, as was also seen in the wild population control results, the avoidance response shown by the flies during choice testing is a specific response to the difference in malathion concentration between the two surfaces.

III - Characterization of mutations: i) Tests for X-linkage. Proof that the trm mutations are in fact X-linked, as the results of screening indicate, was obtained by crossing males of these lines to Canton S females and testing the F_1 males for resistance and avoidance. (Full results are given in Appendix J.)

The avoidance testing results for the F_1 males are summarized in Figure 3.2.5 (avoidance index distributions) and Table 8 below.

TABLE 8. Results of avoidance testing of F_1 males from crosses of trm males to Canton S females.

Male Parent	$\bar{A}$ F_1 male	s.d.
Canton S	0.74	0.16
59cs	0.74	0.19
151cs	0.73	0.18
12cs	0.74	0.21

Compared by anova, the F_1 males show no significant difference in avoidance from each other or Canton S males ($F_{(3,331)}=0.15$, $p>0.2$).

The results from resistance testing of the F_1 males are summarized in Table 9, which lists the KT_{50} values obtained for a dose of 1% malathion administered via vacuum injection. The result listed for males from a cross of MRM-1 male to Canton S female was included as an example of the type of result expected for an autosomal dominant resistance mutation, which MRM-1 is. None of the trm line F_1 males differ significantly from CS males.

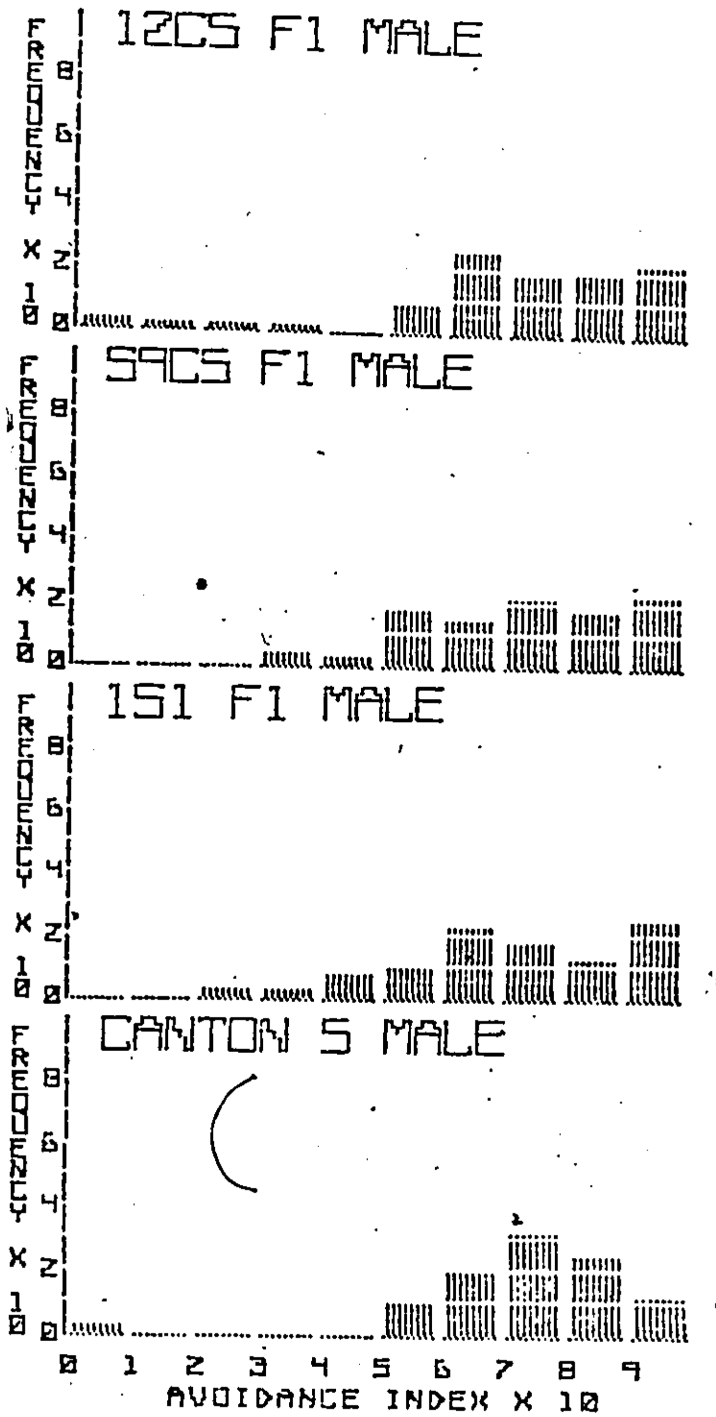


Fig. 3.2.5 avoidance index distributions for males from crosses of mutant males to CS females

TABLE 9. Results of resistance testing of F_1 males from crosses of trm males to Canton S females (1% malathion dose).

Male parent	F_1 KT_{50}(min.)
Canton S	17
59cs	18
12cs	18
151cs	14
MRM-1	32

Both the avoidance and resistance results from the F_1 males of these crosses indicate that the trm mutations are carried on the X-chromosome, since none of the F_1 males share the paternal males' differences from Canton S males, a result explained by the fact that the trm mutations responsible for these differences are on the X-chromosome which was maternally derived in these crosses.

ii) Dominance of mutations: dominance was determined by crossing mutant females to Canton S males and testing the F_1 females for avoidance and resistance. Since these females were heterozygous for the trm mutant and wild-type alleles, the dominance relationship between them will be manifested in the phenotypes of these females. (raw data and comparisons are shown in Appendix J)

The results of avoidance testing of the F_1 females are shown in Figure 3.2.6 (avoidance index distributions) and Table 10. Figure 3.2.7 shows the results

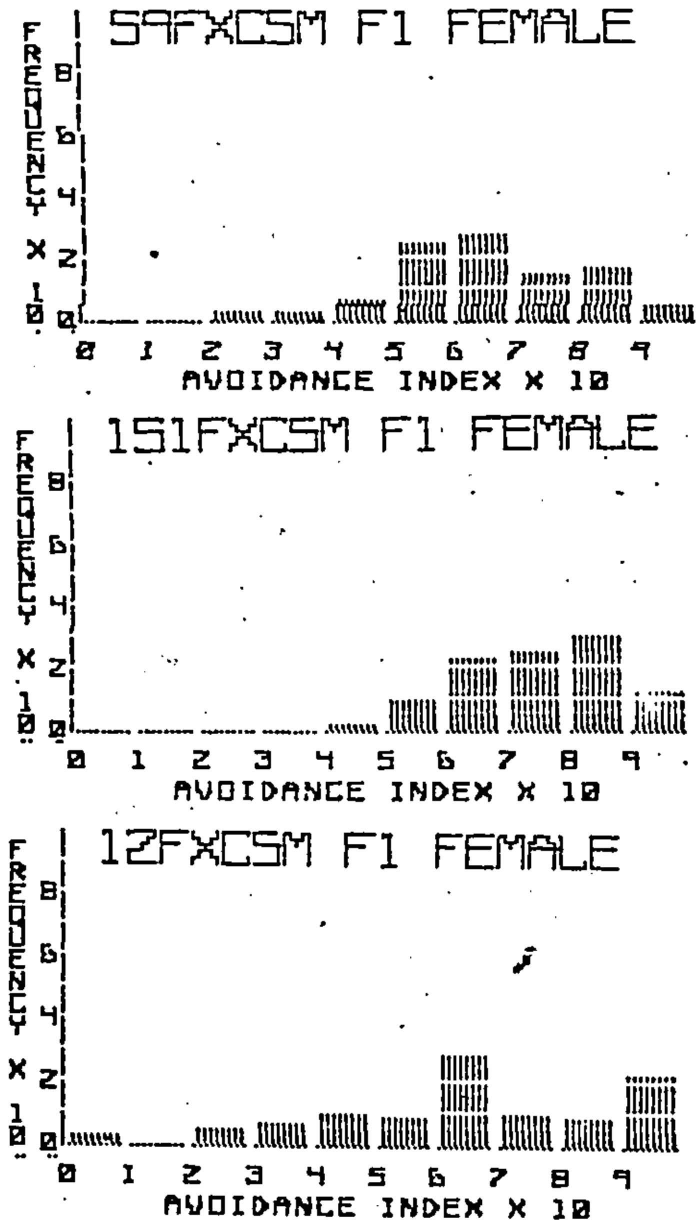


Fig. 3.2.6 avoidance index distributions for female progeny of crosses of mutant females to CS males.

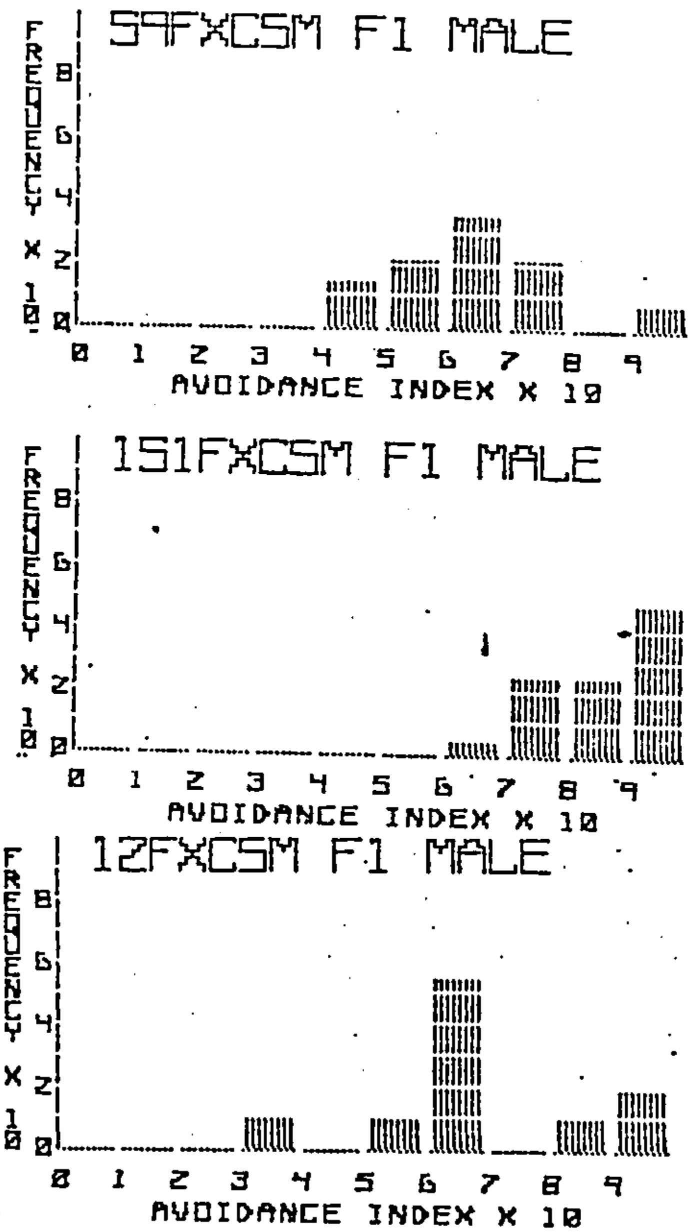


Fig. 3.2.7 avoidance index distributions for males progeny of crosses of mutant females to CS males.

for males from these crosses, none of whom differ significantly in avoidance from males of their parental trm lines (Appendix J).

TABLE 10. Results of avoidance testing and comparisons to parents for F_1 females from crosses of trm females to Canton S males.

Female Parent	$\bar{A}$ Parent	$\bar{A}$ F_1	Parent vs. F_1 d.f.	Parent vs. F_1 F
59cs	0.65	0.63	1,185	0.44
151cs	0.81	0.75	1,71	1.9
12cs	0.69	0.67	1,109	0.12
Canton S	0.78			

Results of anova comparisons between F_1 females and their parental line females are also given in Table 10, and none of these comparisons produced a significant difference ($p>0.1$ in each case). In the case of lines 59cs and 12cs, the F_1 female avoidance results show that these trm mutations are dominant over the wild type alleles with respect to the behavioural response phenotype. Since females of line 151cs do not differ significantly from Canton S females for avoidance, the dominance or recessiveness cannot be determined from the avoidance results for the trm-151cs mutation.

The results of resistance testing of the F_1 females (1% malathion dose) are summarized in Table 11. From these results it can be seen that the heterozygous females from lines 59cs and 12cs parents show the same level of resistance as those parents, showing that the trm mutations carried by these lines are dominant for resistance as well as avoidance. The results for the 151cs heterozygotes show that they are more resistant than their female parents, showing the same level of resistance as Canton S females, hence the trm-151cs mutation is recessive to the wild-type allele.

TABLE 11. Results of resistance testing of F_1 females (1% malathion dose).

Female Parent	KT_{50}(min.)	
	Parent	F_1
59cs	38	42
151cs	15	29
12cs	32	42
Canton S	25	

IV - Mutant Mapping: i) Determination of flanking markers - employing the procedure outlined in the Methods, the F_2 recombinant males were obtained from the trm mutant crosses and tested for resistance (testing temperature was 23-24^oC). The raw data can be found in Appendix K, and the results are summarized as follows:

a) trm-12cs: the average knockdown curve for the ++++ phenotype F_2 males is given in Figure 3.2.8, and it does not differ significantly from the results obtained for 12cs at the same dose (1% malathion), in both cases the KT_{50} is 27 minutes. Thus the F_2 males which received the 12cs X chromosome intact from the parental generation have not acquired any other genes from the marker strain that affect resistance to a degree sufficient to be a consideration in explaining the results of resistance testing of the other F_2 males, although there may be effects upon resistance due to the marker loci themselves.

Individual knockdown curves (1% malathion dose) are shown for the ++vf males in Figure 3.2.8, and the +++f male results are in Figure 3.2.9 (not enough +cvvf males were obtained for testing). Of these two recombinant classes, the +++f F_2 males were the only ones to give stepped knockdown curves of the type indicating split populations for resistance (runs number 3 and 4 in Fig. 3.2.9). Thus it was concluded that the trm-12cs mutation is located on the X-chromosome between the marker loci vermillion and forked.

b) trm-59cs: the average knockdown curve for ++++ F_2 males is shown in Figure 3.2.10, and it does not differ significantly from that obtained for 59cs males at a dose of 1% malathion (KT_{50} values are 26 and 27 minutes respectively).

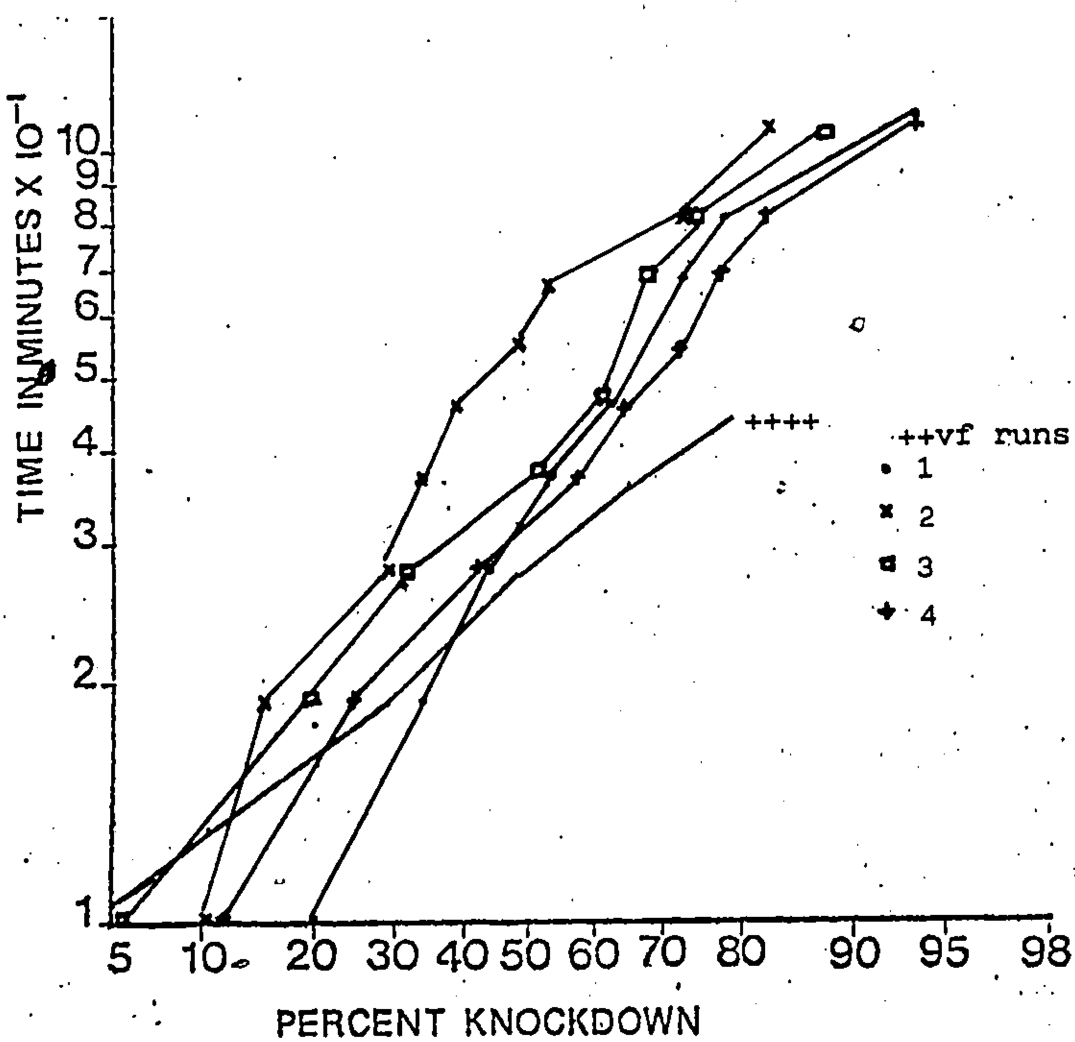


Fig. 3.2.8 individual run knockdown curves for 12cs cross ++vf and ++++ F_2 males - 1% malathion dose.

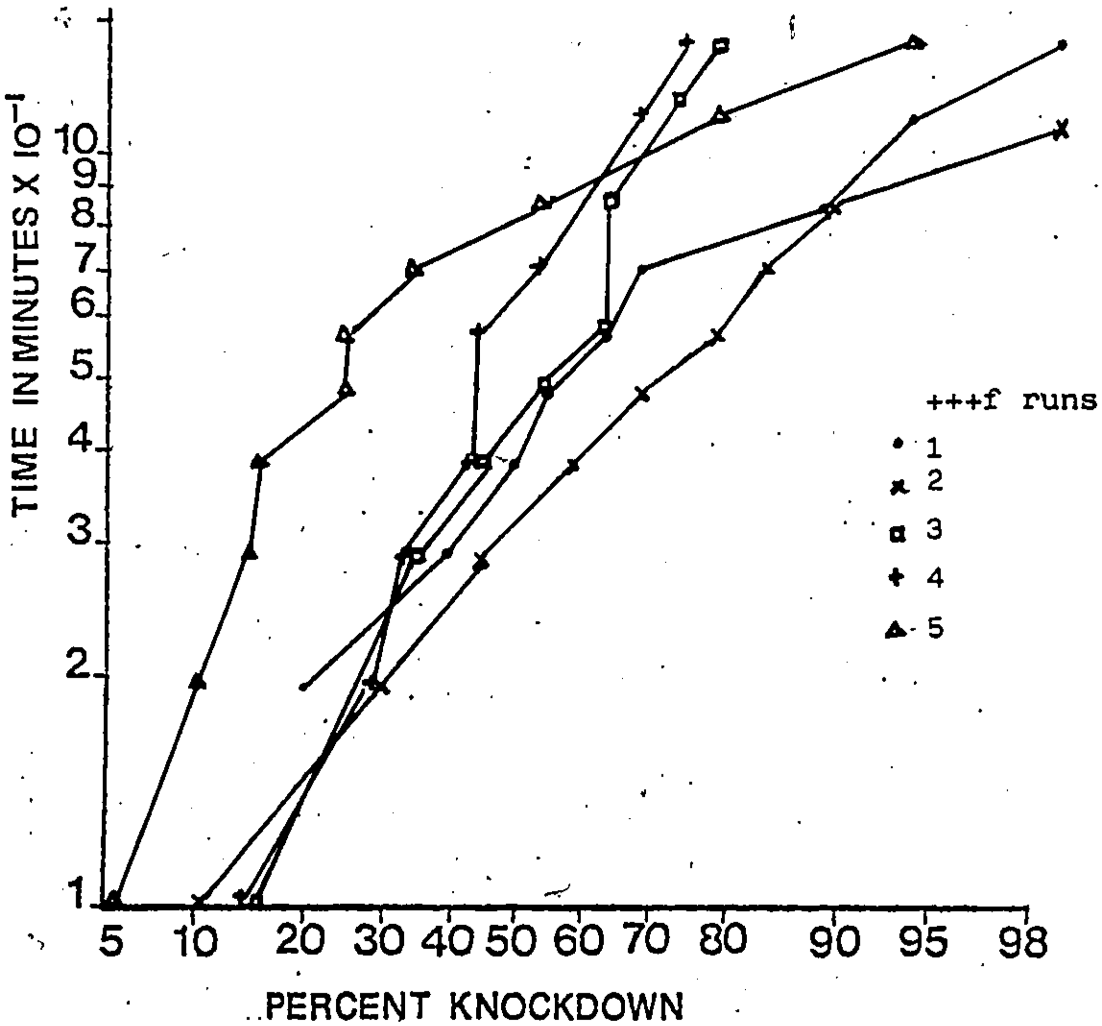


Fig. 3.2.9 knockdown curves for 12cs cross +++f F_2 males - 1% malathion dose.

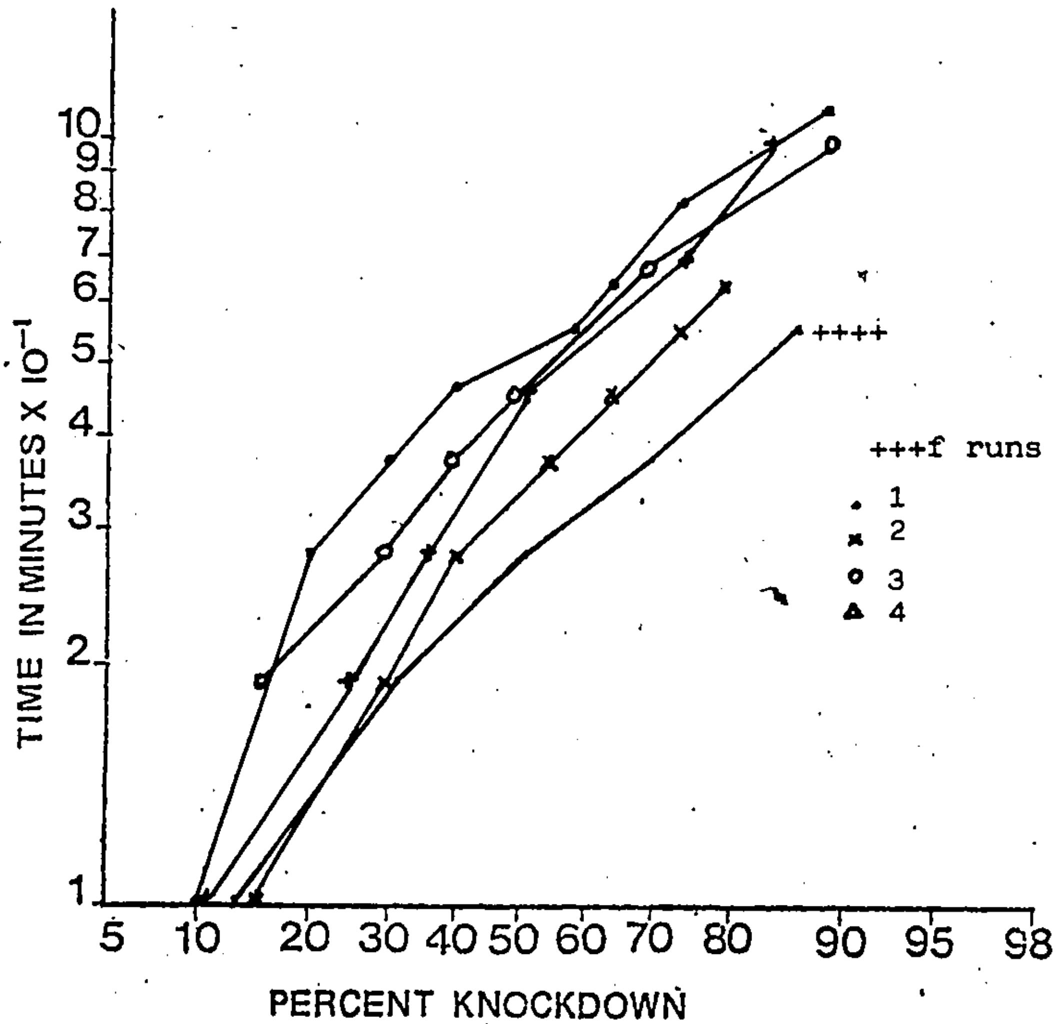


Fig. 3.2.10 individual run knockdown curves for 59cs cross +++f and ++++ F_2 males - 1% malathion dose.

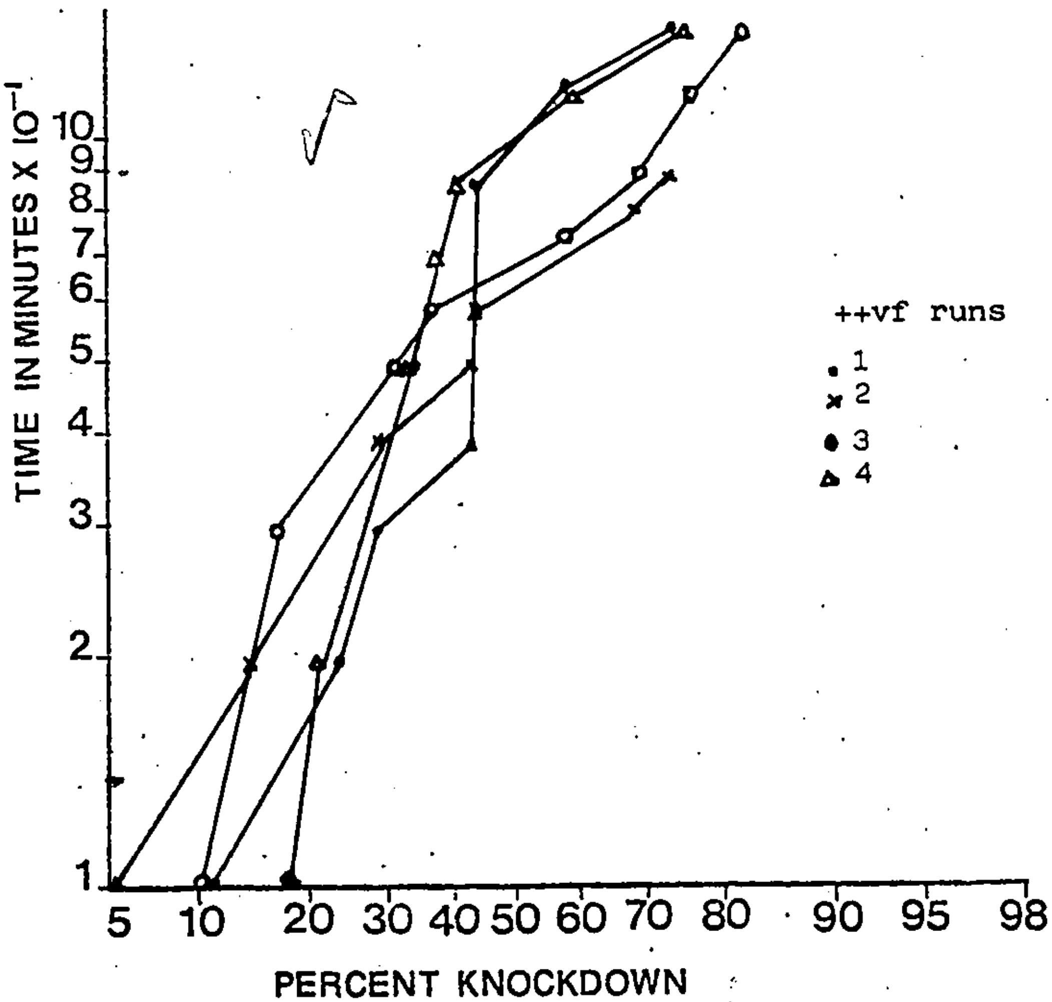


Fig. 3.2.11 knockdown curves for 59cs cross ++vf F_2 males - 1% malathion dose.

The individual run knockdown curves shown in Figures 3.2.10 (+++f F_2 males) and 3.2.11 (++vf F_2 males) for a dose of 1% malathion show that it is only among the ++vf F_2 male results that stepped curves are seen (eg. runs 1 and 2 in Fig. 3.2.11). From these results it was concluded that the trm-59cs mutation is located between the marker loci crossveinless and vermillion on the X-chromosome.

c) trm-151cs: owing to the high sensitivity of 151cs flies to malathion, F_2 males from the 151cs cross were tested first at a dose of 0.5% malathion. The results for this dose are shown in Figures 3.2.12 (++++ and +++f F_2 males) and 3.2.13 (++vf F_2 males). The average knockdown curve shown for the ++++ males does not differ significantly from that obtained for 151cs males at the same dose (KT_{50} values are 22 and 24 minutes respectively).

At a dose of 0.5% malathion, only the +++f F_2 male results show stepped curves (runs 1 and 2 in Fig. 3.2.12). Since the high knockdown times compared to the results for the other mutant crosses may have distorted the results from this cross, the F_2 males were also tested at a dose of 1% malathion, with the knockdown curves shown in Figure 3.2.14.

Again at this dose the ++++ males are not different from their 151cs parents (KT_{50} values are 15 and 13 minutes respectively), and only the +++f F_2 males show stepped knockdown curves (all 3 runs in Fig. 3.2.14).

From these results it was concluded that the trm-151cs mutation is located on the X-chromosome between the markers vermillion and forked.

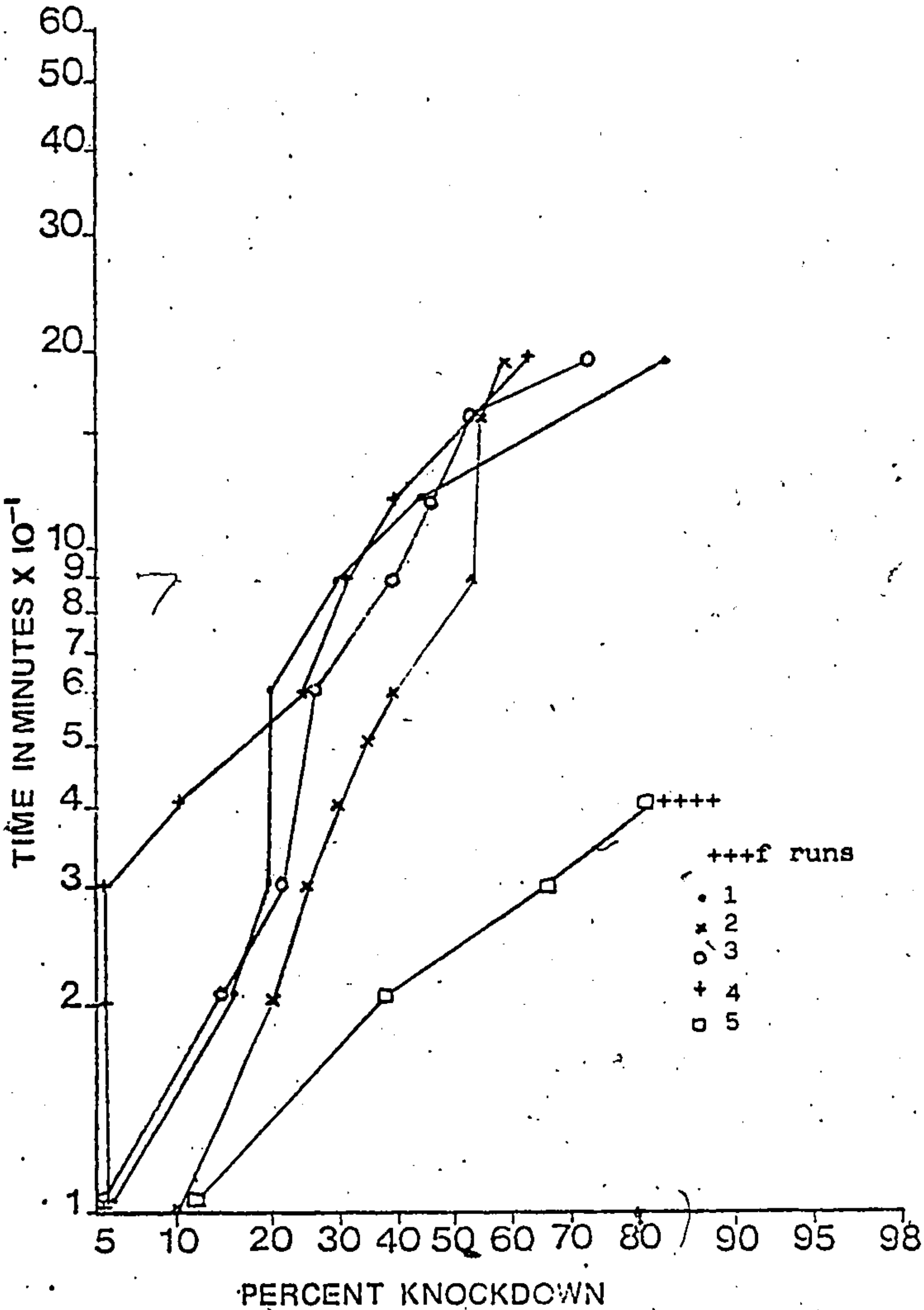


Fig. 3.2.12 knockdown curves for 151cs cross +++f and ++++ F_2 males - 0.5% malathion dose.

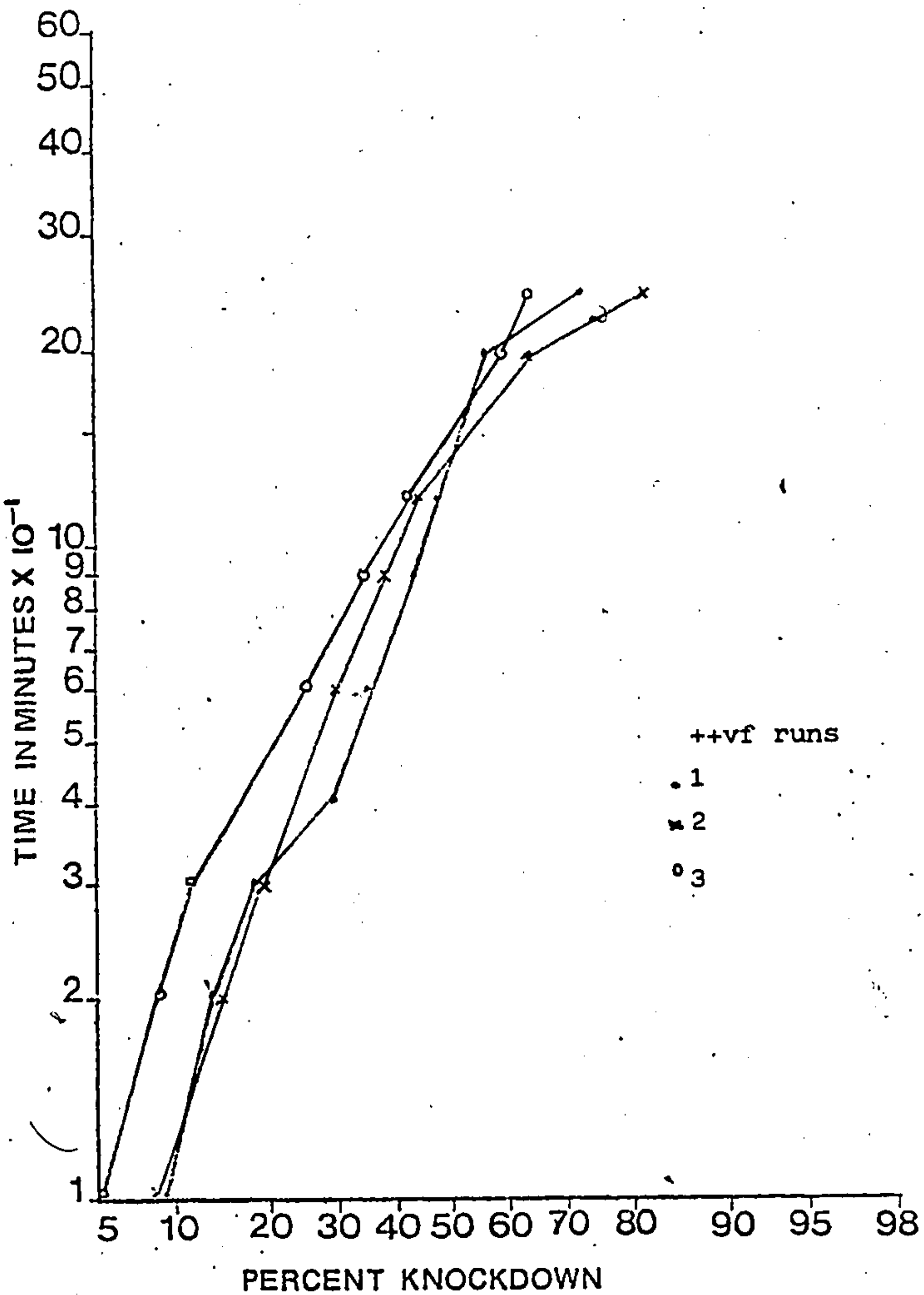


Fig. 3.2.13 knockdown curves for 151cs cross ++vf F_2 males ± 0.5% malathion dose.

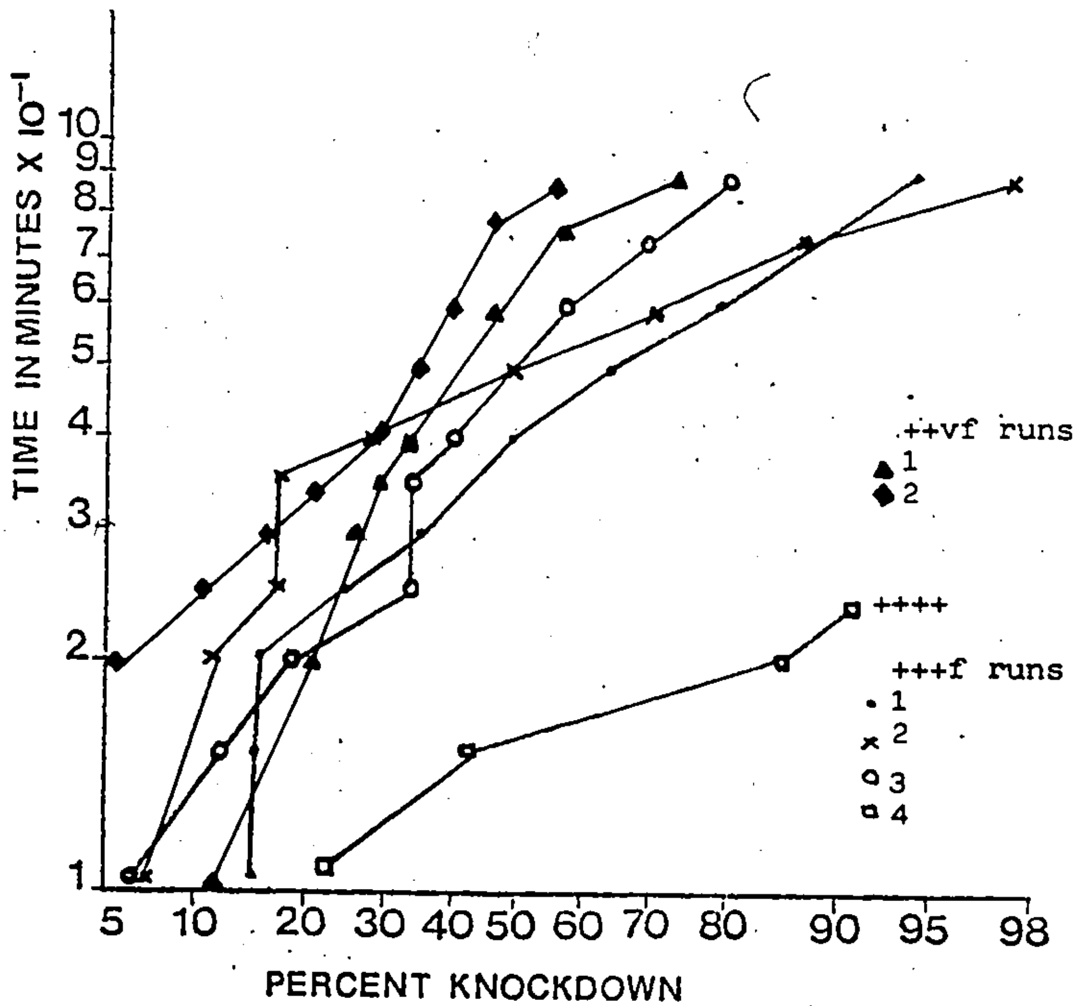


Fig. 3.2.14 knockdown curves for 151cs cross ++++ ++vf and +++f F_2 males - 1% malathion dose.

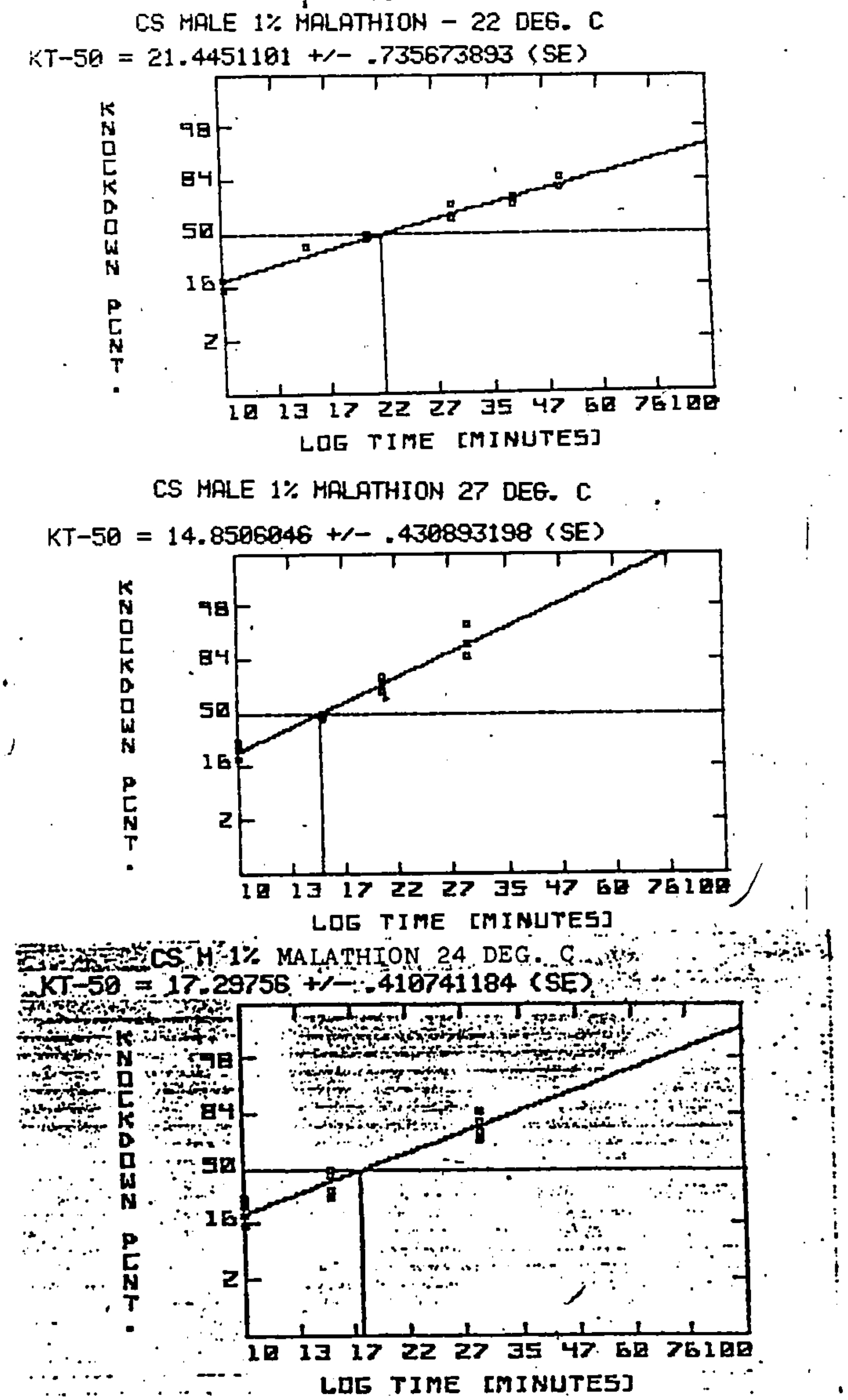


Fig. 3.2.15 effects of temperature on knockdown.

ii) Determination of approximate map locations: as mentioned in the Methods, once it had been determined which class of F_2 male recombinants was a split-population for resistance for each line, males of those classes were crossed to attached-X females to establish lines whose males all carried the X chromosome of their F_2 male parent. In the cases of lines derived from 151cs and 12cs, these F_2 parents were of the +++f phenotype, while for 59cs they were ++vf.

From the average knockdown curves obtained for the F_2 recombinant males, it cannot be determined whether the trm mutations carried by lines 151cs and 12cs increase or decrease the resistance of the +++f F_2 males carrying them, since the curves differ little for males from each of the three crosses (Figure 3.2.16). In the case of line 59cs, however, a comparison of the ++vf male knockdown curves for 12cs and 59cs F_2 males (Figure 3.2.17) suggests that the trm-59cs mutation increases the resistance of those males carrying it (KT_{50} values for 59cs and 12cs ++vf F_2 males are 57 and 39 minutes respectively, the results for 151cs ++vf F_2 males were not used for this comparison since the sample tested at the 1% malathion dose was much smaller than for the other two groups).

Owing to fluctuations in laboratory temperatures during the resistance testing period, the attached-X lines were tested in an incubator kept at temperatures of 26-27°C, which were higher than those used during resistance testing of the F_2 males (23-24°C), hence the knockdown percentages shown in the attached-X line results are generally higher at a given time post injection, due to the effects of these higher temperatures upon knockdown (see Fig. 3.2.15). All raw data for this stage of testing is contained in Appendix K, and the results are summarized as follows:

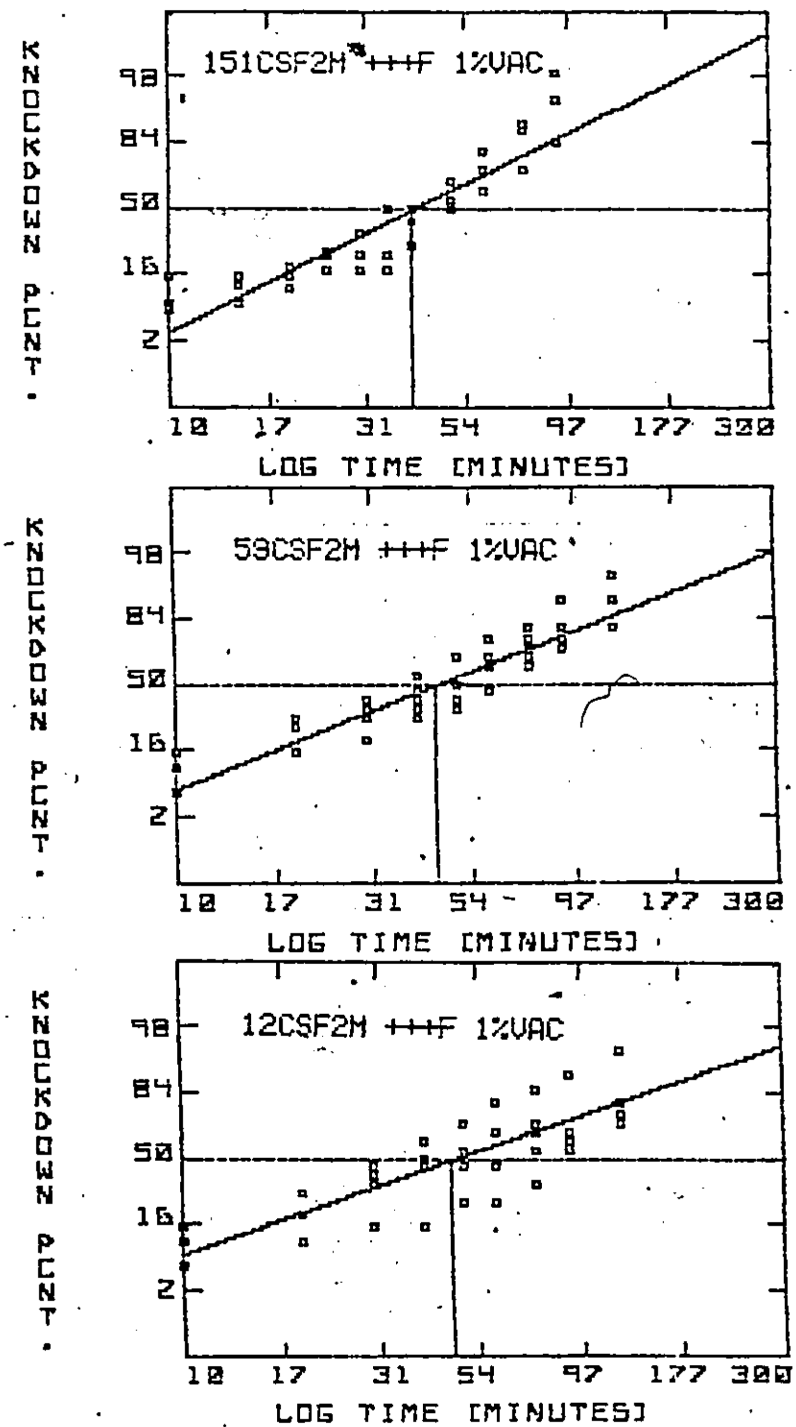
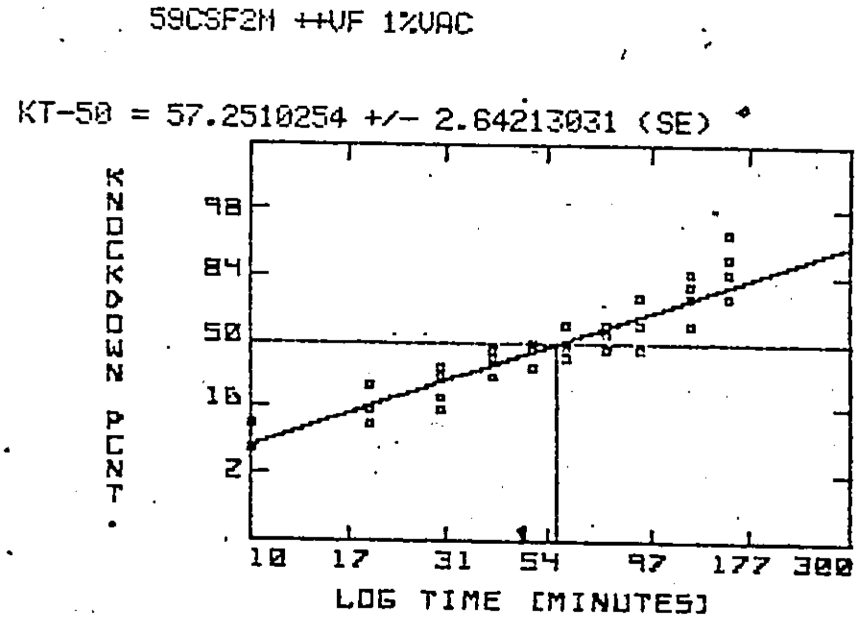


Fig. 3.2.16 knockdown curves for +++f F_2 males from the 3 mutant crosses (1% dose).



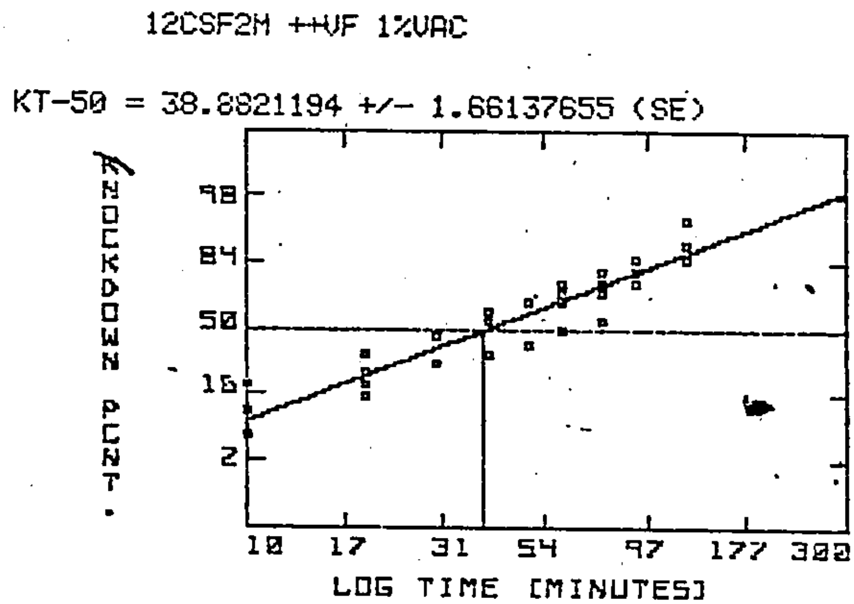


Fig. 3.2.17 knockdown curves for ++vf F_2 males from the 12cs and 59cs crosses - 1% malathion dose.

a) trm-59cs: the attached-X lines derived from ++vf F_2 males from this mutant cross fall into two distinct resistance classes when tested at a dose of 1% malathion. The knockdown curves for these groups are shown in Figure 3.2.18, and the marked differences in resistance are illustrated in the average KT_{50} values for the "sensitive" and resistant groups (28 and 50 minutes respectively. The results of F_2 recombinant male resistance testing, as previously mentioned, indicated that those ++vf F_2 males carrying the trm-59cs mutation are more resistant than those without it, and this conclusion is verified by a comparison of the results for the 59cs ++vf attached-X lines and a ++vf attached-X line derived from the 12cs cross F_2 males (Figure 3.2.18). The 12cs line shows the same level of resistance as the 59cs "sensitive" group lines, and since the 12cs line presumably carries no trm mutation, then neither do the 59cs sensitive lines.

Of 31 attached-X lines tested, 15 were found to be in the "resistant" group, and thus carried the trm-59cs mutation, while the other 16 lines fell into the "sensitive" category. Thus the approximate map location of the trm-59cs mutation can be derived:

$$\frac{15}{31} \times 19.3 \text{ (distance between cv and v)} = 9.3 \text{ map units from v locus}$$

Thus trm-59cs is located at approximately map position 24 on the X-chromosome.

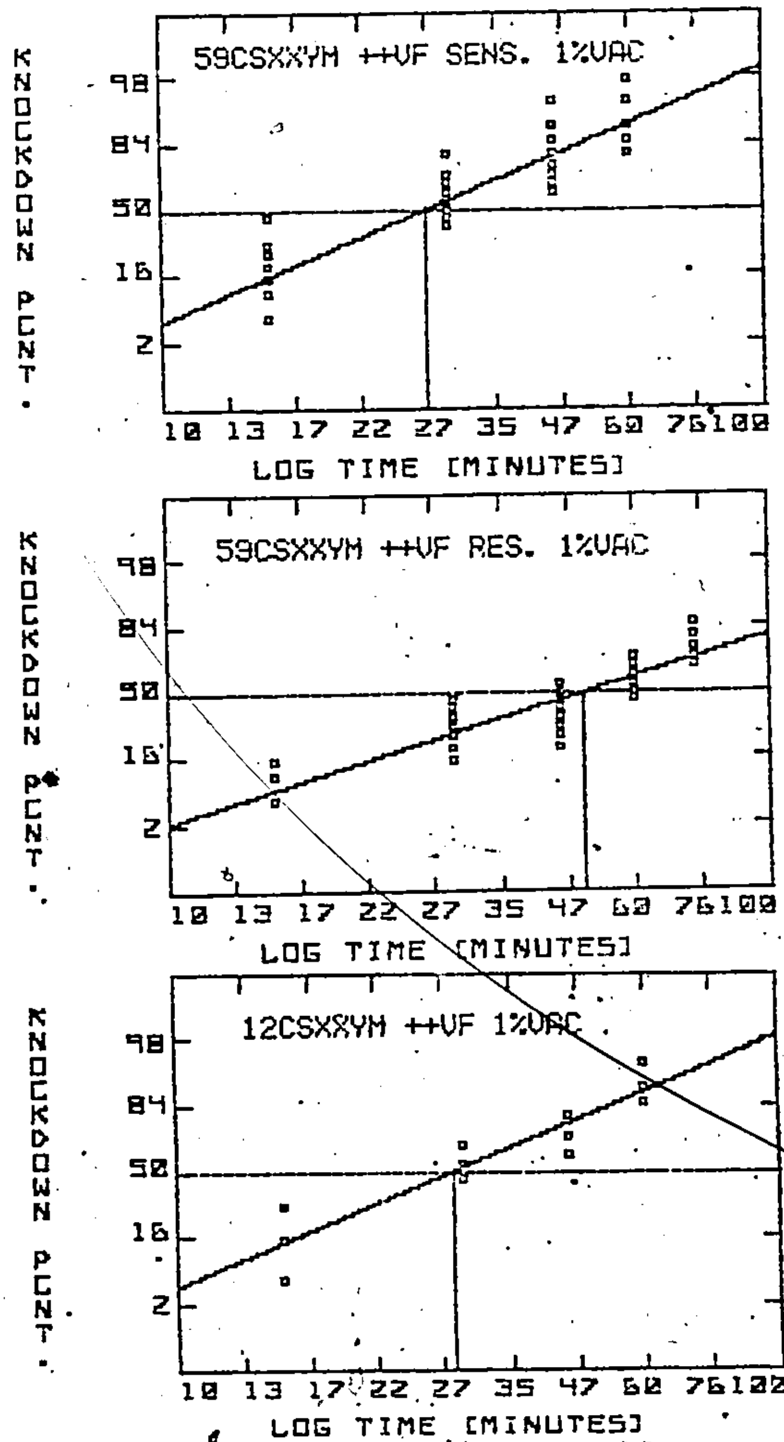


Fig. 3.2.18 knockdown curves for males of attached-X lines indicated.

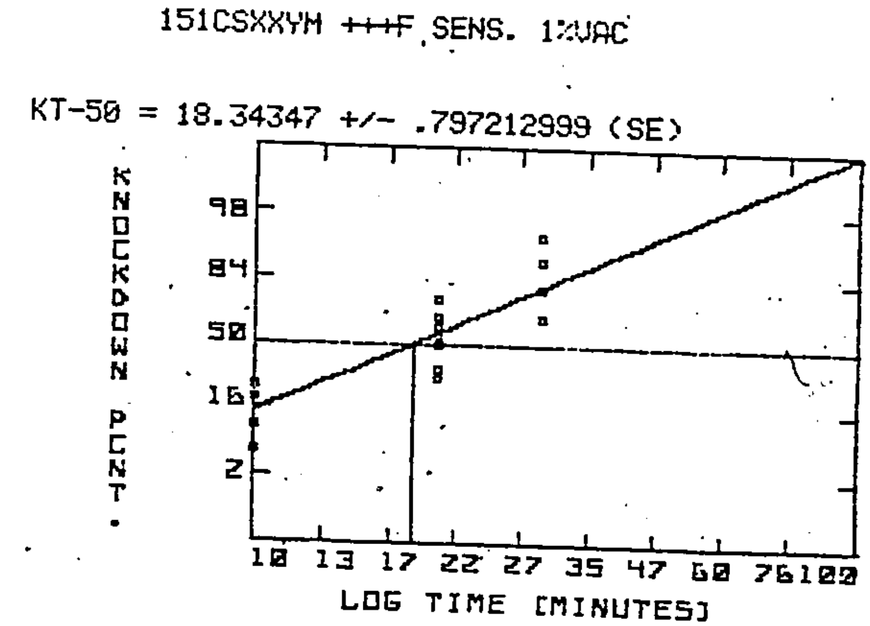
b) trm-151cs: owing to a shortage of +++f F_2 males from this cross, attached-X lines were established using these males and those of the reciprocal recombinant class, ycvv+. In each case the attached-X lines fell into two distinct resistance classes when tested with a dose of 1% malathion, as shown by the knockdown curves in Figures 3.2.19 (+++f lines) and 3.2.20 (ycvv+ lines).

Since, allowing for the effects of temperature differences during testing, the knockdown curve for the "resistant" group of 151cs attached-X +++f line males shows a similar level of resistance to that of the +++f F_2 males from all three mutant crosses (Fig. 3.2.16), it was assumed that this resistant group represents those lines which do not carry the trm-151cs mutation, while the "sensitive" lines do carry it. Using similar reasoning, and comparing the ycvv+ attached-X line knockdown curves to that from ycvv+ F_2 males from this cross (Fig. 3.2.19), the same relationship between sensitive and resistant lines emerges.

Of 30 total attached-X lines tested, 17 were of the +++f phenotype and 13 were ycvv+. Of the 17 +++f lines, 4 were found to be sensitive to malathion, and hence carried the trm-151cs mutation, while of the 13 ycvv+ lines, 7 <u>did not</u> carry this mutation, thus the approximate map location of the mutation is:

$$\frac{4 + 7}{30} \times 23.7 \text{ (dist. from v to f)} = 8.7 \text{ map units from the f locus,}$$

or approximately at map location 48 on the X chromosome.



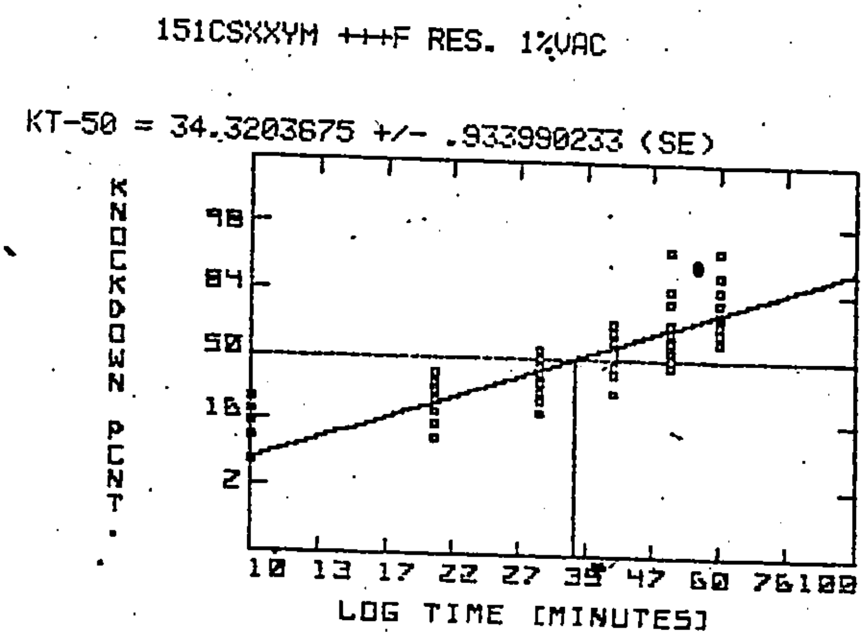


Fig. 3.2.19 knockdown curves for males of attached-X lines indicated.

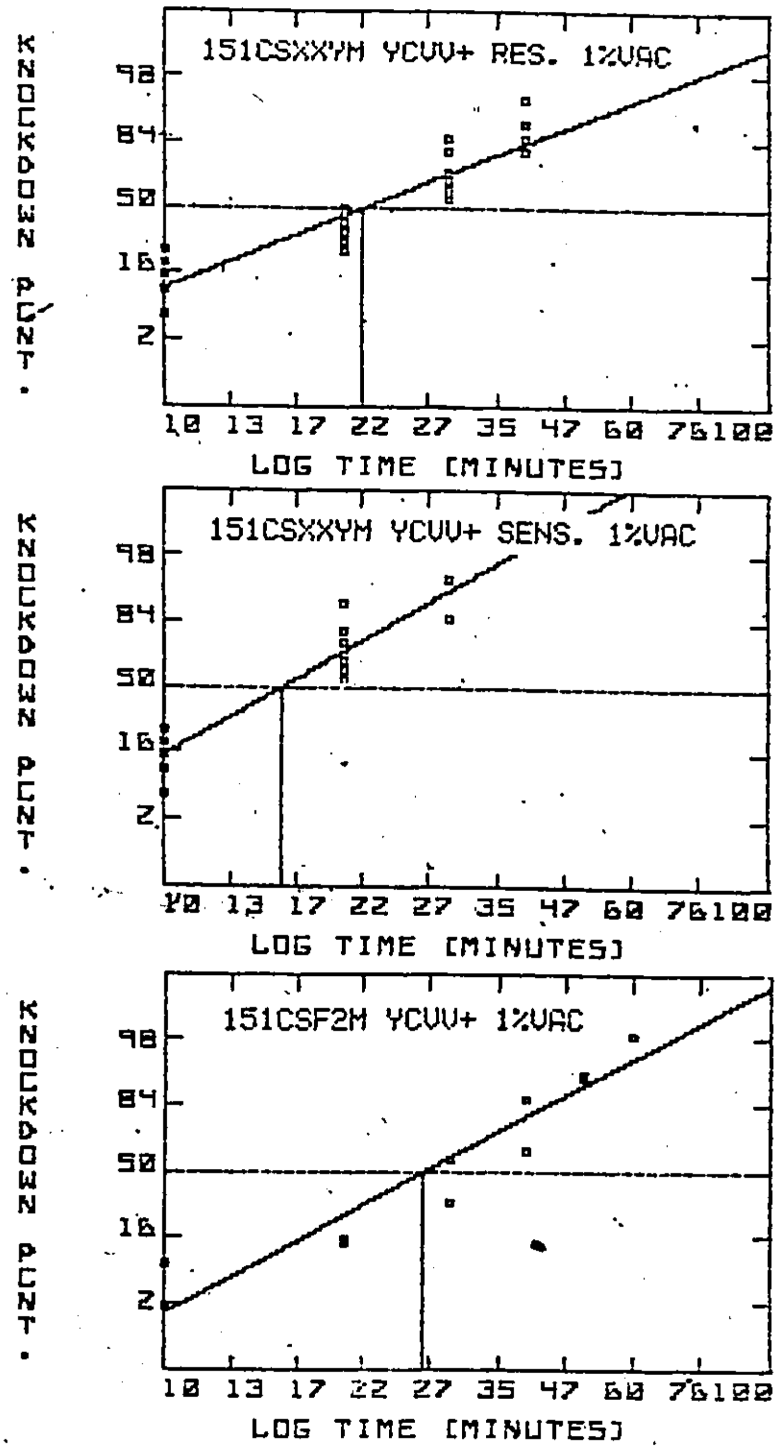


Fig. 3.2.20 knockdown curves for males from 151cs cross F_2 and attached-X ycvv+ groups.

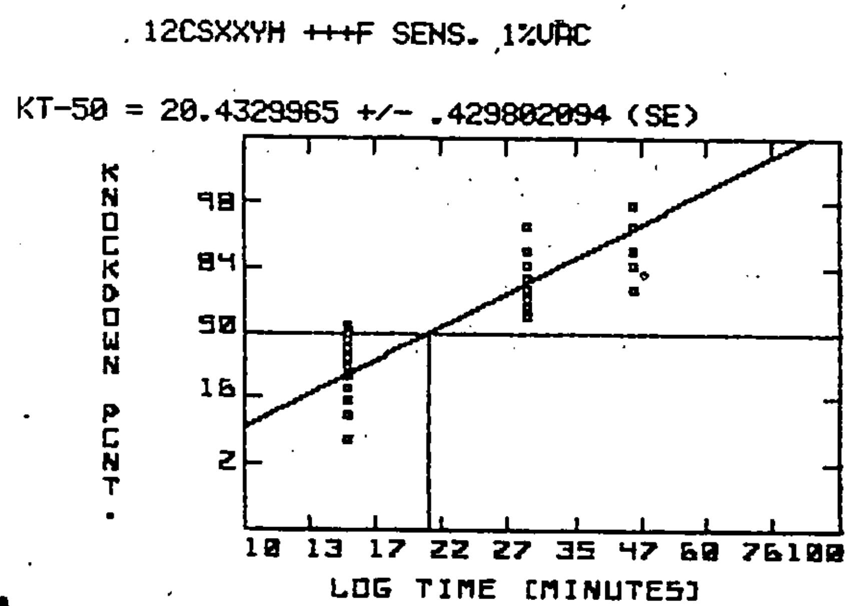
c) trm-12cs: the knockdown curves for the two resistance classes found among the +++f attached-X line males from this cross are shown in Figure 3.2.21. The resistant group shows the same level of resistance as that seen for the +++f resistant group of attached-X line males from the 151cs cross, and hence the 12cs resistant group represents those attached-X lines which do not carry the trm-12cs mutation. Unlike its effect upon resistance in the Canton S strain genetic background, it appears that the trm-12cs mutation causes a decrease in resistance in those +++f males carrying it, as evidenced by the sensitive group of attached-X lines, which presumably all carry the mutation.

This is an unexpected result and merits further discussion. The sensitive group of attached-X lines derived from the 12cs cross F_2 +++f males could have arisen in two ways: 1) the trm-12cs mutation does in fact give rise to lower resistance in +++f males carrying it when they are compared to males carrying the corresponding allele carried by the ycvvf marker chromosome. 2) there is another gene carried by the marker X chromosome which is a different allele than that carried by the 12cs ++++ X chromosome at the same locus, and it is this locus, and not the trm locus, that is responsible for the different resistance classes seen in the +++f F_2 and attached-X line males.

With respect to the second possibility listed above, if another such gene was involved, we would expect to see its effects manifested in the results for the 59cs +++f male F_2 flies in the form of a split-population for resistance as well, but this was not observed. An examination of the +++f F_2 male knockdown curves (1% malathion dose) from crosses with all 3 mutant lines (Fig. 3.2.16) does not show large differences in resistance (KT_{50} values are: 151cs - 40 min.; 59cs - 45 min.; 12cs - 47 min.), however, the fact that some of the 151cs and 12cs F_2 male curves are for split-populations means that the KT_{50} values calculated for them are not likely to be accurate.

In order to get an estimate of the relative resistances of the two resistance classes present among the 12cs +++f F_2 males, the curve for run 2 on Fig. 3.2.9 was assumed to represent the results for a batch of flies mostly of the same genotype, since the knockdown curve is a straight line. This curve is replotted in Figure 3.2.22 and the KT_{50} value is 31 minutes. It is more difficult to arive at an estimate of the resistance of the more resistant sub-population, since the F_2 and attached-X line results show it to be a minority in the F_2 male population. A rough estimate was obtained, however, by taking the upper sections of the knockdown curves for runs 3 and 4 on Fig. 3.2.9 and plotting the points from 60 minutes post injection and up separately, giving an extrapolated KT_{50} value of 54 minutes (Fig. 3.2.22). This value, judging from the results of the split-population test runs described in the mutant mapping section of the Methods, is most likely an under-estimate, since the knockdown percentages used include most or all of the sensitive flies.

These very rough estimates give a general idea of the relative resistances present among the F_2 males, and if we compare the resistant sub-population of 12cs +++f F_2 males to the 59cs +++f F_2 males, all of whom presumably carry the trm-59cs mutation, the latter group shows less



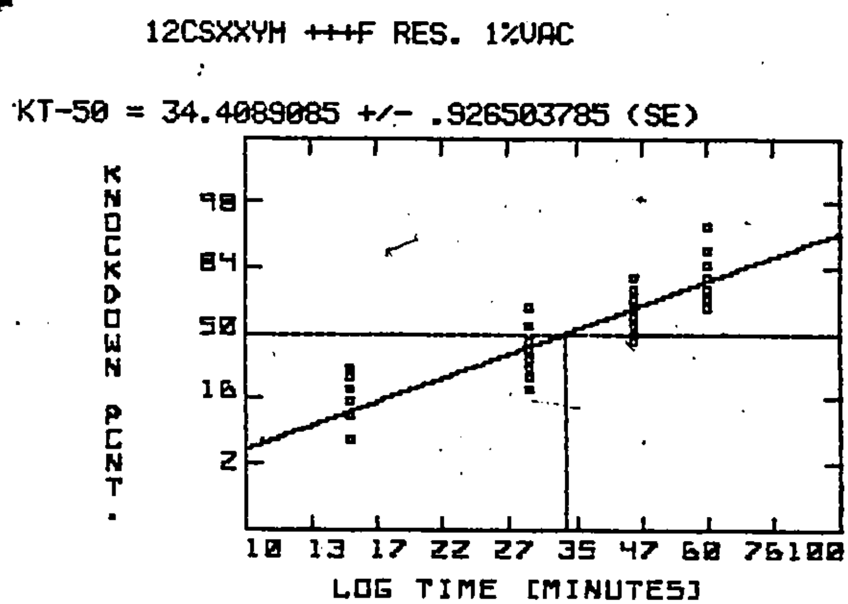
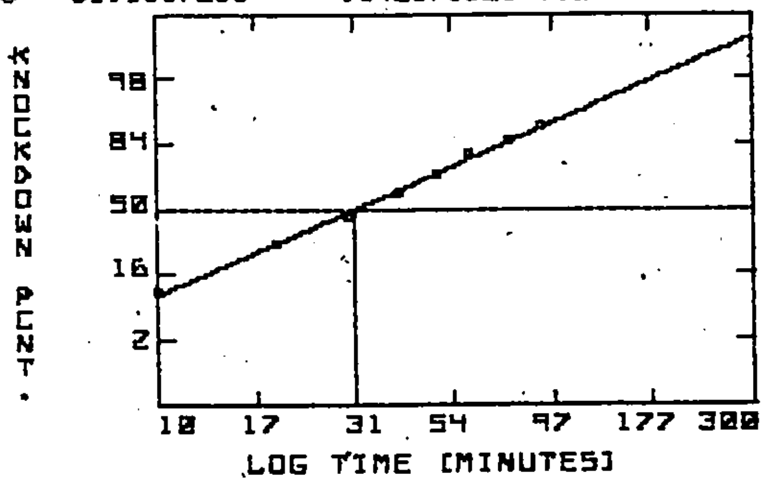


Fig. 3.2.21 knockdown curves for two classes of lines seen among 12cs cross attached-X line males.

12CSF2M +++F RUN 2 1%VAC

KT-50 = 31.1367265 +/- .542576928 (SE)



12CSF2M +++F RUNS 3&4 >60MIN 1% VAC

KT-50 = 53.980227 +/- 5.48645322 (SE)

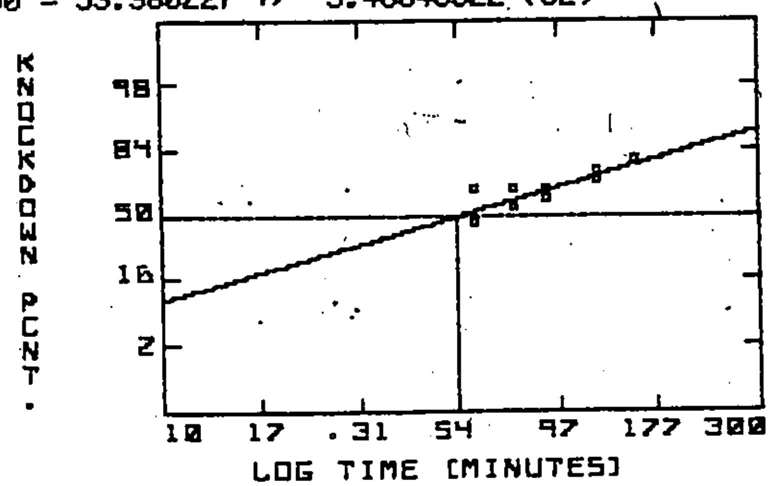


Fig. 3.2.22 knockdown curves for runs indicated of 12cs cross F_2 +++f males.

resistance (Fig. 3.2.16), with a differnce in KT_{50} values of 9 minutes (remember that the 12cs +++f F_2 resistant group KT_{50} is an under-estimate).

Thus in the case of all 3 trm mutations, they appear to interact with the marker locus forked (f) to produce lower malathion resistance in +++f males carrying them, and therefore the conclusion that the sensitive group of 12cs attached-X lines represents those carrying the trm-12cs mutation is the most likely explanation of the results. The fact that the 59cs trm mutation when present with both markers vermillion (v) and forked gives an increase in resistance when compared to ++vf males lacking the mutation must be attributable to epistatic interactions among the three loci which determine the resistance phenotype of the ++vf flies.

Of 29 attached-X lines tested from the 12cs cross, 20 were found to carry the trm-12cs mutation. From this information the approximate map location of the mutation can be calculated:

$$\frac{20}{29} \times 23.7 \text{ (dist. from v to f)} = 16.3 \text{ map units from the f locus,}$$

or approximately at map location 40 on the X chromosome.

From the mapping results, the trm mutations can be located on the X chromosome as follows:

0	13.7	24	33	40	48	56.7
y	cv	trm-59cs	v	trm-12cs	trm-151cs	f

Due to the approximate nature of the map locations calculated, the possibility that trm-151cs and 12cs are allelic cannot be ruled out.

V - Other Mutants: in addition to the trm mutant lines, three other single mutant lines were tested for malathion resistance and avoidance (raw data in Appendix L). These lines were: cyloc (Canton S with yellow mutation) MRM-1 (chromosome 3 dominant resistance mutant), and 127 (isolated in mutant screen).

i) Resistance testing: a) Line 127 - originally isolated in the rough screen of mutagenized attached-X lines, this line was subsequently found not to differ from Canton S for resistance when tested by vacuum injection (see dose-response curves on Figure 3.2.23). In general, 127 male flies have a lower level of activity than Canton S, and when females were made homozygous for the mutation in the 127 line via crosses to the FM6 balancer line, the resulting 127cs line showed very low egg viability, and hence all the results given here are for the males of the original attached-X line. The 127 mutation was not mapped, however its position on the X chromosome was verified via crosses of 127 males to Canton S females, which gave F_1 males indistinguishable from Canton S males for malathion resistance and avoidance.

b) Line cyloc - this is a laboratory stock bearing the mutation yellow (y), which was backcrossed to Canton S for 12 generations to give a line differing very little genetically from Canton S except for the yellow locus. As shown by the dose-response curve in Fig. 3.2.23, males of this line do not differ significantly from Canton S males for malathion resistance.

c) Line MRM-1 - this line was isolated via mass-screening of F_1 progeny of mutagenized flies in the vacuum-injection set-up. It has been characterized by Dr. R. A. Morton as a third chromosome mutation for

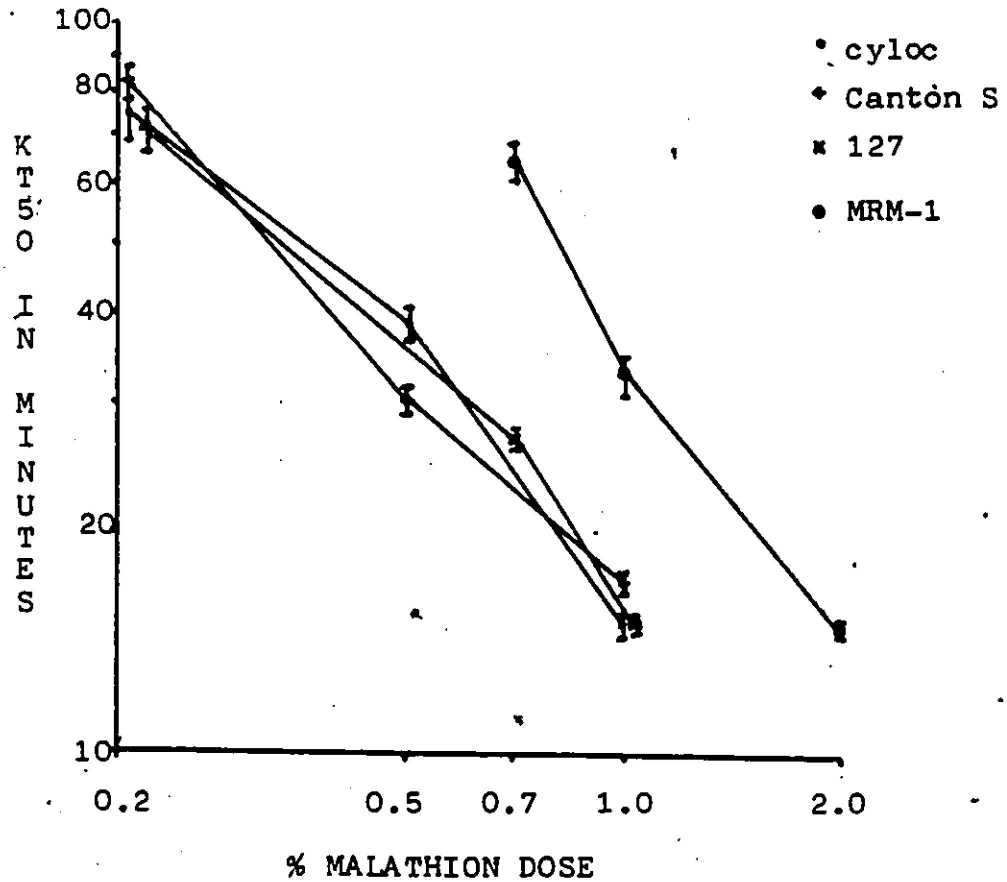


Fig. 3.2.23 dose-response curves for males of lines cyloc, 127, MRM-1 and CS

increased malathion resistance. Figure 3.2.23 shows the MRM-1 male dose-response curve which indicates the magnitude of this resistance increase.

ii) Avoidance Testing: a) Line 127 - analysis of variance comparisons of the individual run results from avoidance testing of 127 males showed much inhomogeneity among them ($F_{(5,148)}=10.03$, $p<0.001$ for a comparison of all runs), with avoidance index mean values ranging from 0.58 to 0.82 (see Appendix L for full results). The 127 flies are generally much less active than Canton S flies (see results of activity testing in section VI following), and the mumbers seen on each surface during avoidance testing are larger than those seen for Canton S. 127 flies also gave anomalous control run results - while the results for the (1%-1%) runs did not differ significantly from those obtained for the other lines (comparison of 1%-1% runs for 127, Canton S and MRM-1 males gave an $F_{(2,117)}$ value of 2.46, $p>0.05$, and an overall $\bar{C}$ value of 0.49), the results of the 127 male (0%-0%) testing differed significantly from the results obtained for Canton S ($F_{(1,122)}=28.7$, $p<0.001$), and the mean control index value for 127 males was 0.35, which is significantly different from the value of 0.5 expected and obtained in testing with the other lines.

When the results for 127 male avoidance testing were pooled and compared to those obtained for Canton S males (via means test), no significant difference was seen ($F_{(1,2.4)}=3.32$, $p>0.05$), however, given the variability in the 127 avoidance run results, this result is not a valid indicator of a lack of difference in avoidance behaviour between these two lines. The average $\bar{A}$ value for the 127 runs is 0.69, which indicates that 127 males do indeed avoid malathion to some degree, and variation among the individual run results is most likely due to the 127 flies' generally low level of activity, and their observed tendency to aggregate on the test surfaces, which may be due to an abnormality in their pheromonal responses, since males were often seen to court other males in this line.

b) Line cyloc - since the avoidance run results for each sex in this line were found to be homogeneous, they were pooled for further comparisons. Comparisons of cyloc males and females with the appropriate sex of Canton S flies showed that in both sexes the cyloc flies showed a stronger tendency to avoid malathion, as indicated by the $\bar{A}$ values:

$\bar{A}$

	Male	Female
Canton S	0.73	0.78
cyloc	0.82	0.95
comparison	$F_{(1,2.3)}=18$	$F_{(1,1.3)}=47$ $(p<0.001)$

c) Line MRM-1 - as with line 127, the avoidance run results for both sexes of MRM-1 flies showed inhomogeneity among the run results (for males $F_{(5,153)}=15.2$, range in $\bar{A}$ from 0.68 to 0.94; for females $F_{(3,107)}=7.9$, range of $\bar{A}$ from 0.74 to 0.91, in both cases $p<0.001$), however, there was no significant difference from Canton S seen in the control run results for this line. MRM-1 males were found to have a higher $\bar{A}$ value than Canton S males when the pooled results were compared ($\bar{A}$ values were 0.86 and 0.73 respectively) but no differences were seen between MRM-1 and Canton S female avoidance results ($\bar{A}$ values were 0.83 and 0.78 respectively). (Comparison F values were: males - $F_{(1,2.3)}=34.9$, $p<0.001$; females - $F_{(1,1.4)}=2.5$, $p>0.1$).

VI - Further Experiments: i) Activity testing: this was done using the countercurrent apparatus technique of Benzer (1967, 1973). The procedure is illustrated in Figure 3.2.24 (taken largely from Benzer 1973), which shows how the apparatus "fractionates" a group of flies (60 flies in this study) which are started in the first tube (a) and allowed to run towards a light (15 cm. away) for 15 seconds (b), before the frame is shifted (c) and the flies are tapped back down into the bottom tubes (d) prior to being allowed to run towards the light again for another cycle (e). At the end of 5 complete cycles, the original group of flies is distributed among the 6 tubes (f), and the distribution of flies among the tubes is an indication of the level of activity and strength of positive phototaxis of the flies tested - i.e. flies with low activity or negative phototaxis will tend to stay in the first tubes, while those with high activity or strong positive phototaxis (normal for these Canton S flies) will end up more in the end tubes. The effects of activity level and phototaxis are distinguished by running the flies in countercurrent tests away from the light source (eg. light at the bottom in Fig. 3.2.24). Under these circumstances, flies with low activity will still show a distribution weighted towards the first tubes, while negatively phototactic flies will end up in the end tubes since they will run away from the light.

The results of activity testing of the mutant lines (raw data in Appendix M) are summarized in Table 12, which contains the cumulative distributions from towards and away from light runs for males. These distributions are shown graphically in Figures 3.2.25 and 3.2.26.

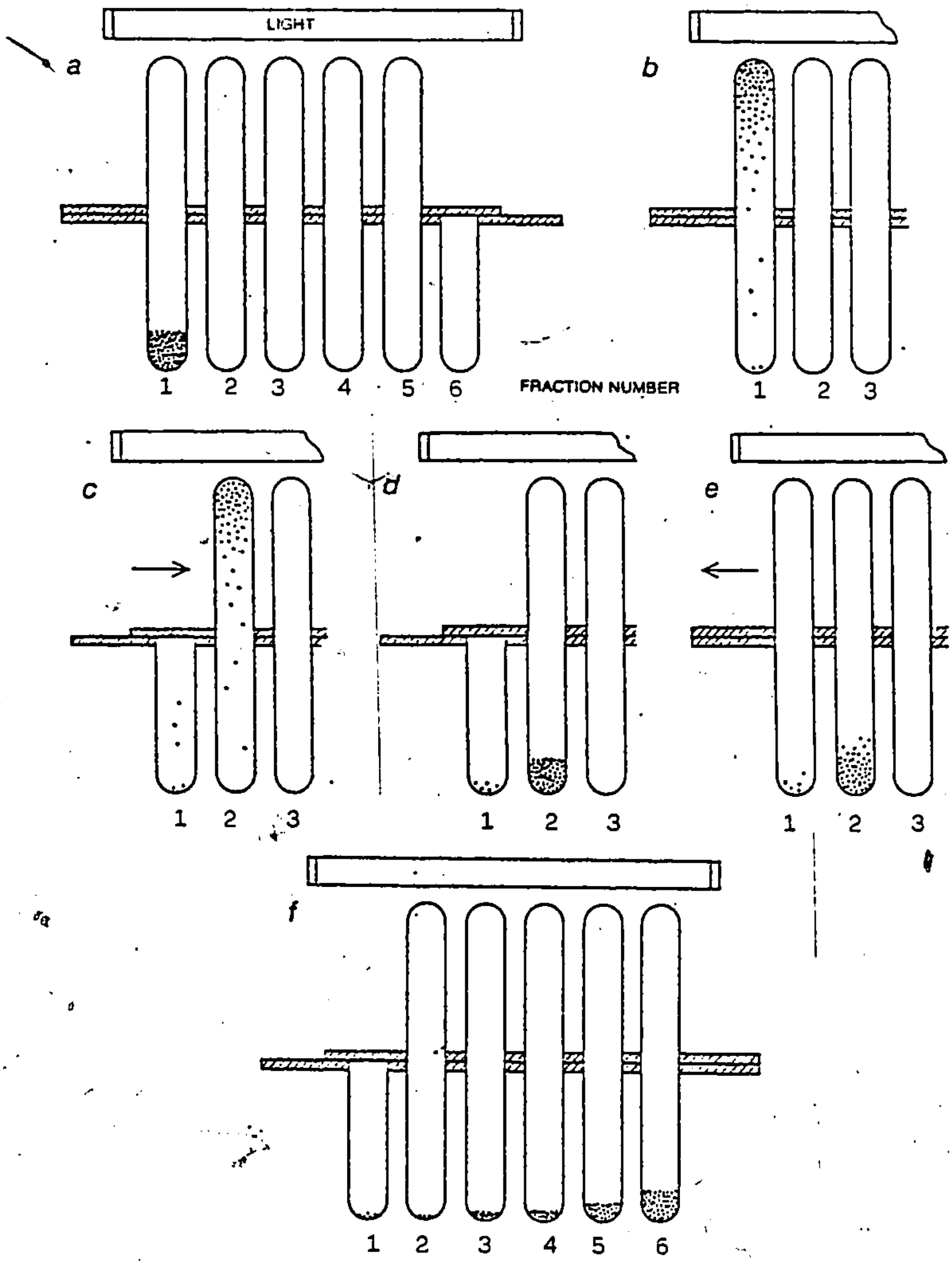


Fig. 3.2.24 countercurrent apparatus and testing procedure.

TABLE 12. Results of male activity testing - cumulative distributions.

	Percentage						
Tube #:	1	2	3	4	5	6	Sample Size
TO LIGHT:							
Canton S	10	17	32	58	82	100	120
12cs	4	10	29	51	77	100	180
151cs	7	9.5	33.5	61.5	89.5	100	120
59cs	2	4	8	16	48	100	120
127	33	39	52	66	77	100	120
MRM-1	9	10	32	52	81	100	120
AWAY FROM LIGHT:							
Canton S	40	62	77	93	100	100	60
12cs	48	73	87	98	100	100	60
151cs	80	90	98	100	100	100	60
59cs	48	67	80	93	100	100	60
127	98	98	100				60
MRM-1	53	82	95	100			60

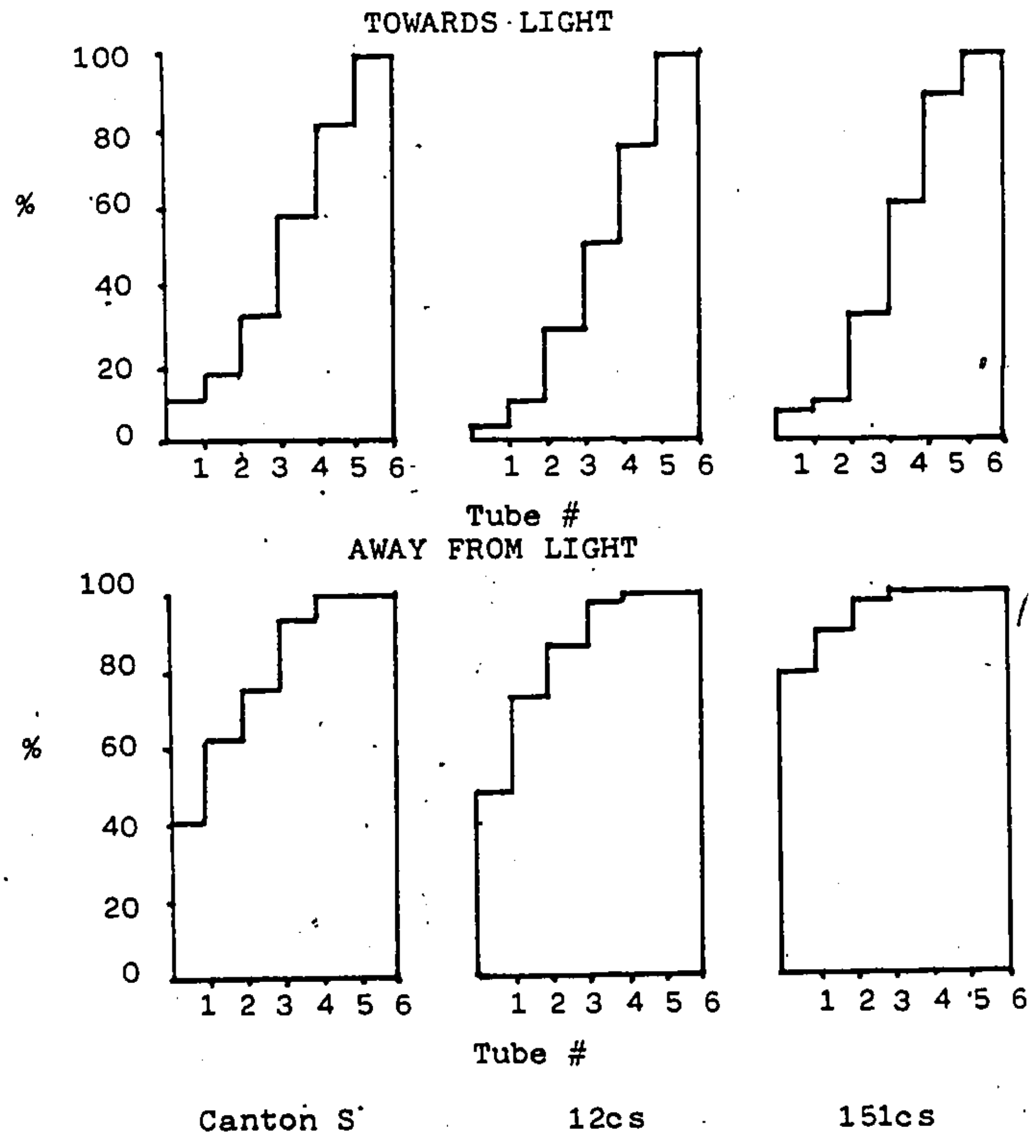


Fig. 3.2.25 results of activity testing.

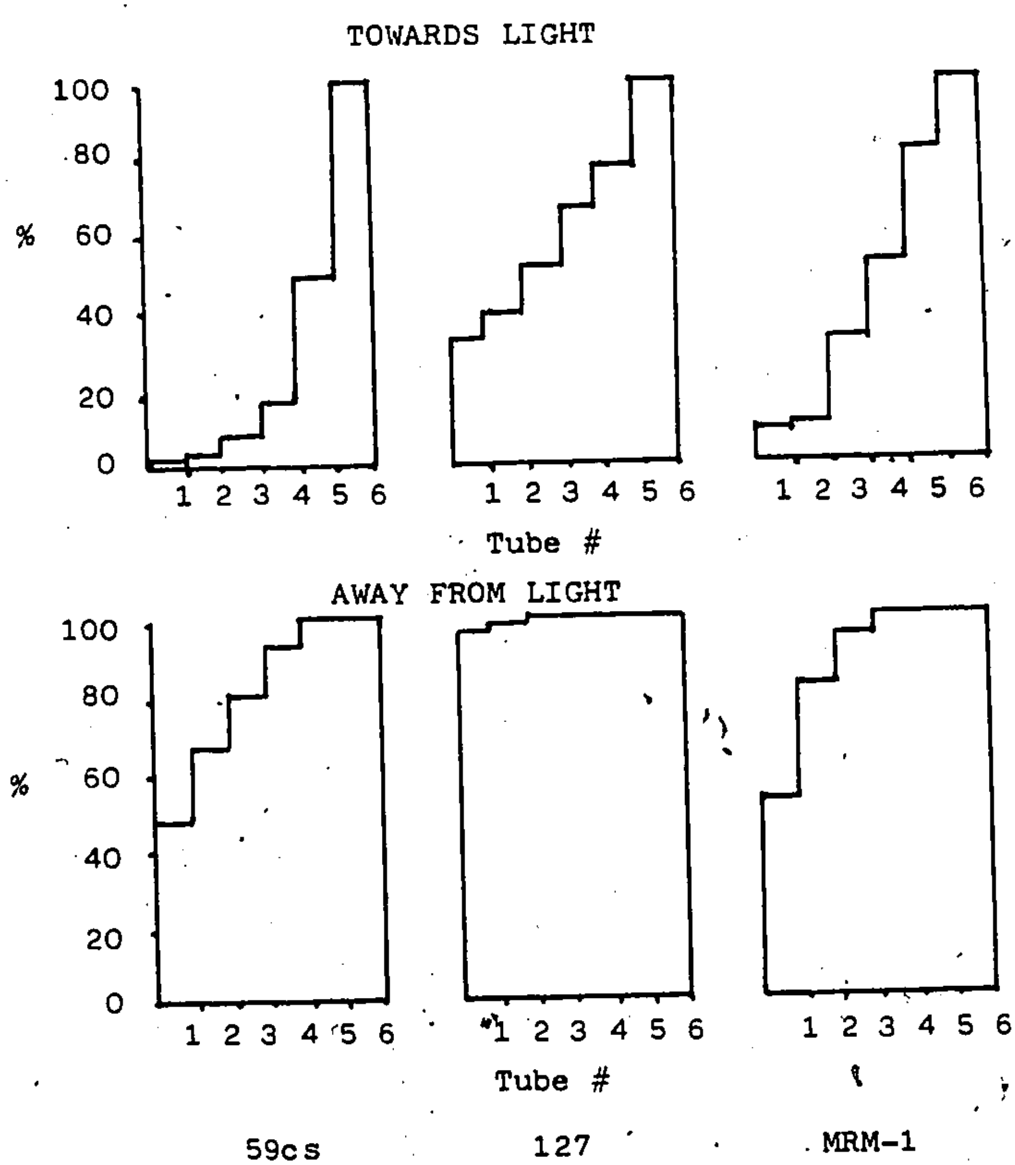


Fig 3.2.26 results of activity testing.

The results of significance testing done between the cumulative distributions of the lines are summarized in Table 13 below. Comparisons were done using the Kolmogorov-Smirnov two sample test used to compare knockdown distributions earlier, and the values of the significant difference percentage, D_{crit} are: for towards light runs - 17.6%; for away from light runs - 24.8%.

TABLE 13. Results of comparisons between cumulative distributions obtained from countercurrent activity testing.

Compared	D_{max} (%) To light	Away from light
Canton S vs.		
12cs	7	11
151cs	7.5	40*
59cs	42*	8
127	23*	58*
MRM-1	7	20

*$p<0.05$

These results indicate that 59cs flies may be more strongly phototactic than Canton S flies, since 59cs flies show a distribution weighted towards the end tubes in runs towards the light, but when run away from the light they show no indication of higher activity than Canton S flies. The most striking result is that for the 127 flies, which show a much lower level of activity than the rest under both testing conditions, indicating that indeed these flies have generally low activity.

ii) Morphological differences: the only differences noted among the trm lines were with respect to size, with flies of 59cs appearing to be larger, and those of line 151cs smaller, than Canton S flies. These size differences are illustrated by the weight differences shown in Table 14. Comparisons by chi-square testing show that 59cs flies are in fact heavier than Canton S flies for both sexes, while 151cs flies are significantly lighter, thus these two trm mutations appear to have effects upon adult size as well (all flies weighed were 5 - 7 days old). Lines 12cs and Canton S showed no significant difference in weight.

iii) Development time and egg survival: results of measurents of the length of time elapsed between egg laying and eclosion of adult flies from pupae, and the percentage of total eggs hatching are shown in Table 15, and the eclosion profiles are illustrated in Figure 3.2.27. The percentages of flies eclosed in the table are percentages of eggs laid, and the total percentages indicate the percentages of eggs that gave rise to adults. The eclosion rate results show no striking differences among the lines, except that 12cs flies appear to eclose a bit earlier than the others. The egg survival results indicate that 59cs flies may be less viable from egg to adult than those of the other lines.

TABLE 14. Weights of mutant flies and results of comparisons to Canton S.

Line		Sample Size	Mean Weight (mgms.)	-M.W. of CS (mgms.)	χ^2	p
Canton S	female	54	1.23			
	male	63	0.87			
59cs	female	60	1.37	0.14	15.9	$<.001$
	male	52	0.98	0.11	13.9	$<.001$
12cs	female	56	1.22	0.01	0.08	$>.05$
	male	34	0.84	0.03	1.03	$>.05$
151cs	female	64	1.13	-0.10	8.1	$<.005$
	male	83	0.74	-0.74	19.4	$<.005$

TABLE 15. Developmental times of trm mutant lines and Canton S flies.

Line		#eggs laid	% flies eclosed Day: 8	9	10	11	12	% Total
Canton S	female	132	0	10	38	2	0.8	99
	male		0	11	25	9	0.8	
12cs	female	184	0	40	11	4	0	97
	male		0	20	12	9	0.5	
59cs	female	136	0	12	37	7	0	85
	male		0	2	18	9	0	
151cs	female	111	0.1	26	23	5	0	94
	male		0	10	23	7	0	

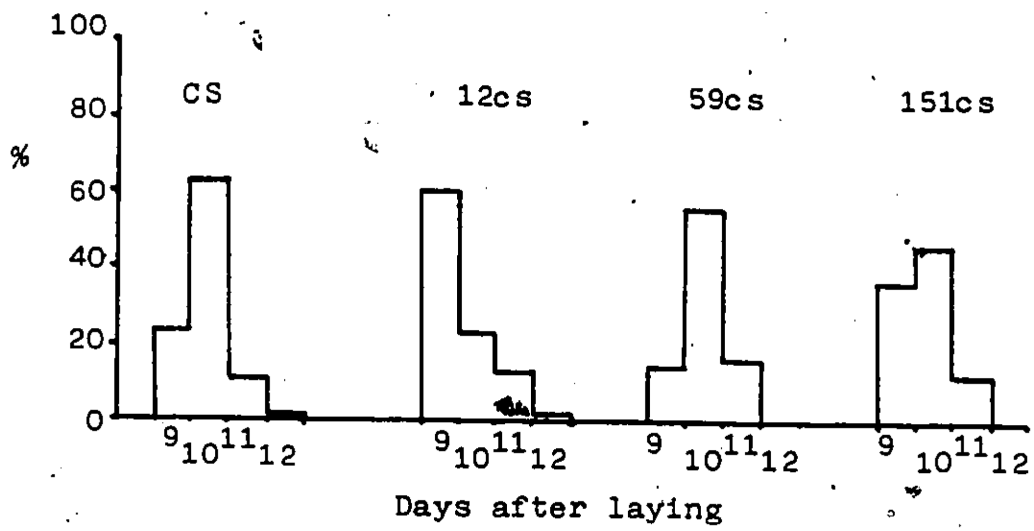


Fig. 3.2.27 rates of eclosion for mutant lines and Canton S.

PART 3 - Methodological Considerations

I - Choice of the avoidance index used: in order to make use of each individual observation made during the avoidance test runs, the number pairs were converted into index values by dividing the number of flies on the control (0%) surface by the total number of flies on both surfaces. This particular method of calculating the avoidance index was chosen for the following reasons:

i) a simple proportion (eg. #on 0%/#on 1%) or an index such as that described by Schreck (1977) (#on control surface - #on treated surface/ #on control) would give a range of values from 0 to infinity, since the number of flies on one or the other surface is frequently 0. For the index chosen in this study, the only case treated as special was when both numbers in a pair were equal to 0, in which case a value of A of 0.5 was assumed, and thus all values of A range from 0 to 1, an interval allowing convenient analysis of the experimental results.

ii) any index which involved the total number of flies being tested would be difficult to calculate, since, in the case of the mutant lines especially, flies were being knocked down during the test runs. Hence an index such as that used by Fuyama (1978) in a similar choice set-up ((#in treated trap - #in control)/#tested) would require that the number of flies knocked down be counted at each observation time, a necessity which would have cut down greatly on the number of observations that could have been made in a given time, as well as complicated the data analysis with no attendant advantage being gained.

iii) within the range of observation times used (up to the time when 60% of the flies were down or t=30 min. after introduction to the chmaber) the values of the avoidance index show only random variation with respect to the mean value (see section following), meeting the first criterion for analysis of variance.

II - Suitability of avoidance data for analysis of variance: the results of the avoidance test runs were tested against the assumptions of analysis of variance as follows:

i) Random sampling of individuals - aside from sex and age, no other deliberate selection among individuals within a test group was made.

ii) Independance of observations - this was tested for via a runs test (Sokal and Rohlf 1969), in which each avoidance index value for a group was expressed as being above (+) or below (-) the mean value for the group, and the number of "runs" (sequences of like sign) was calculated and compared to the number expected by random chance, to give a t value. Figure 3.3.1 shows a plot of the first 100 avoidance run data points in their order of observation (individual runs are separated by arrows) for Canton S males. No obvious pattern emerges from a visual inspection, and the t value from the runs test of -1.01 is less than the $t_{crit.}$ value of $\pm$ 1.96, indicating that the observations do indeed vary randomly, as was seen generally for all groups tested. (Of the mutant lines, only the 151cs male results gave a significant t value, and in a sample of the wild population lines including all of the A lines, only A-15-R males gave a significant t value.)

iii) Homogeneity of variances - as mentioned in the Methods, homogeneity of variances was tested for via Bartlett's test. Within each data set (i.e. for one sex of a line) the individual run data were found to be homogeneous with respect to variance for all lines except

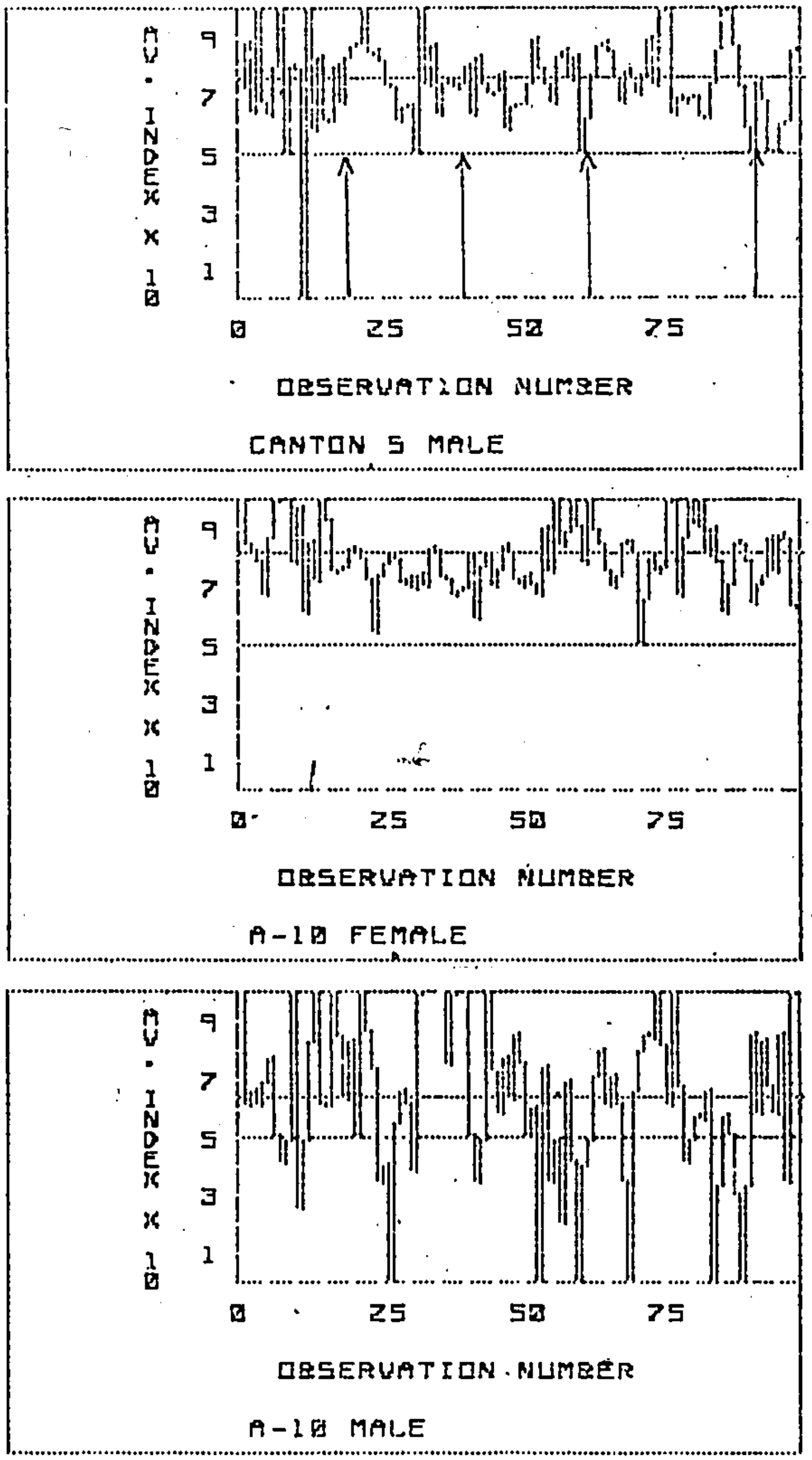


Figure 3.3.1 sequences of A values.

127 and MRM-1, hence homogeneity testing within groups was done via analysis of variance. Comparisons of variance among the pooled group results turned up inhomogeneities in variance between lines and sexes for both mutant and wild population lines. For most of the wild lines the variances between sexes were homogeneous, and hence intersex comparisons were made via anova, however for some wild lines and the mutant lines inter-sex and -strain comparisons could not be done in this manner since the variances of the groups tested varied too greatly.

The differences in variance did not show a discernable relationship to the value of $\bar{A}$, and hence none of the conventional transformations of data removed the heteroscedasticity, which appears to reflect genuine differences in behaviour (eg. compare the results for line A-10 males and females in Figure 3.3.1).

iv) Normality of distribution of error terms - a graphical test (Sokal and Rohlf 1969) of the data from avoidance testing of Canton S males is illustrated in Figure 3.3.2, which shows that the results fit a normal distribution fairly well (i.e. give a straight line on the plot of upper class boundary values versus cumulative frequency of A values falling below them), as the distribution of A values obtained for this line suggests. Most of the avoidance results for other lines tested do not show great differences from normality as indicated by their histograms, except in those cases where the value of $\bar{A}$ is very high, producing a skewed distribution. However, since interstrain comparisons were not done via anova, no attempt was made to normalize the skewed distributions since their skewness had little effect upon intragroup homogeneity testing.

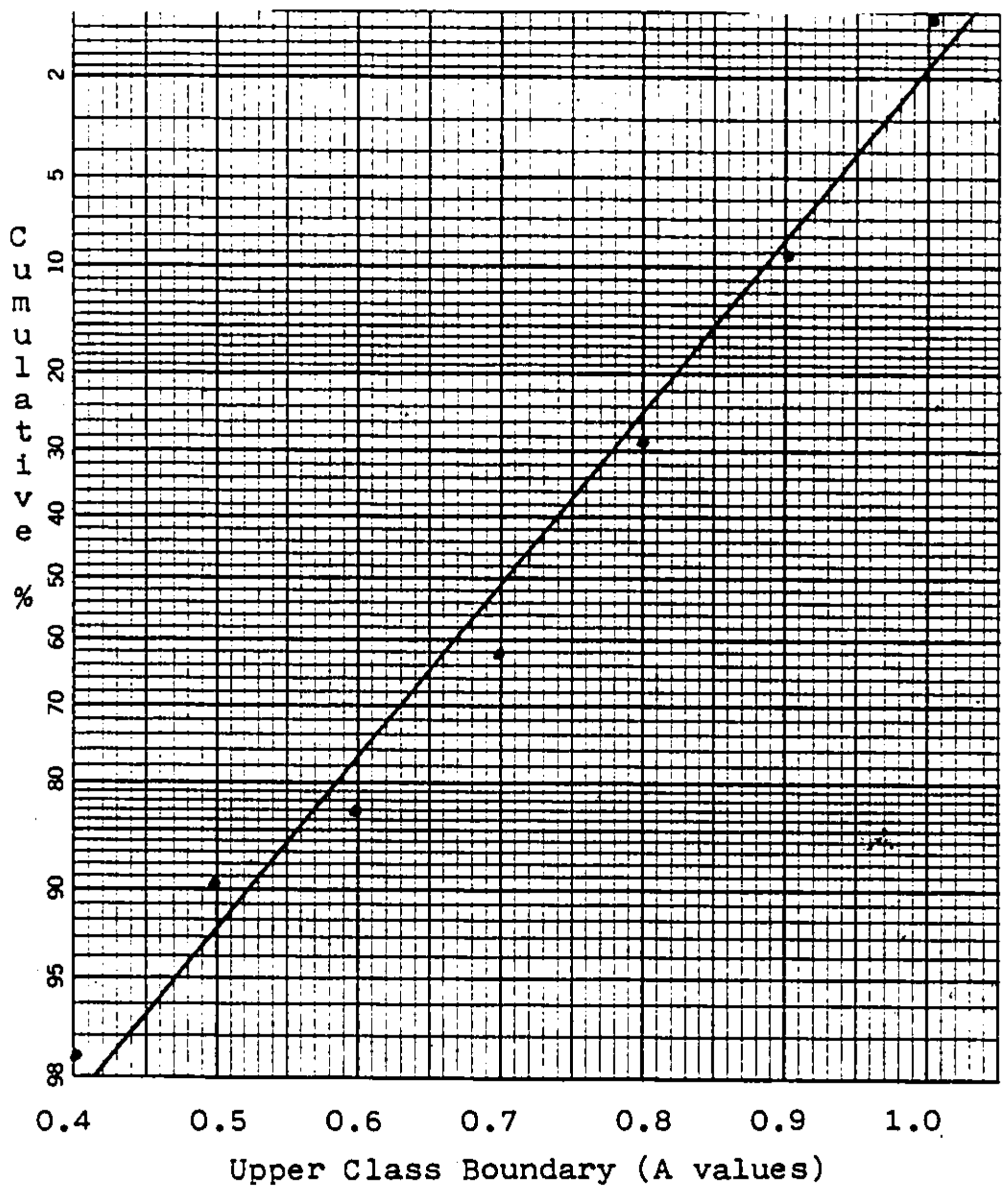


Figure 3.3.2 graphical test for normality of Canton S male avoidance run results.

In summary of the preceding section, the avoidance index data meet all criteria for analysis of variance testing at the intragroup level in most cases, but some intersex and all interstrain comparisons had to be done via the means test since the data among the lines is inherently heteroscedastic.

III - Choice of malathion preparation: the commercial preparation used in this study was chosen for the following reasons:

i) since some of the wild populations may have been exposed to malathion as a selection agent in their natural habitat, it was desirable to assay their responses to malathion in the form in which it is commonly applied, in order to see if any specific responses have been affected by selection.

ii) technical grade malathion (eg. Cythion - Cyanamid Canada Inc.) does not go into solution in water above 145 ppm and forms a very coarse emulsion at higher concentrations. Thus it would be difficult to test flies for their avoidance of malathion concentrations within the usual range of application concentrations (0.3 - 1% by volume) using the technical preparation, while the commercial preparation, with its emulsifyers, forms a fine emulsion at those concentrations.

IV - Effects of malathion concentration on avoidance: the concentration of 1% malathion by volume was chosen for the avoidance assay used in this study because: i) at that concentration Canton S and most of the wild population line flies give normally distributed avoidance index values, allowing run data to be compared via analysis of variance. ii) since Canton S is a laboratory strain which has never been exposed to insecticide, a dose was chosen which would a value of $\bar{A}$ of around 0.75, at the centre of the expected range of $\bar{A}$ values (from 0.5 to 1.0), so that a stronger or weaker aversive tendency in a mutant line would show up (i.e. if a malathion dose was used which gave a high $\bar{A}$ value for Canton S, it would be difficult to detect a more strongly avoiding mutant line).

The relationship between malathion concentration and avoidance is shown in Figure 3.3.3, which gives the avoidance index distributions for Canton S females tested at three different concentrations. The batch of malathion used for these experiments was different ("new batch") and more potent than the one used for all of the other experiments described so far, hence the results for the 1% malathion concentration shown are not directly comparable with those already given. The values of $\bar{A}$ for the three concentrations shown in Fig. 3.3.3 are: 0.1% - 0.6; 0.5% - 0.70; 1% - 0.94.

As the distributions and mean values show, the value of $\bar{A}$ decreases with decreasing concentrations of malathion used in the avoidance test, and the difficulty of picking up differences in avoidance between lines at low testing doses is illustrated in Figure 3.3.4, which gives the A distributions for 12cs and 151cs females at a malathion concentration of 0.1%. Comparisons of the data from 12cs, 151cs and Canton S females (raw data in Appendix N) via the means test gives no significant differences at a concentration of 0.1% ($F_{(2,5.9)}=4.8$), whereas at the concentration used for the main body of experiments, 12cs females show significantly less avoidance than females of the other two lines.

Thus the avoidance response of the flies depends upon the concentration of malathion used to test

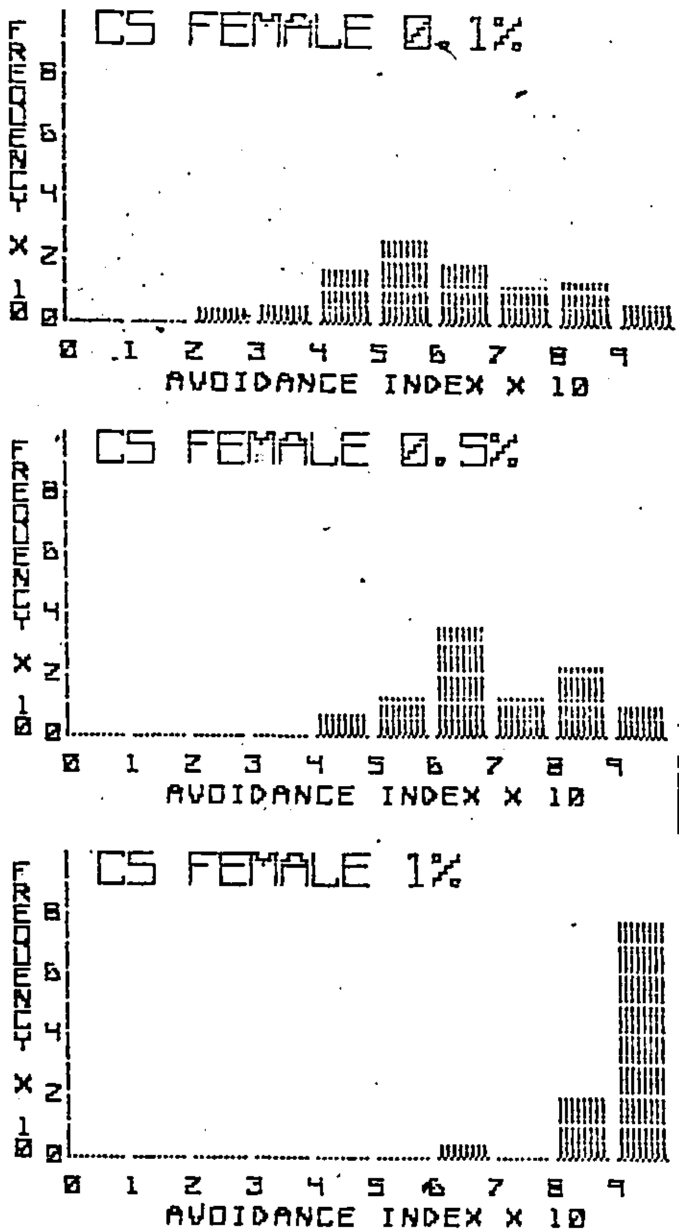


Figure 3.3.3 results of Canton S avoidance testing with 3 different concentrations of malathion (new batch).

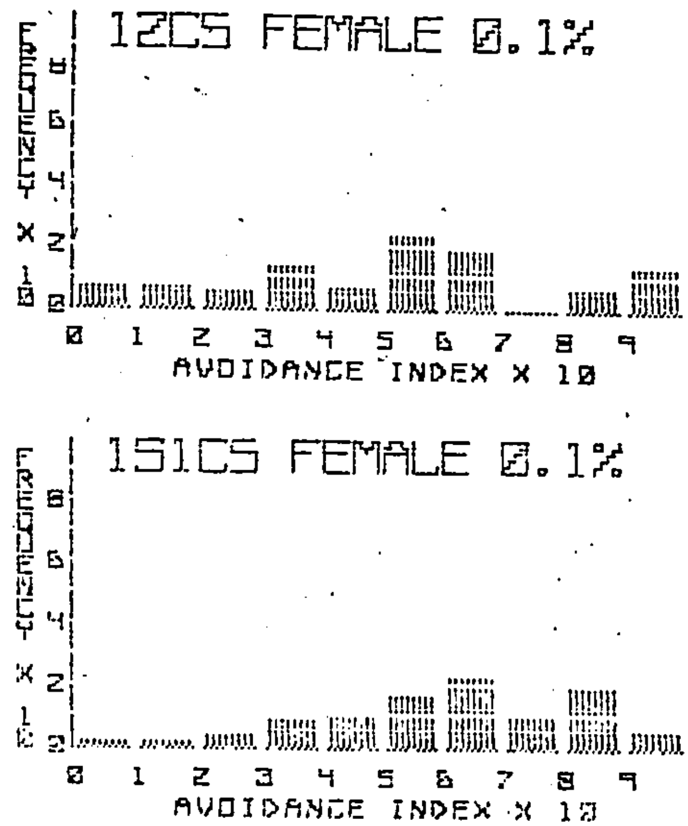


Figure 3.3.4 results of avoidance testing with 0.1% malathion.

for it, and the concentration employed in this study was chosen so that relative differences in avoidance would show up among the mutant lines. With respect to the wild population lines, the 1% malathion concentration appears to be useful in separating them into discrete groups with respect to avoidance, and while the members of the extremely strongly avoiding group may be separable under different test conditions, for this study such a separation was not considered to be necessary.

V - Knockdown during avoidance testing: i) wild population lines - as has already been mentioned, scoring in the avoidance test runs ceased at 20 minutes after the flies were introduced to the chamber or when 60% had been knocked down, since, as was shown earlier in this section, within this interval the avoidance index values varied randomly. For the wild population lines the knockdown consideration was not important in most cases. Table 16 lists the percentages of flies down at t=20 min. for the wild line avoidance runs, and these results show that for only one line, B-7, was the 60% knockdown cutoff point used.

The rate of knockdown is fairly consistent from run to run within these lines, but since knockdown during avoidance runs is most likely affected by the avoidance response, resistance, and other behavioural factors (such as general activity level), it was not expected that it would turn out to be a factor of much use in characterizing the flies' response to malathion. This expectation is borne out when we look at the correlations between the percentages of flies down at t=20 min. in avoidance runs and the values of $\bar{A}$ for all lines (from the graph

TABLE 16. Knockdown percentages at t=20 min. in wild line avoidance runs.

Line	Run	% down F	M
A-4	A	20	22
	B	14	20
	C		20
A-5	A	40	36
	B	28	44
	C		48
A-6	A	16	20
	B	14	14
	C	12	16
A-7	A	12	14
	B	12	8
	C		19
A-8	A	22	36
	B	18	34
	C	40	60
A-9	A	44	30
	B	46	34
	C		34
A-10	A	12	8
	B	46	26
	C	10	34
	D		16
A-11	A	26	50
	B	34	52
	C	46	60
	D	60	
A-12	A	20	32
	B	14	24
	C	10	

Line	Run	% down F	M
A15R	all	5	5
A-16	A	10	16
	B	8	26
	C	19	
	D	26	
A-17	A	6	24
	B	14	22
	C	12	32
B-2	A	18	12
	B	14	10
	C		14
B-4	A	24	22
	B	30	24
B-7	A	>60	>60
	B	>60	>60
	C	46	>60
B-8	A	12	10
	B	4	12
	C		24
B-10	A	40	52
	B	40	58
	C		60
B-13	A	0	16
	B	8	6
	C		8
B-16	A	20	20
	B	22	30
	C	26	48

Line	Run	% down F	M
B-17	A	20	48
	B	18	34
	C	20	22
J-8	A	6	16
	B	6	34
J-11	A	16	12
	B	16	14
	C	6	10
J-19	A	0	10
	B	8	8
V-3	A	12	16
	B	16	28
V-11	A	8	8
	B	4	20
	C		10
V-31	A	20	36
	B	34	32
	C	14	

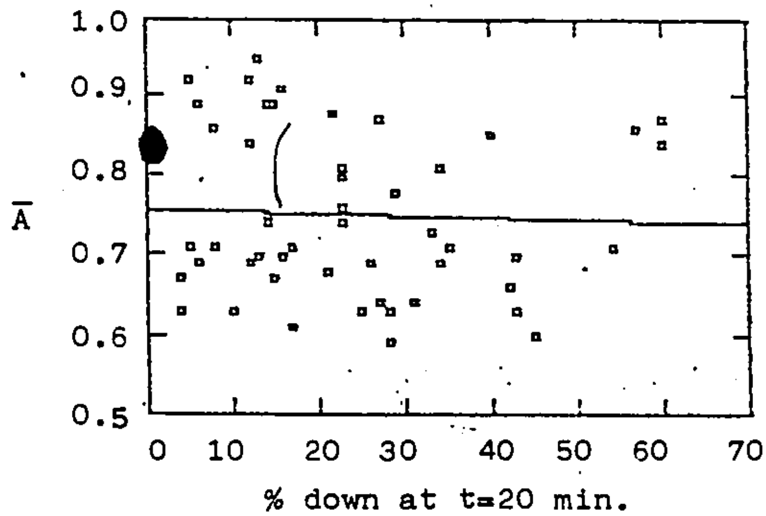


Fig. 3.3.5 relationship between knockdown and avoidance for wild population lines (males + females) in (0%–1%) runs.

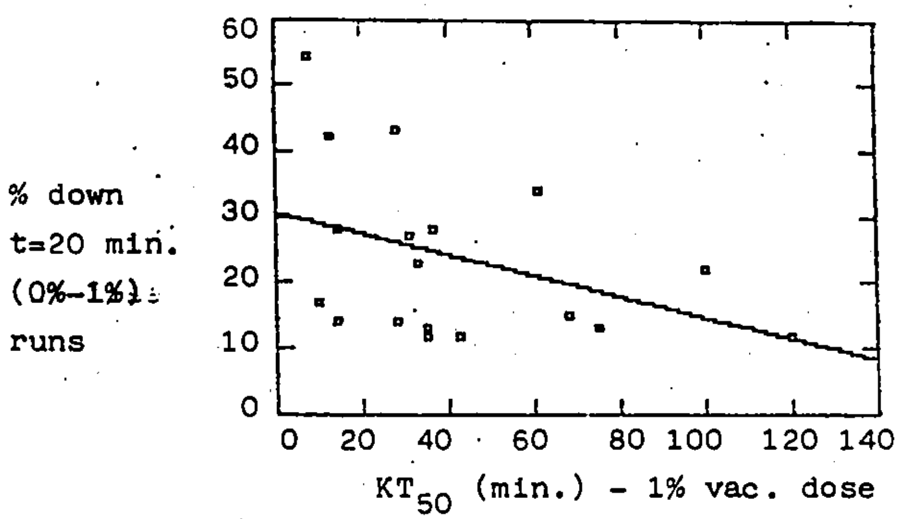


Fig. 3.3.6 relationship between knockdown in avoidance testing and vacuum injection assay resistance testing.

in Figure 3.3.5, the value of the correlation coefficient, r = -0.039, df=50), or KT_{50} values for a dose of 1% malathion vacuum adminstered for those lines for which this was determined (shown in Figure 3.3.6 - the value of r = -0.38, df=16), neither of which is significant.

ii) mutant lines - Table 17 lists the knockdown data for the mutant line avoidance runs. Unlike the results for the wild population lines, the 60% knockdown cutoff point was employed for most runs, and there is considerable run-to-run variation in knockdown rate. This inter-run variation and the lack of any obvious relation between avoidance run knockdown and any of the other characteristics assayed for these lines makes any further analysis of avoidance test knockdown likely to be unprofitable. The variation in knockdown rates may be due to factors which were uncontrolled for in the experiments, such as microenvironmental conditions within the chambers, but since the avoidance data from the runs showed no significant differences within each group, these uncontrolled factors do not seem to be as important as those factors (fly age, temperature, time of day when run, illumination, etc.) which were controlled with respect to the results of the avoidance assay itself.

iii) knockdown in control runs - Tables 18 and 19 list the knockdown data for the (1%-1%) control runs for the wild population and mutant lines respectively. As with the avoidance run knockdown data, there is no correlation between control knockdown and resistance as assayed via vacuum injection (between the % of flies down at t=10 min. in 1%-1% runs and the KT_{50} for a 1% vacuum-applied dose of malathion, r = -0.398, df=12, p 0.05 for wild lines) and there is considerable inter-run variation within each group, presumably due to the same unknown

TABLE 17. Knockdown vs. time for mutant line avoidance runs.

Line	Time (min.):		5'	8'	10'	13'	15'	18'	20
151cs	male	A	4	8		16		19	21
		B	16	25		30			
		C	10	20	31				
	female	A	7	15	18	24	28	30	
		B	7		30				
		C		6	14	21	26	30	
12cs	male	A	2	14	21	25	31		
		B	6	19	26	28	31		
		C	2	9	19	23	27	30	
		D		15	25		30		
		E		18	28	30			
	female	A	3	9	13	17			21
		B	1,	6	8	13			19
59cs	male	A	2	7	10	16	25	29	
		B	2	9	14	20	29	32	
		C	2	12	19	25	29	31	
		D	3	13		26	30		
	female	A.		4		8	15	20	
		B		4		20	31		
		C		1		12	20		24
CantonS	male	A	5	14		17		22	
		B	4	12		16		19	
		C	4	7		12		17	
		D		11		19		22	27
		E		14		23			30
	female	A		10	21		28		
		B			17		26		
		C			16		25		

TABLE 18. Knockdown vs. time for wild population (1%-1%) control runs.

Line			% down															
		Time (min.):	5	'	'	8	'	'	11	'	'	14	'	'	17	'	'	20
A-6	female	A																0
		B								2								8
	male	A													4			22
		B								2				12				40
A-7	female	A					2			6			10			26		30
	male	A					2			10			14			24		38
A-8	female	A						2			4		10					40
	male	B						4			16		38					60
A-10	female	A			10		22			52								
		B			6				28			50		60				
	male	A			30		46			60								
		B			4				22			48		60				
A-11	female	A	12		22		48		60									
	male	A	10		24		38		50			60						
A-12	female	A						2				14		22			56	
		B										24			30			40
	male	A						22			54							
		B										28			32			40
A-15	female	A												6				14
		B										4						6
	male	A										4				12		
		B										4			10			24
J-11	female	A										4			14			18
		B										2			12			14
	male	A										2			22			26
		B										6			32			48

TABLE 19. Knockdown vs. time for mutant line (1%-1%) control runs

Line		% down Time(min.): 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
151cs male	A	9	14		20	31											
	B	8			16	21		26	30								
	C	7			13		19	30									
12cs male	A	6			16	31											
	B	10			20	31											
	C	6			13	30											
59cs male	A	11			24	31											
	B	0			5	10		18		24			30				
	C	2			13		24		27		30						
CS male	A	6			17	25		32									
	B	9			16	26		30									
	C	2			15	25		30									

factors that gave rise to the inter-run variations in the mutant line avoidance testing results. Since the control runs combined for all groups show no significant differences with respect to the values of the control index ($\overline{C} = 0.5$), the differences in knockdown rates between these runs, while they may be indicative of real differences among lines, do not appear to be worth any further discussion.

PART 4 - The Avoidance Response and Its Relationship to Resistance

I - Behavioural determinants of differences in avoidance index values: in order to provide a clear understanding of what the differences in avoidance index values among lines actually mean in terms of fly behaviour, comparisons were made between the mean avoidance index values for the wild population lines and the mean values for numbers of flies actually seen on the test surfaces during the avoidance and control runs. The values used for these comparisons are listed in Table 20, and were taken from the data in the appropriate appendices.

i) relationship between $\bar{A}$ and the mean total number of flies on both surfaces per observation for wild line avoidance runs: this relationship is shown graphically in Figure 3.4.1, and the correlation is highly significant ($r=0.62$, $df=50$, $p<0.001$), indicating that lines showing higher values of $\bar{A}$ also tend to show more flies actually on the test surfaces than those lines with low $\bar{A}$ values.

ii) relationship between $\bar{A}$ and mean total number of flies on the 0% surface in avoidance runs: this relationship is graphed in Figure 3.4.2, and the correlation is also highly significant ($r=0.72$, $p<0.001$), indicating that higher avoidance is accompanied by greater numbers of flies on the malathion-free surface.

iii) relationship between $\bar{A}$ and the mean total number of flies on the 1% surface during avoidance runs: graphed in Figure 3.4.3, this relationship gives a significant negative correlation ($r= -0.56$, $p<0.001$), indicating that the higher the value of $\bar{A}$ is, the lower is the number of flies going onto the malathion-treated surface.

TABLE 20. Numbers of flies on surfaces (means) for avoidance and control runs with wild populàtion lines.

Runs:		(0%-1%)			(1%-1%)	(0%-0%)	
Line		#on 0%	#on 1%	0%+1%	Total #on	Total #on	$\bar{A}$
A-4	male	5.9	2.7	8.6			0.81
	female	3.9	1.5	5.4			0.61
A-5	male	4.3	1.6	5.9			0.70
	female	4.7	1.6	6.3			0.69
A-6	male	5.0	2.1	7.1	6.2	9.4	0.71
	female	5.0	1.8	6.8	4.4		0.74
A-7	male	4.0	1.6	5.6	4.2	6.4	0.75
	female	4.7	1.9	6.6	4.2		0.69
A-8	male	3.8	2.5	6.3	6.3	6.4	0.63
	female	4.3	2.4	6.7	4.9		0.64
A-9	male	5.8	3.4	9.2			0.64
	female	5.7	3.7	9.4			0.60
A-10	male	4.3	2.3	6.6	7.0		0.63
	female	7.8	2.2	10	4.9		0.8
A-11	male	3.3	1.7	5.0	6.8	8.6	0.71
	female	4.0	2.2	6.3	5.0		0.72
A-12	male	3.6	2.5	6.1	5.7	9.3	0.59
	female	5.1	2.3	7.4	7.8		0.67
A15R	male	14.0	1.2	15.2	6.9	9.8	0.92
	female	6.9	2.7	9.6	4.9		0.71
A-16	male	3.8	2.0	5.8			0.68
	female	3.5	1.6	5.1			0.70
A-17	male	6.5	2.8	9.3			0.69
	female	3.7	1.7	5.4			0.70
B-2	male	22.9	1.6	24.5			0.92
	female	18.2	1.6	19.8			0.91

Table 20 cont.

Runs:		(0%-1%)			(1%-1%)	(0%-0%)	
Line		#on 0%	#on 1%	0%-1%	Total #on	Total #on	$\bar{A}$
B-4	male	8.0	2.0	10.0			0.81
	female	7.9	1.2	9.1			0.87
B-7	male	7.0	1.4	8.4			0.84
	female	10.7	1.8	12.5			0.87
B-8	male	7.9	1.0	8.9			0.89
	female	6.8	2.1	8.9			0.86
B-10	male	7.2	1.2	8.4			0.86
	female	9.1	1.7	10.8			0.85
B-13	male	3.8	2.2	6.0			0.63
	female	4.0	2.4	6.4			0.63
B-16	male	3.4	1.3	4.7			0.73
	female	5.2	1.9	7.1			0.74
B-17	male	8.1	3.2	11.3			0.71
	female	10.1	2.7	12.8			0.78
J-8	male	4.3	2.7	7.0			0.62
	female	3.7	1.5	5.2			0.69
J-11	male	8.3	1.6	9.9	6.9	8.2	0.84
	female	7.0	2.1	9.1	5.8		0.70
J-19	male	8.0	3.5	11.5			0.71
	female	6.7	3.5	10.3			0.67
V-3	male	6.3	0.7	7.0			0.88
	female	8.0	1.2	9.2			0.89
V-11	male	25.0	1.3	26.3			0.95
	female	12.0	1.5	13.5			0.89
V-31	male	11.7	2.9	14.6			0.81
	female	6.5	3.0	9.5			0.76

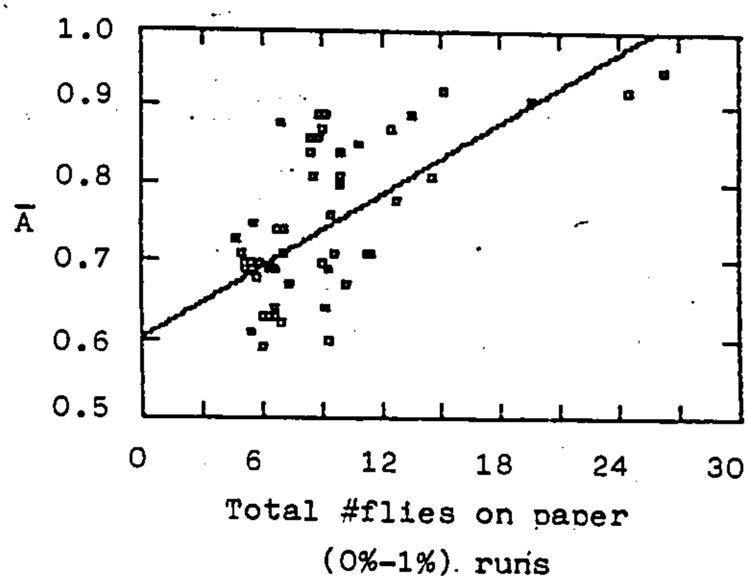


Figure 3.4.1 relationship between $\bar{A}$ and number of flies on test surfaces - wild population lines.

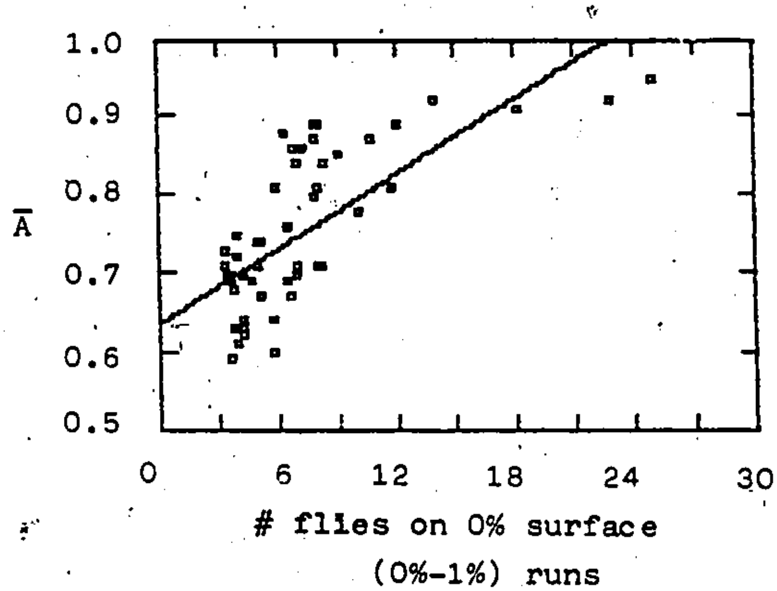


Figure 3.4.2 relationship between $\bar{A}$ and number of flies on control surface in avoidance runs - wild population lines.

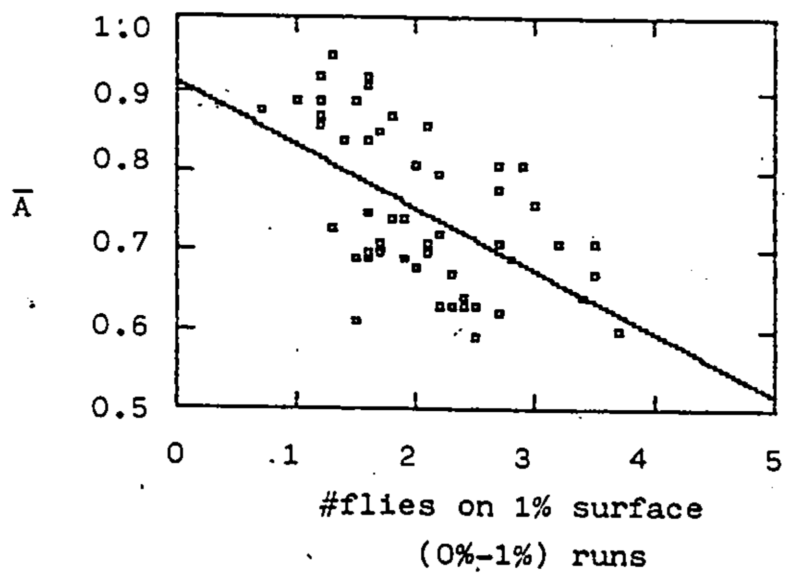


Figure 3.4.3 relationship between number of flies on treated surface and $\bar{A}$ - wild population lines.

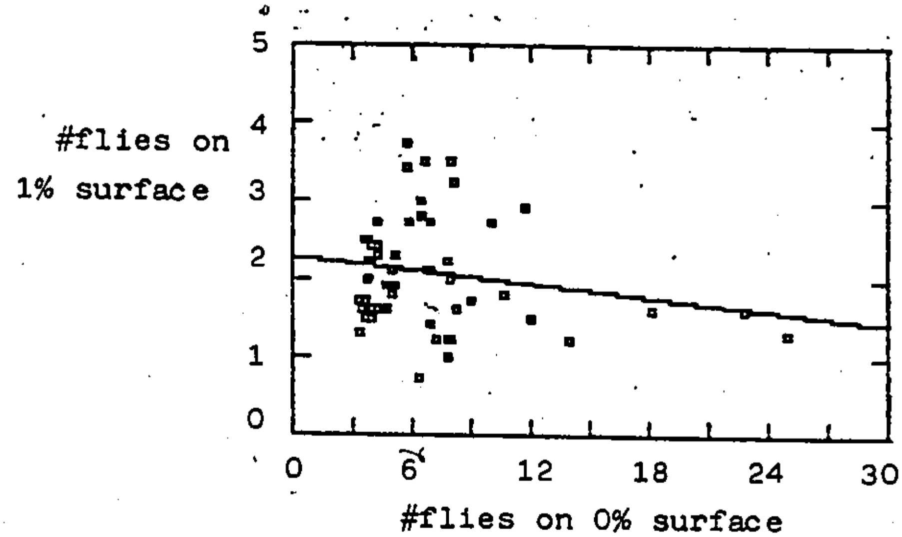


Figure 3.4.4 relationship between numbers of flies on treated and untreated surfaces in avoidance runs - wild population lines.

While at first glance the above correlations way appear to be trivial, since they involve correlating the avoidance index and the values of its components, they do in fact lead to conclusions not derivable a priori from the avoidance index values themselves. For example, it is not necessary for the total number of flies on the test surfaces to change in order to give widely different values of $\bar{A}$ (eg. in Table 20, line A-9 females have a mean total #of flies on surfaces 9.4, and for B-8 males this mean value is 8.9, yet their respective $\bar{A}$ values are 0.6 and 0.89 - the same numbers of flies on the surfaces are distributed differently between the two different kinds). Similarily, an increase in the value of $\bar{A}$ need not be accomplished by increasing the number of flies on the untreated surface exclusively (eg. the $\bar{A}$ value for V-3 males is 0.88 and that for J-19 females is 0.67, yet the mean #flies on the 0% surface are 6.3 and 6.7 respectively), or by only decreasing the number of flies on the 1% surface (compare lines B-2 and A-5 on Table 20), and the correlations between these quantities and $\bar{A}$ indicates that both vary in the manner already mentioned to give rise to the range in avoidance behaviour seen among the lines, although the mean numbers of flies on either surface are not correlated with each other (Figure 3.4.4, $r=-0.18$, $p>0.05$).

The fact that the value of $\bar{A}$ is negatively correlated with the number of flies actually going on the malathion treated surface, and positively correlated with the number going on the untreated surface is not surprizing - since presumbaly not going on the treated surface in preference of the other is what avoidance is all about. The positive correlation between $\bar{A}$ and the total number of flies on either surface raises a question, however. As has already been mentioned, there is no a priori reason to predict that the lines which have the highest values of $\bar{A}$ should also show the highest total numbers of flies going on the test surfaces. In order to examine further why this should be, the results from the avoidance and control runs were compared, and the results of these comparisons are listed in Table 21.

In the table, the first three correlations listed merely show that the correlations noted for the entire set of wild population lines given earlier hold also for the group of lines for which controls were done. The comparisons with the results of the (1%-1%) and (0%-0%) control runs show generally that no set of results is an indicator of how flies of a particular line will behave under other test circumstances. Specifically, the lines which show the strongest avoidance do not have less affinity for the surfaces in the (1%-1%) control runs than the less strongly avoiding lines, and the higher numbers of flies seen for the strongly avoiding lines in the (0%-1%) runs do not derive from these flies having a higher overall affinity for 0% test surfaces in general, as shown by the lack of a significant correlation between the mean number of flies on that surface for the (0%-0%) and (0%-1%) runs.

Thus in the avoidance (0%-1%) test situation, not only is the probability of flies going on the untreated surface increased and the probability of them being found on the malathion treated surface decreased, but the presence of the treated surface affects the flies' affinity for the untreated surface in general,

TABLE 21. Results of comparisons between various parameters listed in Table 20.

Compared	Correlation Coefficient	d.f.	p
#on 0% in (0%-1%) runs vs. $\bar{A}$	0.83	14	<0.001
#on 1% in (0%-1%) runs vs. $\bar{A}$	-0.77	14	<0.001
#on 0% + #on 1% vs. $\bar{A}$	0.83	14	<0.05
Total #on (1%-1%) runs vs.:			
1) Total #on (0%-0%) runs	0.51	5	
2) Total #on (0%-1%) runs	0.49	5	
3) $\bar{A}$	0.33	5	
4) #on 1% in (0%-1%) runs	-0.09	14	>0.05
Total #On (0%-0%) runs vs.:			
1) Total #on (0%-1%) runs	0.48	5	
2) $\bar{A}$	0.28	5	
3) #on 0% in (0%-1%) runs	0.47	5	

(A bracket groups the d.f. entries of both lower groups under p >0.05.)

increasing it in the case of the strongly avoiding lines to levels higher than those expected from (0%-0%) control runs (eg. see results for A-15-R males in Table 20). For some strongly avoiding lines, this shift in affinity resulted in a steady accumulation of flies on the 0% surface during the avoidance runs, as shown in the graphs in Figure 3.4.5 for B-2 males and V-3 females, both of which show significant deviation from independance for observations in the runs test (t_s=-4.9 and -4.13 respectively, $p<0.05$). This tendency of the flies to find the untreated surface and stay there is not the general explanation for the overall correlation between $\bar{A}$ and the mean #flies on the test surfaces however, since runs testing of the most strongly avoiding lines (A-15-R, B-2, B-4, B-7, B-8, B-10, J-11, V-3, V-11) shows up only 4 instances of significant deviation from independance of observations in avoidance runs (A-15-R male, B-7 female, and the two already mentioned) out of 18 groups tested. Thus most lines did not show the accumulation of flies on the untreated surface during the course of avoidance runs, and typical results of avoidance testing for these lines are shown in Figure 3.4.6.

A definitive explanation of the correlation between $\bar{A}$ and the mean numbers of flies on the surfaces during avoidance runs would require further experimentation (i.e. is there a direct physiological effect of malathion on fly activity - the results of the 1%-1% control runs do not support that hypothesis), leading into areas beyond the scope of this study. From the foregoing discussion, however, a simple explanation for the correlation suggests itself. Looking at the mean numbers of flies on the test surfaces for the two classes of control runs, if the results of the (0%-0%) runs

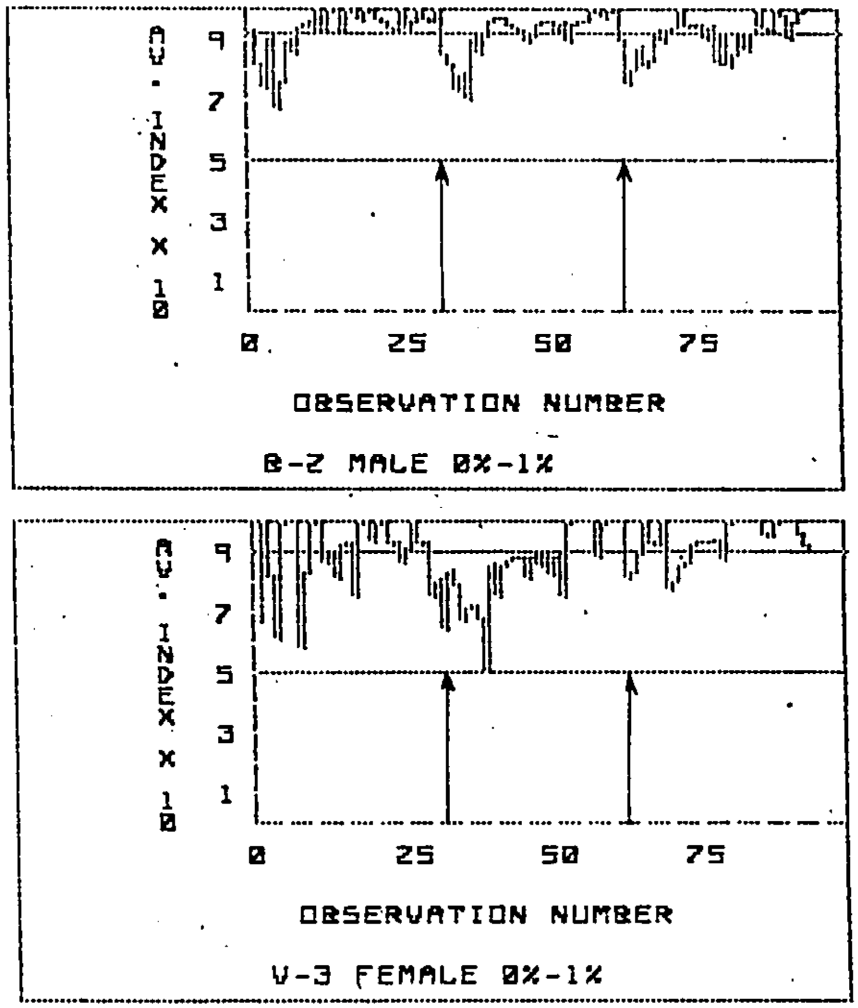
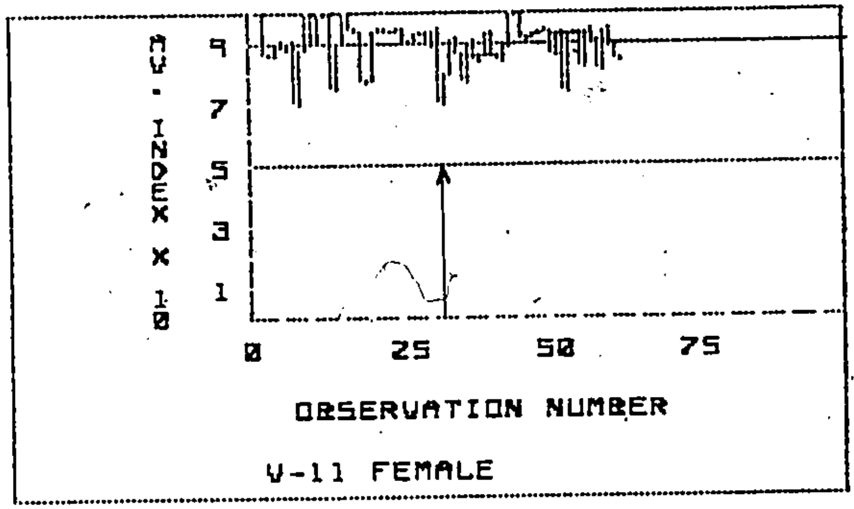


Figure 3.4.5 avoidance index values in sequence of observation for two lines showing gradual accumulation of flies on control surface during runs.



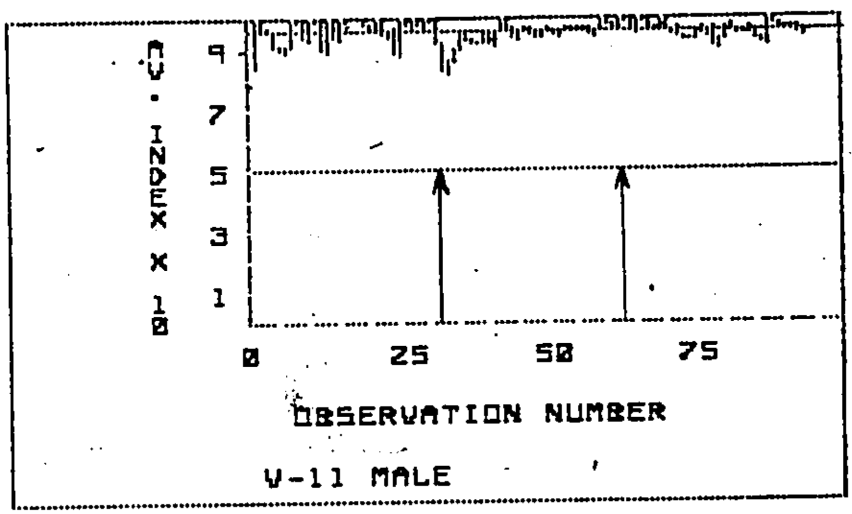


Fig. 3.4.6 avoidance index values in sequence for two typical lines.

represent the basic affinities of the flies for the test surfaces, this affinity is reduced if the surfaces are treated with malathion in each instance (see Table 20), an indication of what might be called the "basic" avoidance response - behaviour which leads flies finding themselves upon a treated surface to leave that surface and tend not to go back onto it. In the avoidance run results, the effects of this basic avoidance response show up among the lines with low to moderate strength of the avoidance response, but as the value of $\overline{A}$ increases, a second type of behaviour emerges, which might be called the "secondary" avoidance response, which causes flies encountering the treated surface not only to avoid it, but to attempt to disperse as far as possible from it - in the case of the avoidance runs to the untreated surface.

Since all of the wild population lines tested show some level of positive avoidance of malathion, the basic avoidance response is a common characteristic, and the value of $\overline{A}$, while it is also determined by the strength of this basic response, appears to be largely determined by the strength of the secondary avoidance response. Both responses represent specific responses of the flies to malathion, and do not simply arise from differences among lines in affinity for the test surfaces in non-choice situations, and there may be many factors involved in determining the strength of each response, a consideration to be taken up again in the discussion.

II - Correlation of resistance and avoidance:

i) wild population lines - the realtionship between the mean value of the avoidance index and KT_{50}

for a dose of 2% malathion for the lines tested fully for resistance (both sexes) is shown graphically in Figure 3.4.7, and from the graph a value of the correlation coefficient was calculated, $r=0.13$, which is not significant ($df=14$, $p>0.1$). Thus among these lines there is no correlation between physiological resistance and avoidance (no significant correlations were obtained when the sexes were done separately: for males $r=0.33$; for females $r=0.14$; in both cases $df=6$, $p>0.1$).

If the results from resistance testing with a 1% malathion dose for males of lines B-2, V-3, V-11, V-31 and the A series lines (except A-15-R, which does not give 50% knockdown at this dose) are plotted against their values of $\bar{A}$, a significant correlation does emerge (see Figure 3.4.8, $r=0.75$, $df=9$, $p<0.01$). Thus among these lines, the most resistant lines also tend to show the strongest avoidance response.

ii) mutant lines - the relationship between resistance and avoidance in these lines is illustrated in Figure 3.4.9 (KT_{50} for 0.2% dose vs. $\bar{A}$). The value of $r=-0.79$ ($df=6$, $p<0.05$) which indicates a significant -ve correlation between the strength of the avoidance response and the level of resistance. This relationship holds when the sexes are considered separately as well (Figures 3.4.10, 3.4.11), in each case $r=-0.98$ ($df=2$, $p<0.05$).

It is interesting that the relationships between resistance and avoidance are in opposite directions for the mutant and wild population lines, however it must be remembered that in the latter group, variation is most likely due to genetic differences at many loci, while the mutations are single gene differences, a point to be considered further in the Discussion following.

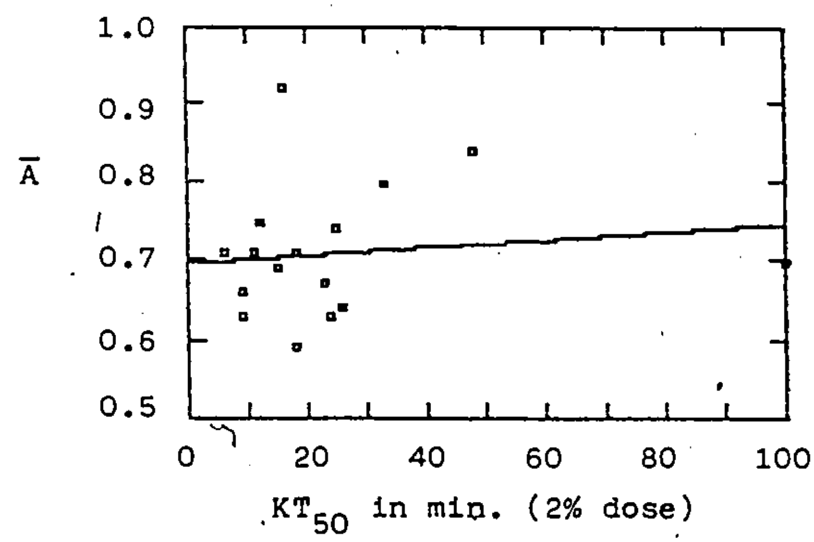


Fig. 3.4.7 relationship between resistance and avoidance - wild population lines.

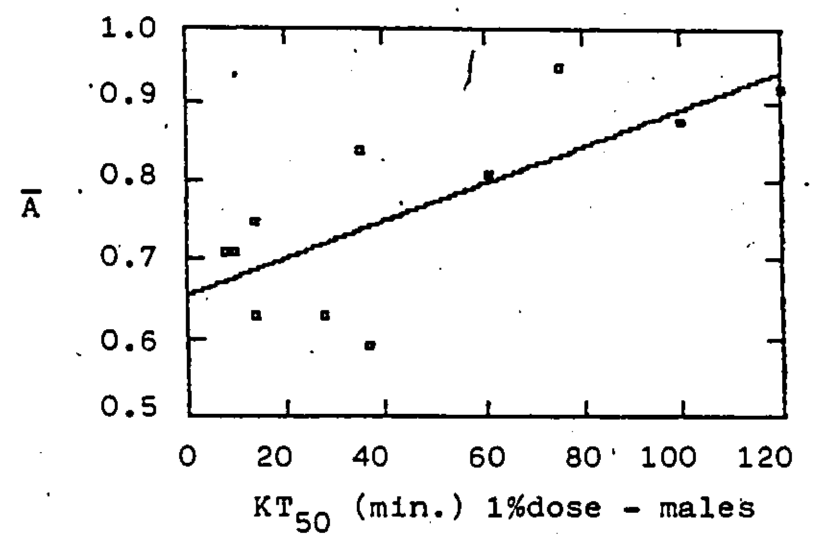


Fig. 3.4.8 relationship between resistance and avoidance for males of wild population lines.

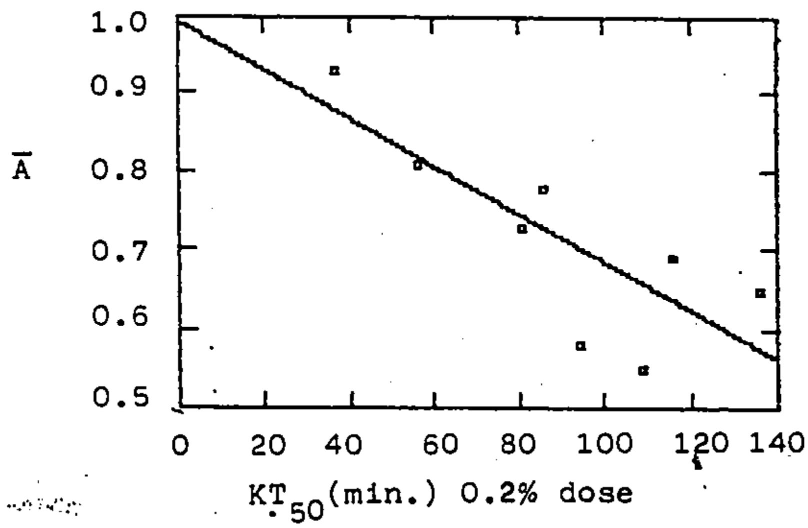


Fig. 3.4.9 relationship between avoidance and resistance for males and females of the trm lines and Canton S.

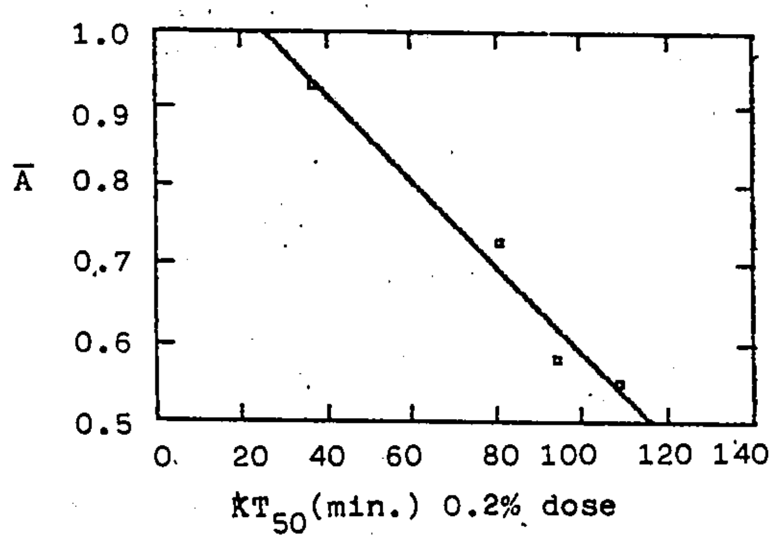


Fig. 3.4.10 relationship between avoidance and resistance for males of trm lines Canton S.

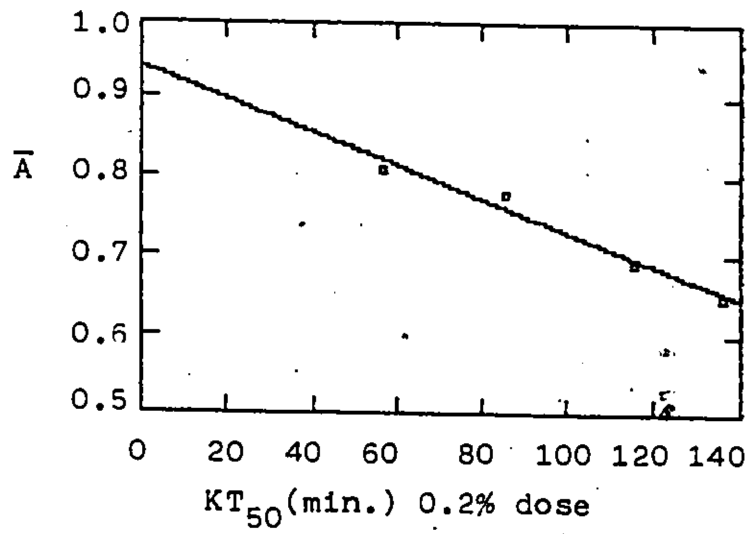


Fig. 3.4.11 relationship between avoidance and resistance for females of the trm lines and Canton S.

DISCUSSION

I - Review of Results: i) The avoidance index and the avoidance response - the results of the control runs show that the avoidance behaviour seen in the choice (0%-1%) runs is a specific response to malathion. As was pointed out in section 3 of the Results, the values of the avoidance index given for the various lines are not absolute, but relative indicators of the strength of the flies' behavioural response to malathion. The testing dose of 1% malathion by volume was chosen because it gave a good spread of $\bar{A}$ values for both the wild-caught and mutant lines, and this dose is also approximately equal to the recommended application dose for the insecticide preparation used in this study, a fact of some importance with regards to considerations of possible selection effects upon the natural populations.

ii) Wild population lines - a) ranges of responses - the lines sampled from the wild populations show a wide range of resistance to malathion (Results Part 1 section I), consistent with the observations of Morton and Singh (1982). The range in resistances is illustrated in Fig. 3.1.1 (page 32), and is shown by a comparison of lines A-11 and A-15-R, which can be separated by a discriminating dose of 1% malathion, that will kill all of the A-11 flies in 60 minutes, yet give a total mortality of less than 10% for A-15-R flies.

A similar wide range in levels of response is seen in the results of avoidance testing of these lines (Results Part 1 section II), with this range being illustrated in Figure 2.5 (page 23). All lines avoided malathion to some degree, and they can be grouped according to their values of $\bar{A}$ into avoidance response classes which cover the entire range of $\bar{A}$ values from 0.6 to greater than 0.9, with an overall average $\bar{A}$ value of 0.75.

b) genetic bases of responses - 1) intrastrain differences - there were significant differences found in several lines between the sexes for resistance (Table 1) and avoidance (Table 2). In the case of resistance, where the sexes differed the female was the most resistant in each instance, and the resistances of the sexes were found to be strongly correlated (Fig. 3.1.6). For avoidance, the intersex differences followed no similar pattern (ranked by $\bar{A}$ values, six lines showed males greater than females, 4 showed the opposite relationship), and there was an overall positive correlation between male and female $\bar{A}$ values (also in Fig. 3.1.6).

2) interstrain differences - the results of the testing of progeny from the crosses of selected wild population lines (Part 1 section III of the Results) for resistance indicate that the F_1 and F_2 flies tend to resemble the least resistant parent. The results for crosses between line A-11 and lines J-11 and A-10 show no segregation of resistance genes in the F_2 generation, hence differences among these lines for resistance are presumably due to differences at several genetic loci, with no apparent maternal or sex-linked factors. The results from crosses with line A-15-R show that this line carries a recessive autosomal major gene conferring high resistance to malathion, but this locus is not responsible for the high avoidance shown

by A-15-R males, as shown by the results of the F_3 selected progeny, all of whose parents were resistant to a dose of 1% malathion, and hence were homozygous for the high resistance allele.

Since there was no method available for detecting split populations for differing strengths of avoidance, the F_2 generation from the wild population crosses were not tested for avoidance, and the only major gene effects which could have been detected for this trait would be those due to dominant loci, which would show up in the F_1 results. These results, however, do not give any definite indication of major gene effects, in fact the F_1 progeny in general show lower $\overline{A}$ values than either parent, suggesting that however many loci exist which have an effect upon the avoidance response, those which decrease it tend to be dominant over those which increase its strength.

c) relationship between resistance and avoidance: a comparison of the resistance and avoidance testing results for the lines which had complete resitance profiles determined for them showed no significant correlation between the two responses, however, comparison of $\overline{A}$ values and knockdown (1% malathion dose) for males of these lines and the highly-avoiding lines did give a significant positive correlation, indicating the very strongly avoiding lines are also highly resistant.

d) comparison of lines sampled from different localities: the wild population lines were sampled from 3 localities: urban Hamilton (A and B lines), Jordan orchard (J lines) and Vineland orchard (V lines). The samples from the latter two populations are too small to allow any definitive conclusions to be made, but it is interesting to note that the V lines as a group are among the most strongly avoiding and highly resistant of all lines tested (eg. average $\overline{A}$ for A&B lines = 0.74, for V lines =0.87; mean KT_{50}'s for 1% dose are 25 and 75 min. respectively), consistent with what would be expected if the V lines come from an area where selection pressure from malathion is high.

iii) Mutant lines: a) avoidance and resistance testing - screening and subsequent testing of the progeny of EMS-treated males led to the isolation and characterization of three X-linked mutations affecting malathion resistance, which were designated as trm mutants (for toxin response- malathion). The mutations' effects and map locations are as follows:

trm-59cs - flies carrying this mutation show increased resistance to malathion over wild type (Canton S), and decreased strength of the avoidance response. The mutation is dominant to the wild type allele for both phenotypic characteristics, and it was mapped to a location of approximately 24 centimorgans from the end of chromosome 1.

trm-12cs - the phenotypic effects upon resistance and avoidance of this mutation are the same as those of trm-59cs, and trm-12cs is also dominant to the wild-type allele, mapping to approximately 40 centimorgans from the end of chromosome 1.

trm-151cs - flies carrying this mutation show decreased malathion resistance compared to Canton S and increased avoidance in the males, while homozygous females show slightly but not significantly higher avoidance that Canton S females. trm-151cs is recessive to the wild-type allele (for resistance effects) and was located at approximately map position 48 on chromosome 1.

Three other mutant lines were also tested, with the following results:

yellow - located at map position 0 on chromosome 1, this mutation gave increased avoidance to malathion without affecting resistance in the Canton S genetic background.

MRM-1 - an autosomal (chromosome 3) mutation which confers high resistance; flies carrying this dominant mutation were also seen to have elevated $\overline{A}$ values, however no consistent results were obtained for avoidance testing in this line.

127 - an EMS induced mutation isolated in an attached-X line which has no effect upon resistance but a general effect upon behaviour (low activity, unpredictable responses during avoidance and control runs, male-male courtship) and fecundity (homozygous line had very low fertility).

b) activity testing - the results of activity testing for the trm lines and Canton S showed no difference in activity level, although trm-59cs flies appear to be more strongly positively phototactic than the other lines.

c) morphological differences - the only phenotypic effects upon morphology seen for the trm mutations were upon the size of adult flies, with flies carrying the trm-59cs allele being larger (heavier) and flies with the trm-151cs allele being smaller (lighter) than Canton S flies. trm-12cs flies showed no significant morphological differences from Canton S flies.

d) developmental rate - results of measurements of developmental time indicate that 12cs flies may develop slightly faster than the other trm lines and

Canton S, but no gross differences were seen among the lines.

e) relationship between resistance and avoidance in trm lines - there is a significant negative correlation between resistance and avoidance among the trm lines and Canton S, both overall and within each sex. As resistance increases, the strength of the avoidance response decreases within this group (Results Part 4).

iv) The nature of the avoidance response: from the discussion in Part 4 of the Results, it was concluded that the malathion avoidance behaviour of the flies can be thought of as consisting of two components: 1) the basic avoidance response, which is common to all lines and is seen as a result of flies leaving the treated surface and tending not to go back upon it during avoidance test runs; and 2) the secondary avoidance response, which results from the flies not only showing the basic response, but also dispersing as far as possible from the treated surface. While there appear to be genetic differences in the strengths of both types of response showing up in the study (eg. most of the differences among the trm lines seem to be due to differences in the strength of the avoidance response), most of the results from the strongly avoiding lines from the wild populations can be attributed to increases in the intensity of the secondary response, as indicated by the positive correlation between the value of A and the numbers of flies going onto the test surfaces during avoidance runs.

II - Discussion of Results - i) Effectiveness of the response assays used: since the aim of this study was to combine practical and evolutionary considerations of the phenomenon (problem) of insect resistance to insecticides, it is appropriate that some mention be made of the potential practical value of the assays that were developed during its course.

a) the vacuum-injection resistance assay - as discussed in the Methods section, the main reason the vacuum-injection technique was developed was to provide a way of measuring the resistance of Drosophila in which the behaviour of the flies could not affect their uptake of malathion. Of course, many methods have been de-veloped for the topical application and injection of insecticides (eg. see Busvine 1957) but most of them would have been tedious and difficult with an insect as small as D. melanogaster. These methods would also have been slower (important, considering the thousands of flies tested in this study), and would have made the detection of split populations difficult due to the intrusion of many sources of experimental error.

Since most mechanisms of insecticide resistance involve metabolism of the insecticides (discussed below), it is likely that resistance measured via the vacuum injection assay will correlate with the resistance measured by other means, and in order to verify this assumption, the results of this assay were compared to those from a feeding assay for various selected lines in Pluthero, Singh and Threlkeld (1982) as follows:

The feeding assay involved recording the knockdown as the flies fed on medium containing 50ppm of malathion (as described in Singh and Morton 1981). The times to 50% knockdown (KT_{50}) for a dose of 10% malathion adminstered by vacuum injection and a dose of 50ppm in the feeding test were determined for each sex of 13 selected and unselected (for resistance) lines derived from the wild populations used in this study.

Approximately 100 flies of each type were tested, and the doses were chosen to illustrate the range of resistances existing among the lines.

The relationship between the resistance measured via the two methods is shown in Figure 4.1. The correlation coefficient between the KT_{50} values measured by the two methods is 0.86 (df=23, $p<0.001$), which indicates that resistance as measured via the vacuum injection assay is comparable to that measured by other means.

While most of the selected lines tested via the feeding assay had approximately the same level of avoidance (90% of $\bar{A}$ values were beteeen 0.65 and 0.8), the results for lines with low $\bar{A}$ values (0.5 - 0.6) suggest that their KT_{50} values from the feeding test are lower than would be expected from their vacuum-injection assay results, thus there may be an effect of behaviour upon resistance as assayed by feeding, an effect which could have been manifested more strongly among the wild population and mutant lines used in this study, whose $\bar{A}$ values cover a wider range.

b) avoidance assay - while it was not directly compared to any of them, it is reasonable to say from a comparison of the results of control and choice runs that the avoidance assay used in this study gives as good an idea of the insects' behavioural responses to the insecticide as any of the other methods used to assay these characteristics in the literature (see Methods section for references). The intent of this assay was not simply to measure the "irritability" of the flies (eg. as measured in mosquitos by counting the number of "takeoffs" from a treated surface), but to give them a choice between a malathion

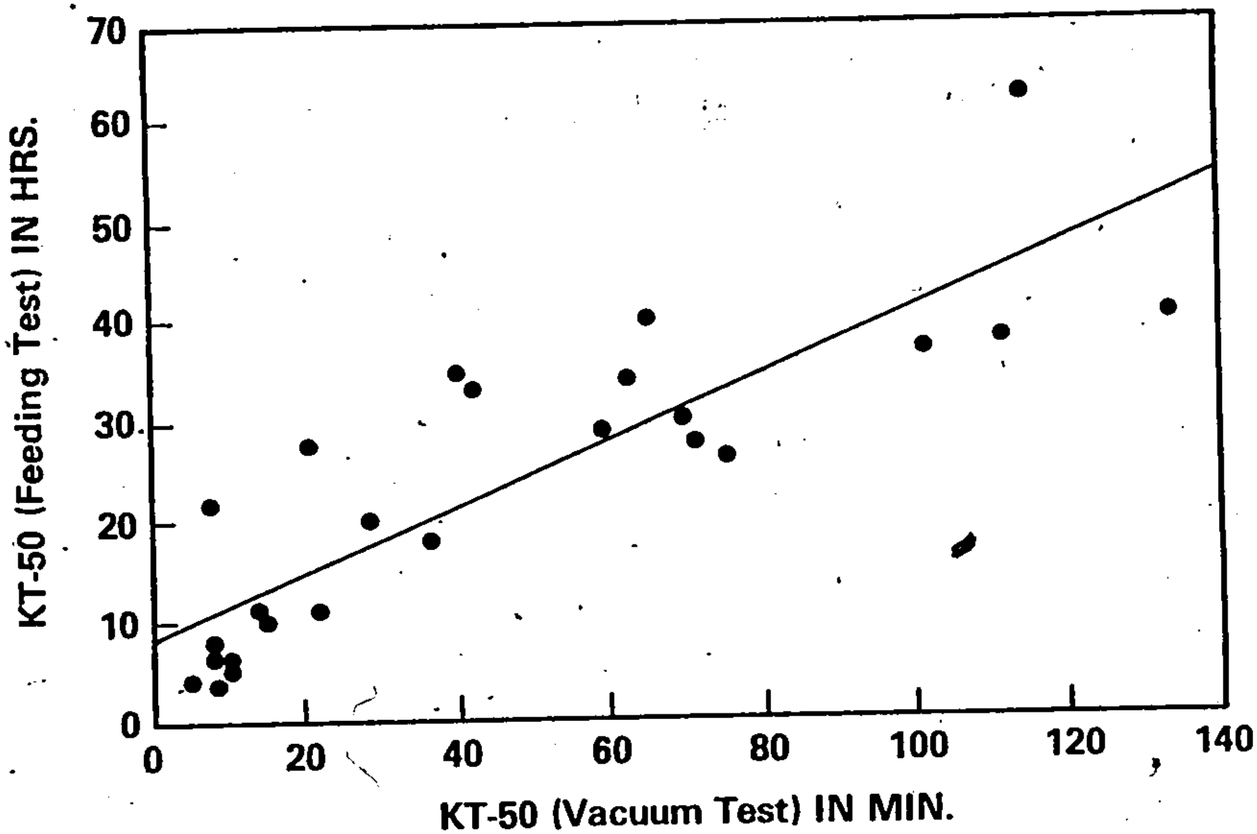


Fig. 4.1 comparison plot of resistances from two assays.

treated and an untreated surface in order to see which they prefer.

The testing chambers were designed with two large surfaces in close proximity to facilitate this choice, and direct observations and results of runs testing of the avoidance run results showed that the flies constantly moved around the chambers' inner surfaces, and hence were indeed making a choice as to where they ended up with respect to the test surfaces.

As a result of using the surface choice set-up, the results from avoidance testing suggest that the behavioural response to malathion showed by the flies actually has two componenents. The main response - which would appear to be the same as the "irritability" measured by others, and the secondary dispersion response. While these hypothesized response components explain the results fairly well, they may be little more than heuristic concepts, requiring further experimentation to elucidate their nature. Here, as in several other instances during the course of this study, the opportunity arose for a new experimental direction to be taken, however it was felt that such a diversion of effort would merely result in the research getting bogged down in the type of particulars that are peripheral to the main thrust of the overall study.

In summary then, it seems fair to say that the avoidance assay employed in this study gives a reasonable indication of the flies' responses to malathion at the behavioural level. Whether the responses actually assayed have any significance with respect to the responses flies would show to malathion in their natural surroundings is not known from direct testing, however, the fact that the avoidance testing results suggest both irritation and dispersal components to the behavioural response is an indication that avoidance in the natural and laboratory situations are likely to be comparable phenomena.

ii) Resistance - a) wild population lines: in selection experiments insecticide resistance usually behaves as a quantitative trait (eg. for D. melanogaster Dapkus and Merrell 1977; King and Somme 1958; Oshima 1958; Singh and Morton 1981; Crow 1954) and yet it has been shown that major genes coding for enzymes and proteins are involved in the breakdown of insecticides (for reviews see Georghiou 1972; Tsukamoto 1969; Plapp 1976). The resolution of this apparent paradox lies in the fact that most of the selection schemes used in these experiments, depending upon the methods of insecticide application employed, could have acted simultaneously on several possible basic mechanisms of resistance (eg. physicochemical, behavioural, morphological), each of which have many genes affecting them (eg. those coding for the many enzymes involved in detoxification pathways - Perry and Agosin 1974). Most of these genes probably produce quantitative differences (Falconer 1981) in resistance within the population, and account for much of the observed response to selection in the experiments, but it is also not unusual for populations to also contain genes segregating within them which produce qualitative differences in resistance.

The results of resistance testing of the wild population lines used in this study indicate that genetic variation of both types exist within these populations. The major gene for resistance isolated in the A-15-R subpopulation is unusual in that it is recessive, while such genes are typically dominant (Plapp 1976).

Aside from this major gene, however, the results of the wild line crosses indicate that the differences in resistance seen among the lines are largely due to the effects of many different loci. This conclusion is borne out by the results of selection for malathion resistance which was done upon mixtures of these lines (Singh and Morton 1981, Pluthero, Singh and Threlkeld 1982), which showed that large increases in resistance can be produced via selection, with approximately 20% of the elevation in resistance apparently being due to the fixation of major genes such as the one found in line A-15-R.

The wide range in resistance seen among the lines from the Hamilton population (A and B lines) does not betoken the existance of strong selection pressure from malathion having been imposed upon it, and thus this population gives a good indication of the range of resistances existing in populations relatively unchallenged by insecticide. While the sample size is small, the uniformly high resistance of the Vineland (V lines) population flies may be a result of malathion use in the orchard from which this population was sampled, which may have selected for flies showing high levels of resistance.

b) mutant lines: the parental wild-type stock within which the trm mutations were induced, Canton S, has been used in laboratory genetics studies for over fifty years, and hance has never been exposed to any of the modern insecticides in the natural environment. Its level of resistance to malathion is similar to that of the most sensitive lines among the wild population flies tested in this study. As far as is known, the trm mutations described in this study are the first

X-linked malathion resistance factors to be isolated in D. melanogaster. Kikkawa (1967) isolated several X-ray induced alleles of a locus at map location 64.5 of chromosome II in D. melanogaster (Hikone strain) which affected resistance to the organophosphate insecticide parathion. All of the loci examined by Kikkawa that affected resistance were found to be dominant, as are the trm-59cs and 12cs mutations in this study, although his methods of isolation would not have picked up recessive mutations.

X-linked mutations were screened for in this study because: 1) no bias in favour of dominant mutations would exist in the screening procedure, allowing recessive alleles such as trm-151cs to be isolated. 2) X-linked loci are useful for other genetic analysis techniques, such as mosaic studies. The effects of the trm mutations upon resistance are fairly large ($\pm$ 50% change in KT_{50} value of Canton S), producing differences between lines comparable to those seen between some of the wild population lines, which presumably differ at several loci.

Since the main interest of this study was the resistance-avoidance interaction, no investigation was made into the mechanisms of malathion resistance affected by the trm loci. The only phenotypic effects noted for these genes besides their effects upon resistance and avoidance were upon adult size (trm-59cs larger, 151cs smaller, than Canton S), which may have a direct effect upon resistance to a small degree (Brown 1958, Perry 1958, Bochnig 1960), but is not likely to be the main reason for the resistance differences, since if size was a strong determinant of resistance, we would expect the intersex differences to be much greater than the inter-strain differences in resistance, since typically the weight differences between males and females of the same line are over three times as large as the differences between the same sex of different lines.

The trm mutations in general did not have any effect upon developmental rate, and the possible slight effect of trm-12cs is in the opposite direction to what would have been expected from other studies, which have shown a relationship between increased resistance (to DDT) and late emergence (Pimentel at al. 1951).

Since the vacuum-injection method was used to apply malathion in this study, the resistance mechanisms at work in the mutant lines most likely involve metabolism of the insecticide or susceptibility of the target sites (the target enzyme, acetylcholinesterase, is coded for by genes on chromosome III), as opposed to other uptake resistance mechanisms such as reduced cuticular penetration.

The trm-151cs mutation is interesting in that it has a negative effect upon resistance, and is also recessive, which suggests that it may cause a defiency in some factor (enzyme) required for the metabolism of malathion. The trm mutations as a group, along with those studied by other workers, serve to point out yet again the large number of loci in the genome which have a potential effect upon the insects' response to insecticide, and it may be that further work will show that, as suggested by Kikkawa (1967), most of the variation seen in natural populations for resistance is the result of the superimposed qualitative effects of alleles at many loci, as opposed to truly quantitative gene effects.

iii) Avoidance - a) wild populations: as mentioned in the Introduction, the irritability of mosquitos to DDT has been shown to be modifiable by selection (Gerold and Laarman 1964, 1967). The wide and continuous range in the strength of avoidance to malathion seen among the wild population lines in this study and the lack of any apparent major gene effects upon avoidance point to the importance of quantitative genetic effects upon this characteristic within the populations sampled. The avoidance response has been experimentally reduced in strength via selection upon mixtures of the wild population lines (Pluthero, Singh and Threlkeld 1982), which is further support for this conclusion. The results of the crosses indicate that genes which lower the strength of the avoidance response are dominant to those which raise it, which is similar to what is seen among the trm lines.

The cross results thus suggest that lines with strong malathion avoidance are homozygous for recessive alleles of several loci affecting this trait, and thus the avoidance responses seen in the lines tested may have little in common with their originally captured parents, but instead are determined by which of the avoidance modifying alleles were present originally and have subsequently been fixed within the inbred strains, or are still segregating within them (the high variances in A values seen in some lines may be due to several different genotypes for avoidance-affecting loci existing within them, which are not resolvable by the assay used).

Given this consideration, the results of avoidance testing suggest that the frequency of high-avoidance alleles is highest in the Vineland population represented by the V lines, each of which shows a strong avoidance response. This may be the result of malathion selection pressure in the orchard setting, as was hypothesized as an explanation for the high resistance also seen in these lines. This hypothesis is weakened by the small sample size involved, and also because we do not know for certain if the ranges of responses seen in the study have any significance with respect to possible selective pressures to which the populations may have been exposed. Since the malathion dose used in the assays are within the range of the recommended application doses however, it is likely that the results of this study do give an indication of the responses of wild population flies to the types of selection pressure from malathion they are likely to encounter.

b) mutant lines: the relative strength of the avoidance response shown by the Canton S flies is slightly higher than the mean of the wild population sample, and thus it might be said to be in the "typical" range. As with their effects upon resistance, the trm mutations have large effects upon avoidance, with the trm lines spanning the entire range of $\bar{A}$ values seen in the wild populations.

In lines 127 and cyloc, we have examples of genes with a general effect upon activity affecting the avoidance response, but the effects of the trm mutations upon avoidance are apparently specific for avoidance, since no effects upon activity were found for them. The results for the MRM-1 line, which did not give consistent results in the avoidance assay, suggest that either the mutation responsible for the increased resistance of this line has an unprediactable effect upon avoidance behaviour, or the line used for study has other genes segregating within it which affect this characteristic.

iv) The relationship between resistance and avoidance - in the Introduction, several studies were mentioned which descrived relationships between resistance and behavioural response to insecticides. For malathion, conflicting results were obtained with two different species; one (Euxesta notata - Hooper and Brown 1965) showed a decrease in resistance accompanied by an increase in irritability, while houseflies, both wild-caught and selected in the laboratory, showed strong avoidance accompanied by high resistance. Since each of these studies, and those reported for other species and insecticides, involved lines which had been subjected to natural selection (eg. the S and R strains of houseflies studied by Kilpatrick and Schoof 1959) or artificial selection (Schmidt and LaBrecque 1959), the differences between lines were undoubtedly due to genetic differences at several loci. The relationships between resistance and avoidance seen in the studies reporting high resistance to be accompanied by high avoidance most likely arose due to the effects of selection acting upon both responses - i.e. in wild populations subjected to spraying, there would be selection for individuals who could survive the initial exposure (highly resistant) and limit subsequent uptake of insecticide (avoidance of treated surfaces).

In the study which reported increased irritability accompanied by lowered resistance (Hooper and Brown 1965) selection was done by placing larvae in tubes with insecticide-impregnated paper at one end - hence selection was largely for increased avoidance alone, and the decrease in resistance seen may be a genuine pleiotropic effect of the genes conferring high irritability. Indeed, Hooper and Brown suggest that the relationship seen, and another similar one noted for a DDT-resistant strain of Aedes aegypti which showed less irritability than a susceptible one, "would be expected if resistance involved detoxication of the absorbed insecticide before it could stimulate the afferent nerves of the legs" and thus trigger the aversive response.

Since the physiological and behavioural responses of insects to insecticides are modifiable by changes at many different genetic loci, it is likely that genes can have effects specifically upon one response which override the effects of loci modifying the other - i.e. genes coding for detoxifying enzymes may reduce the probability of insecticide reaching the afferent nerves, but other genes could still give an increase in irritability via other sensory modalities, or by increasing the sensitivities of the afferent neurons to whatever insecticide does reach them. This fact, coupled with the possibility that many resistance mechanisms would not affect the detection of insecticide by the nervous system, would lead us to expect that the relationship between resistance and avoidance seen by Hooper and Brown would be rarely seen in natural or laboratory selected populations.

Experiments done with mixtures of the wild population lines used in this study have shown that avoidance and resistance can be altered independantly by selection, and thus each characteristic is affected by many genes segregating within the wild population, as the results from testing the individual lines indicate. Thus the lack of a correlation between resistance and avoidance in the 8 lines for which complete resistance profiles were determined is not surprising, and the positive correlation which emerges for the males of lines tested for resistance at the 1% dose illustrates how such a correlation between resistance and avoidance can arise. These lines can be placed into two groups, one with relatively low resistance and moderate avoidance strength (showing no correlation between responses within the group), and the other with strong avoidance and high resistance. The high resistance and avoidance response strengths of the latter group are much more likely to be attributable to selection having acted to increase both responses (the fact that most of them are V lines supports this) than to the pleiotropic effects of high-resistance genes alone. Thus the results reported in the earlier studies are not contradictory, but rather arise from the different effects of selection upon the populations under investigation in each case.

The only cases where we would expect to see the type of negative relationship between the two responses hypothesized by Hooper and Brown would be those in which one response can be altered independantly of the other, and thus the effects of, for example, changing the level of resistance upon avoidance could be seen. The single response could be altered by a special selection scheme or, as was done in this study, via the indiction of single mutations.

Since the trm mutations were screened and isolated according to their effects upon the physiological response to malathion of the flies, they provide an ideal opportunity to measure the effects of genes which alter this response upon the behavioural response. The Canton S line within which these mutations were induced has never "seen" malathion or any other insecticide, and hence is not likely to posess any specialized (i.e. selected) adaptations for responding to such substances on a physiological or behavioural level, as indicated by its relatively low resistance and "average" avoidance response, when compared to the wild population lines.

The negative correlation seen between resistance and avoidance in the trm and Canton S lines suggests that changes in resistance due to single gene differences have a pleiotropic effect upon the avoidance response of a type similar to that proposed by Hooper and Brown for the genes they selected for. If the presence of malathion on the treated surfaces in the avoidance test chamber is detected by the flies according to its effects upon the nervous system, then there may be a threshold concentration which, once it is reached in the tissues surrounding the afferent neurons, initiates an aversive response. The existance of such a threshold is suggested by the results of avoidance testing with different concentrations of malathion (Results, Part 3), with lower concentrations presumably requiring longer contact times to cause malathion concentrations within the flies to reach the threshold value, as seen by the lessening of the strength of the avoidance response with decreases in the concentration of malathion on the treated surface.

Thus there are two likely mechanisms by which the trm mutations alter malathion resistance and also avoidance: 1) if the mutation alters the rate of malathion detoxication, it will also alter the rate at which the avoidance response threshold is reached at the afferent neurons, thus altering the time required to initiate the aversive response; 2) if the mutation alters the susceptibility of the target enzyme (acetylcholinesterase) to malathion, it may also alter the response threshold concentration itself, since such a change is likely to alter the sensitivity of the afferent nerves to malathion.

Whatever mechanisms may be involved, the trm mutations show that a gene which alters the physiological tolerance towards a substance can also have a specific effect upon the behavioural response to that substance. The results for the other mutant (eg. the cyloc line, which had increased avoidance with no change in resistance) and wild population lines show that each response also has genes acting specifically upon it, which may obscure the effects of the pleiotropic genes such as those of the trm type. The effects of trm-type genes thus show up only if: 1) they are the main genetic cause of differences between lines for a response, or 2) if selection has acted only on one response, as seen in the experiments of Hooper and Brown.

III - Evolutionary perspectives: as I have already mentioned, the adaptations insects have developed to deal with human efforts to control them provide some of the best examples of recent and current products of the process of natural selection which, by working "solely by and for the good of each being" (Darwin 1859) has produced the complex, immense living system blanketing the Earth. In the course of efforts to simplify this complexity and produce local situations which they can handle, through the replacement of natural ecosystems with monocultural farming, men have found that they cannot escape the complexities of nature after all, since the world is teeming with opportunistic species ready to move in and take advantage of even the most ambitiously planned human disruption.

Thus the study of how adaptations arise and are perpetuated is important in two respects: first, there is the necessity in every questioning mind for the answers to the riddle of what causes create and maintain order in a world which shows no visible impediments to a slide into chaos; and secondly, as a creature whose only advantage over others is the ability to alter the world around him, man has need of the knowledge which will guide him in making changes of the greatest benefit, or at least allow him to steer clear of disruptions which lead to catastrophe.

The war between man and insect that has taken place over the last few decades has pitted the inventiveness of the former against the potential for developing adaptations of the latter. The main weapons of humanity have been the biocides - chemicals of ever-increasing sophistication which have each in their turn been succeeded by newer ones as they fell victim to their own noxious side-effects or became obsolete as the genetic and developmental systems of their targets produced individuals resistant to them.

Insect resistance to biocides has been studied mainly because of its practical significance, not because it is an interesting evolutionary phenomenon. Since the selection pressure from biocides which gives rise to resistant insect races is typically very severe, it is not likely to provide a good example of the type of gradually acting forces which are generally considered to give rise to new species, although current theories suggest that rapid speciation may not be a rare occurrance at all (Gould 1982). When the totality of the possible adaptive responses of insects to the disruptions caused by biocides are considered, however, they become interesting from an evolutionary study perspective because they allow: 1) an investigation of how the different aspects of an overall adaptation are related, and 2) they may provide an accelerated look at how much more slowly acting processes of adaptive change occur.

Thus, while some aspects of the insect response to biocides have not traditionally been considered to be important from a practical application perspective, such as the behavioural response, (since insecticide is typically applied in such a manner as to either negate the effects of behavioural responses eg. spraying of mosquito breeding ponds, or make them unimportant, eg. if insects avoid sprayed crops, the insecticide has accomplished its purpose) all aspects of the adaptive responses are important for evolutionary studies, and some may also prove to have an eventual practical application (eg. does avoidance aid in maintaining residual populations which can re-invade an area treated with rapidly-degrading compounds such as organophosphate insecticides).

The results of investigations done in this study with wild populations and the results of selection experiments done with the same populations show that the different aspects of the biocide response have largely separate genetic bases and that they respond independantly to selection. There are also indications of a large amount of genetically determined variability in each response, making it likely that in D. melanogaster and similar species, selection for biocide responses (or similar traits) involves changes at many loci, with most of these changes having small individual effects.

In the trm mutations, however, we have a class of genes which, acting alone, have a co-ordinated effect upon the total response. While such genes may not be important for the development of adaptations in species with populations posessing a reservoir of genetic variation among loci affecting the traits to be changed by selection, there may be instances where trm-type genes are important in developing adaptations: 1) providing they are not peculiar to Drosophila (unlikely), trm-type mutations may be a main source of genetic variation in some species (eg. those with little genetic variation in general); 2) for some types of adaptations, single gene changes may be very important.

An example of the second instance listed could be those adaptations which allow phytophagous insects to exploit new hosts. In many respects the war between man and insects parallels the evolutionary struggle between plants and the insects which would exploit them that has been going on for millions of years. Like humans, plants employ a wide range of anti-insect chemicals which either repel, inhibit or destroy would-be parasites (Maugh 1982). Thus insect adaptations to man-made and plant-produced inhibitory substances are likely to be similar and may arise via similar mechanisms.

The exploitation of new hosts has been suggested to be an important phenomenon for facilitating rapid sympatric speciation (Bush 1975, 1974) involving few genetic changes. Instances of rapid speciation have been reported (in Hawaiian moths - Zimmerman 1960, in laboratory population of D. paulistorum - Dobzhansky 1972), which suggest that the creation of new species does not require major genetic changes; and cases where new host races have emerged rapidly (in the hawthorn fly - Bush 1974, in diprionid sawflies - Knerer and Atwood 1973, in Drosophila - Heed, 1971) suggest that the adaptations required to exploit new host may not require major genetic changes either, however the processes of host range expansion and speciation have not been definitely linked, since speciation also requires that reproductive isolation arise between populations on the "new" and "old" hosts. If this reproductive isolation can arise due to ecological factors (i.e. reduced probability of insects on different host species encountering each other and mating), or in response to divergent selection pressures acting on populations specialized for different habitats, then the adaptation can lead directly to speciation, a process that has been called "adaptive speciation" (Bush 1982).

It has been argued that single or few gene changes with major effects are not important in the evolution of natural populations (Lande 1981), and they would be insufficient to preadapt individuals to a new niche (eg. host) (Mayr 1963), since such adaptations would involve both behavioural changes in host preference and the ability to exploit the new niche (i.e. survive in it). The primacy of allopatric models of speciation (Dobzhansky 1970, Mayr 1963) rest upon the assumptions that such adaptive changes arise slowly and that their components (habitat selection behaviour, physiological adaptations, etc.) are not under the control of single genes which can produce large qualitative effects in these components. The results from the wild and selected populations of D. melanogaster discussed in this study provide examples of the type of broad-based genetic variation which would be expected to be involved in selection acting to produce adaptations during allopatric speciation or under laboratory selection (eg. Wasserman and Futuyma 1981).

The ease with which major genes for resistance can be found in natural populations, however, does not allow the ruling out of their possible role in giving rise to new adaptations, and there is evidence that shifts in host use require little in the way of genetic, karyotypic or morphological changes (eg. in the hawthorn fly, Rhagoletus - Bush 1974).

Thus the question becomes, can single gene changes produce significant adaptations? With regards to the host-choice situation, the two components of the adaptive response would be: 1) host discrimination behaviour, and 2) the ability to survive on the new host. If, as it seems likely, host discrimination depends upon chemosensory cues, then the demonstrated effects of single gene changes upon detection and response (attraction or repulsion) to chemical stimuli (Kikkuchi 1973, Falk and Atidia 1975) mean that single gene changes can be important in altering this behaviour (single mutations have been shown to have large effects upon sensory systems in general - for reviews see Pak 1975, Ward 1977). Single gene changes may also have large effects upon survival on host plants, as evidenced by the many examples of major gene effects upon biocide resistance, and also where specific genes have been linked to survival on specific hosts (Hatchett and Gallun 1970).

According to the model proposed by Bush (1974), shifts in host species by insect parasites could occur in situations where the old and new hosts are interspersed if: 1) a single gene change gives a change in host choice behaviour and the insects are able to survive on the new host (cases where survival is equivalent or better on normally non-exploited hosts are known - eg. Wasserman and Futuyma 1981); or 2) if larvae cannot survive on the new host, then there must be a mutation at a locus affecting survival as well before the host switch can be successful.

What little evidence there is for single gene changes controlling host choice (Huettel and Bush 1972) is ambiguous, and the model involving such a change leading to speciation has been described as entailing "such a concatenation of unlikely events that it is implausible" (Futuyma 1979). For example, Mayr (1963) points out that for the type of host change proposed to lead to speciation it requires greater fidelity to a single host than is commonly seen - i.e. the mutation allowing the population on the new host to survive must not only permit survival on it but uniquely adapt individuals carrying the mutation to the new host alone. Cases such as the first one listed above (single gene change in host choice behaviour) would be unlikely to give rise to reproductive isolation since nothing stops the insect from switching back and forth between hosts.

The most likely situation which would give rise to sympatric speciation via host change would be the second listed above, where the individuals have genetic preadaptations both for choosing and surviving upon the new host, If the gene responsible for improved survival on the new host also decreases survival on the old host (eg. the results of most selection studies show that factors which increase biocidal resistance are generally deleterious in a biocide- free environment and are lost quickly when selection is relaxed), then there will be selection pressure acting upon the old and new host races to limit interbreeding, and speciation may occur.

The main problem with this model as put forth by Bush is that if two mutations are required, it is difficult to see how they can ever occur together, since the host-response gene is useless without the survival gene, and the survival gene itself lowers fitness in individuals living on the original host, hence it is not likely to establish itself in the population (especially if, like most resistance genes, it is dominant) with a sufficient freqency to make co-occurance of the two mutations likely.

A mutation like the trm-59cs or trm-12cs alleles, however, eliminates this problem of the unlikelyhood of co-occurring mutations, since one genetic change can alter both the behavioural and survival components of the adaptive response in the desired fashion. Of course, it is far from proven that such a mutation could actually give the necessary behavioural and physiological changes that allow host changes in the natural situation, and such changes may still not be significant with respect to speciation. In the trm-type mutations, however, we do have evidence that major gene changes may in some instances prove to be adaptive, and may also be sufficient in and of themselves to preadapt individuals to a new niche, leading possibly to sympatric speciation, but more likely to the niche expansion of a single species.

The role of the trm-type mutations in the evolution of natural populations is unknown, and it may be that these creations of laboratory manipulations have no evolutionary significance, however, it seems to me that such potentially useful means of producing coordinated changes in adaptive responses at one genetic stroke would not have been neglected by a nature whose complexity and inventiveness have shown themselves again and again to thwart, cajole and mystify mankind.

IV - Summary: in conclusion, the results of this study, other than the educational benefits its execution conferred upon its perpetrator, can be condensed as follows:

i) the more neglected of the two components of the adaptive response of insects to biocides, the behavioural (avoidance) response was investigated and found to be made up of a basic aversive and a secondary dispersive component.

ii) natural populations were found to posess a large amount of genetically determined variation in both physiological and behavioural responses to malathion. There is also evidence that these characteristics have been affected by selection in the local populations.

iii) the relationship between avoidance and resistance in natural populations indicates that both responses are independantly modifiable by selection.

iv) a class of X-linked mutations was induced which show a negative correlation between resistance and avoidance. These mutations may prove useful in further studies of resistance and avoidance mechanisms (eg. via genetic mosaic studies), and they may also represent a class of mutations which provide co-adapted changes in responses that may prove to be important in some evolutionary situations.

v) practical methods (already published) for assaying avoidance and resistance were developed, which may prove themselves to be useful in studies of economically important insects.

APPENDIX A

Data Analysis Procedures: data obtained from the resistance and avoidance assays was analyzed via a series of computer programs which were written in the Applesoft Basic language and run on an Apple II Plus computer. The program descriptions are as follows:

1) Resistance data analysis program: largely written by Dr. R. A. Morton, this program was used to take raw data from vacuum injection resistance assay runs and: a) convert individual run results into cumulative percentage knockdown distributions and plot them (probability scale) opposite the log. time post-injection to give knockdown curves; and b) calculate the KT_{50} value and its standard error for each curve (for sample outputs of this program, see Appendix H following).

2) Avoidance data analysis programs: the raw data from avoidance test runs was filed on magnetic diskettes and analyzed as follows:

a) one program was used to take the raw data files and convert each observation into an avoidance index value. The runs within a group (same strain and sex) were compared for homogeneity via Model II single-classification analysis of variance with unequal sample sizes (Sokal and Rohlf 1969, p. 208). The avoidance index values within each group that was found to have its run results homogeneous were pooled and stored as index data files.

b) before any further analysis was done, the index file results were compared via a program which performed Bartlett's test for homogeneity of variances (Sokal and Rohlf 1969, p. 370 - the raw data files were also compared via this test before homogeneity testing). If the variances were found to be homogeneous, the index files were comapred via anova in a program similar to the one described above, and if the variances were found not to be homogeneous, the index results were comapred via a program which performed a means test (test of equality of means of two samples whose variances are unequal, Sokal and Rohlf 1969, p. 374).

c) the index results were tested for randomness of sequence via a computerized runs test above and below the median (Sokal and Rohlf 1969, p. 628).

d) in addition to these programs, others were used to print out the avoidance index distribution histograms, run observation sequences and raw and processed data readouts found throughout the text and appendices.

3) Correlations: the correlation plots seen throughout the text were produced by a program which takes the data to be compared and plots them, determines the best line through the points via linear regression, and calculates the value of the product moment correlation coefficient, r (Sokal and Rohlf 1969, p. 508).

APPENDIX B

Wild population resistance testing data: the results of individual vacuum-injection assay runs for various doses of malathion used on the wild population lines are listed on the pages following. After the raw data, the knockdown curves are given for each sex, strain and dose. These curves were plotted using the mean percentage knockdown values listed in the raw data, and were plotted on log-probability paper, then traced onto the graphs shown for clarity (note that the time scales are not the same in each instance). In most cases, the points on the dose-response curves shown in Figures 3.1.1 and 3.1.2 in the Results section were obtained directly from the raw data, and where readings at the appropriate time (t=15 min. post injection) were not available, knockdown values were extrapolated from the knockdown curves.

The temperature during testing was 23°-24°C, the relative humidity was approximately 30% and all flies were aged 4-7 days at the time of testing. Unless otherwise indicated, the sample size per run was 20 flies.

Table B.1 - knockdown results for a dose of 1% malathion.

Line		Time: (min.)	10	15	20	25	30	35	40	50	60	70	80	90	100	110	170
		#down															
A-6	f	1.	3	6	8	9	10		13	14							
		2.	0		5	8	11	15	16	17	18						
		3.	1	5	7	9	11		12	14							
		4.	1	4	9	11	12		15	17	18						
		5.	0	2	5	10		12	13	15	18						
		6.	2	3	7	9	10	11	12	14	16						
	%		5.8	20	34	47	53	63	68	76	88						
	m	1.	1	5	10	13	15	16	18								
		2.	2	4	7	10	13	14	16								
		3.	5	8	13	16	17	18	19								
		4.	4	9	12	15	18	19	20								
		5.	2	8	11	14	18	19	20								
	%		14	34	52	68	81	86	93								
A-7	f	1.	2	2	4	7	8		9	12							
		2.	2		4		6	8	9	12							
		3.	1	5	6	8	9		11	12	14						
		4.	0	1	2	4	5		9	10	12						
		5.	2	4	5	7	8		9	10	11						
	%		7	16	21	30	36	40	47	56	62						
	m	1.	7	12	13	17											
		2.	9	15	19												
		3.	8	16	18												
		4.	8	15	19												
		5.	8	13	17												
		6.	5	11	15	17											
	%		38	68	84												
A-8	f	1.	2	4	7		10		15								
		2.	3	7	9		11		15								
		3.	2	3	5	7	9		14	17							
		4.	2	4	5	7	9		13	17							
		5.	3	5		8		10		15							
		6.	1	3		8		12		16							
		7.	1	4		9		12		17							
	%		10	21	33	39	49	57	72	84							

Table B.1 continued

Line		Time: (min.)	10	15	20	25	30	35	40	50	60	70	80	90	100	110	170
			#down														
A-8	m	1.	4	4	6	7	9	12	13	16							
		2.	4	7	8	11	13	14	14	17							
		3.	3	7	8	9	11	12	15	18							
		4.	2	2	7	10	12	15	16	19							
		5.	1	4	8	10	11	12	14	17	18						
		6.	1	4	7	8	12	13	13	15	17						
		7.	3	5	7	9	11	13	14	15	16						
	%		12	23	36	47	57	65	71	84							
A-10	f	1.	1		5		9		11	13		16					
		2.	1		5		7		10	13		15					
		3.	2		6		11		12	13		16					
		4.	1	4	7	10	11		12	13	15	17					
		5.	2		8	9	11	12	13	14	15	16					
	%		7		31		49		57	65		80					
	m	1.	3	12	17	18	19										
		2.	5	10	15	13	18										
		3.	4	12	13	16	17										
		4.	7	11	14	16	17										
		5.	6	12	14	16	17										
	%		25	57	73	83	88										
A-11	f	1.	8	11	14	17	18										
		2.	7	12	15	17	18										
		3.	8	13	17	19											
		4.	6	11	14	16	17										
		5.	6	12	15	18											
		6.	5	13	16	18											
	%		33	60	76	88											
	m	1.	14	19	20												
		2.	13	17	19												
		3.	11	14	17												
		4.	13	17	19												
		5.	13	18	20												
		6.	13	18	20												
		7.	12	16	18												
	%		64	85	95												

Table B.1 continued

Line			#down														
		Time: (min.)	10	15	20	25	30	35	40	50	60	70	80	90	100	110	170
A-12	f	1.	0		3		4			5		12		17			
		2.	0		1		5		7	10	11						18
		3.	0		0		1		3	5							17
		4.	0		3		5		6			8	9	10	11		15
		5.	1		2		6		8	9		13	15	16			18
		6.	1		3		5		7	7		9	11	13	14		19
	%		2		10		22		31	35		53		61			87
	m	1.	1	4		7		8		11	14						
		2.	2	7		10		12		14	15						
		3.	2		8		10		12	13	14		14	15	16		
		4.	3		5		7		11	13	14		15	16	17		
		5.	2	5		8			9		14	16					
		6.	2		5		9	9	10	15		13	14	14	15		
		7.	4		6		11	11	11	12		14	14	15	16		
	%		11		30		46		55	66			71	75	80		
J-11	f	1.	0	3	5		8		9	12	13	16					
		2.	3	5	6		9		13	16	18	19					
		3.	0	2	4		8		10	13	16	18					
		4.	0	3	5		10		13	15	17	19					
	%		4	16	25	44			56	70	80	90					
	m	1.	3	6	7		8	11	12	15	17			18		20	
		2.	5	7	9		12		13		14		16			19	
		3.	3	4	6		7		10	10	12	12	14	15		17	
		4.	3	4	5		6		8	10	11	12	13			16	
		5.	1	3	5	7		9	11	12	13	15				18	
	%		15	24	32		41		54	59	68		72			90	

Table B.1 continued - results for males only (tested at 25°C.

Line		#down									
	Time: (min)	10	30	60	90	120	130	180	210	240	270
V-3	1.	0	1	3	7	11	12	14	15		17
	2.	0	1	6	10	12		18			19
$\overline{\%}$		0	5	23	43	58		80			90
B-2	1.	0	2	4	7	9	10	11		12	
	2.		2	3	8	9	12	15		16	
	3.		5	6	8	10	12	13		14	
$\overline{\%}$			15	22	38	47	57	65		70	
V-11	1.	1	2	7	11	12		14		18	
	2.	1	5	7	12	15		17		19	
	3.		7	10	12	13	14	16		17	
$\overline{\%}$			23	40	58	67		78		90	
V-31	1.	2	3	7	10	13	15	/15 total			
%		13	20	47	67	87	100				

Table B.2 - results for testing at a dose of 2% malathion.

Line			Time: (min) 5	7	9	11	13	15	17	19	23	25	30	35	40	50	60	70	150
			# down																
A-6	f	1.		2			5			8		12	13		17	18			
		2.		2			4			9		13	15		16	18			
		3.		3			4			6		8	8		12	14			
		4.		3			4			8		8	10		13	16			
		5.		3			4			8		10	11		13	15			
	%			13			21			39		51	57		71	81			
	m	1.	4		9			16		18									
		2.	2		8			16		18									
		3.	2		8			16		19									
	%		13		42			80		92									
A-7	f	1.			6			9		12		15							
		2.			4			9		13		15							
		3.			6			10		14		16							
		4.			5			10		15		17							
		5.			4			10		15		17							
	%				25			48		69		80							
	m	1.	2			10		15		17			19						
		2.	1			10		13		17			19						
		3.	3			6		10		15			19						
		4.	4			5		10		17			20						
		5.	3			10		13		16			18						
	%		13			41		61		80			95						
A-8	f	1.	1					3				9		14	17				
		2.	1					3				11		18	20				
		3.	2					2				7		13	16				
		4.	2					4				8		15	17				
		5.	1					4				16		18	20				
	%		7					16				51		78	90				
	m	1.	2			4		11				15			19				
		2.	1			4		9				13			19				
		3.	0			2		8				10			16				
	%		5			17		47				63			90				

Table B.2 continued

Line			# down Time: (min) 5	7	9	11	13	15	17	19	23	25	30	35	40	50	60	70	150
A-10	f	1.	2		3							9		10		11	14		
		2.	2		3							9		11		13	17		
		3.	1		2							10		13		16	19		
	%		8		13							47		57		67	83		
	m	1.	5		11			15		18									
		2.	4		11			14		17									
	%		23		55			73		88									
A-11	f	1.		7		15		17											
		2.		8		16		17											
		3.		8		17		18											
		4.		9		15		18											
		5.		8		15		18											
	%			40		78		88											
	m	1.	7		17			20											
		2.	10		18			20											
		3.	8		17			19											
		4.	8		16			17											
	%		41		85			94											
A-12	f	1.	1		4			7		10		11		12		15	17		
		2.	1		3			7		10		12		14		16	19		
		3.	2		4			7		8		10		11		13	15		
	%		7		18			35		47		55		62		73	85		
	m	1.			2					12			17		19				
		2.			4					15			19		20				
		3.			3					11			16		19				
	%				15					63			87		97				
J-11	f	1.			3			5		7			11		15				
		2.			5			11		15			18		20				
		3.			1			5		10			16		19				
	%				15			35		53			75		90				

Table B.2 continued

Line		Time: (min)	5	7	9	11	13	15	17	19	23	25	30	35	40	50	60	70	150
		# down																	
J-11	m	1.				4				11			16		19				
		2.				3				11			13		19				
		3.				3				11			16		18				
		4.				8				17			19		20				
	$\bar{\%}$					23				63			83		95				
A15R	f	1.						2				5			5		6	8	12
		2.						3				5			7		8	9	12
	$\bar{\%}$							13				25			30		35	43	60
	m	1.								5					8		10		15
		2.								7					9		13		19
		3.								7					11		14		16
	$\bar{\%}$									32					47		62		83

Table B.3 - results of resistance testing with a dose of 4% malathion.

Line			# down																
		Time: (min)	5	7	9	11	13	15	17	19	23	25	30	35	40	50	60	70	150
A-6	m	1.	1	3		7		16		18									
		2.	2	8		13		17		19									
		3.	1	7		9		14		17									
		4.	1	6		14		18		20									
	%		6	30		54		81		94									
	f	1.	2	3		7			15	18									
		2.	3	5		9			15	17									
		3.	2	8		12			16	18									
		4.	5	10		15			19	20									
		5.	2	3		6			14	17									
	%		14	29		49			79	90									
A-7	m	1.	3		11		18												
		2.	4		13		20												
		3.	2		11		16												
		4.	3		16		19												
		5.	3		13		18												
	%		15		64		91												
	f	1.	3	7		13			19										
		2.	2	8		13			18										
		3.	5	9		15			19										
		4.	6	12		15			20										
		5.	5	8		16			19										
	%		21	44		72			95										
A-8	m	1.	0			6			11	14		18							
		2.	1			4			12	16		19							
		3.	0			4			8	10		16							
		4.	1			7			12	15		20							
	%		3			26			54	69		91							
	f	1.		6			10			17		19							
		2.		2			8			15		17							
		3.		5			11			18		20							
		4.		2			9			17		20							
		5.		1			11			17		18							
	%			16			49			84		94							

Table B.3 continued

Line			Time: (min) 5	7	9	11	13	15	17	19	23	25	30	35	40	50	60	70	150
			# down																
A-10	m	1.	5	7			14		17										
		2.	3	4			15		19										
		3.	1	4			15		19										
	$\bar{\%}$		15	25			73		92										
	f	1.	4	7			17		19										
		2.	6	9			19		20										
		3.	6	9			17		18										
		4.	4	8			16		18										
	$\bar{\%}$		25	41			86		94										
A-11	m	1.		13	19	20													
		2.		9	14	18													
		3.		7	15	17													
		4.		12	18	20													
	$\bar{\%}$			51	83	94													
	f	1.	9	10		19													
		2.	8	12		19													
		3.	8	11		18													
		4.	9	13		20													
		5.	8	18		20													
	$\bar{\%}$		42	64		96													
A-12	m	1.	0		3				17		20								
		2.	1		5				19		20								
		3.	1		5				13		19								
		4.	1		3				14		18								
	$\bar{\%}$		4		20				79		96								
	f	1.	2		4			10		14		18							
		2.	1		3			12		16		19							
		3.	2		3			9		12		16							
	$\bar{\%}$		8		17			52		70		88							

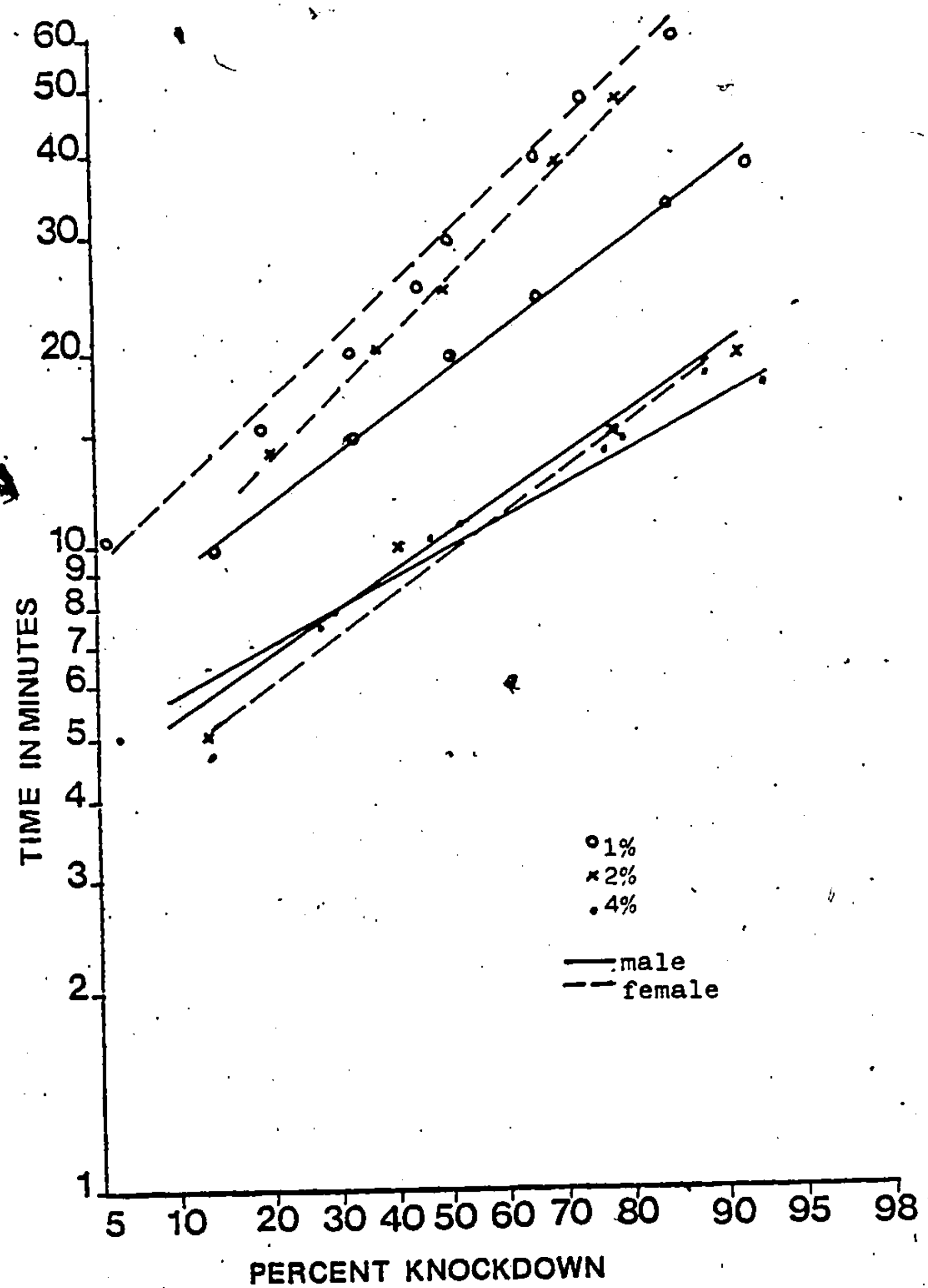
Table B.3 continued

Line		Time: (min)	5	7	9	11	13	15	17	19	23	25	30	35	40	50	60	70	150
			# down																
J-11	m	1.	2		9		14		18										
		2.	2		10		16		19										
		3.	1		5		14		19										
	%		8		40		73		93										
	f	1.	1	3		7		14		19									
		2.	1	5		11		16		19									
		3.	2	4		9		12		17									
		4.	2	4		7		13		18									
		5.	1	5		13		18		20									
	%		7	21		47		73		93									
A15R	m	1.	0		3			6		10			15		18	19			
		2.	1		2			4		8			13		15	16			
		3.	0		1			4		8			15		17	18			
	%		2		10			27		43			72		83	88			
	f	1.	1		2			2		3			5		8	10			19
		2.	1		1			2		3			6		8	10			18
		3.	2		3			6		8			10		12	14			19
	%		7		10			17		23			35		47	57			93

Table B.4 - results of resistance testing at doses indicated.

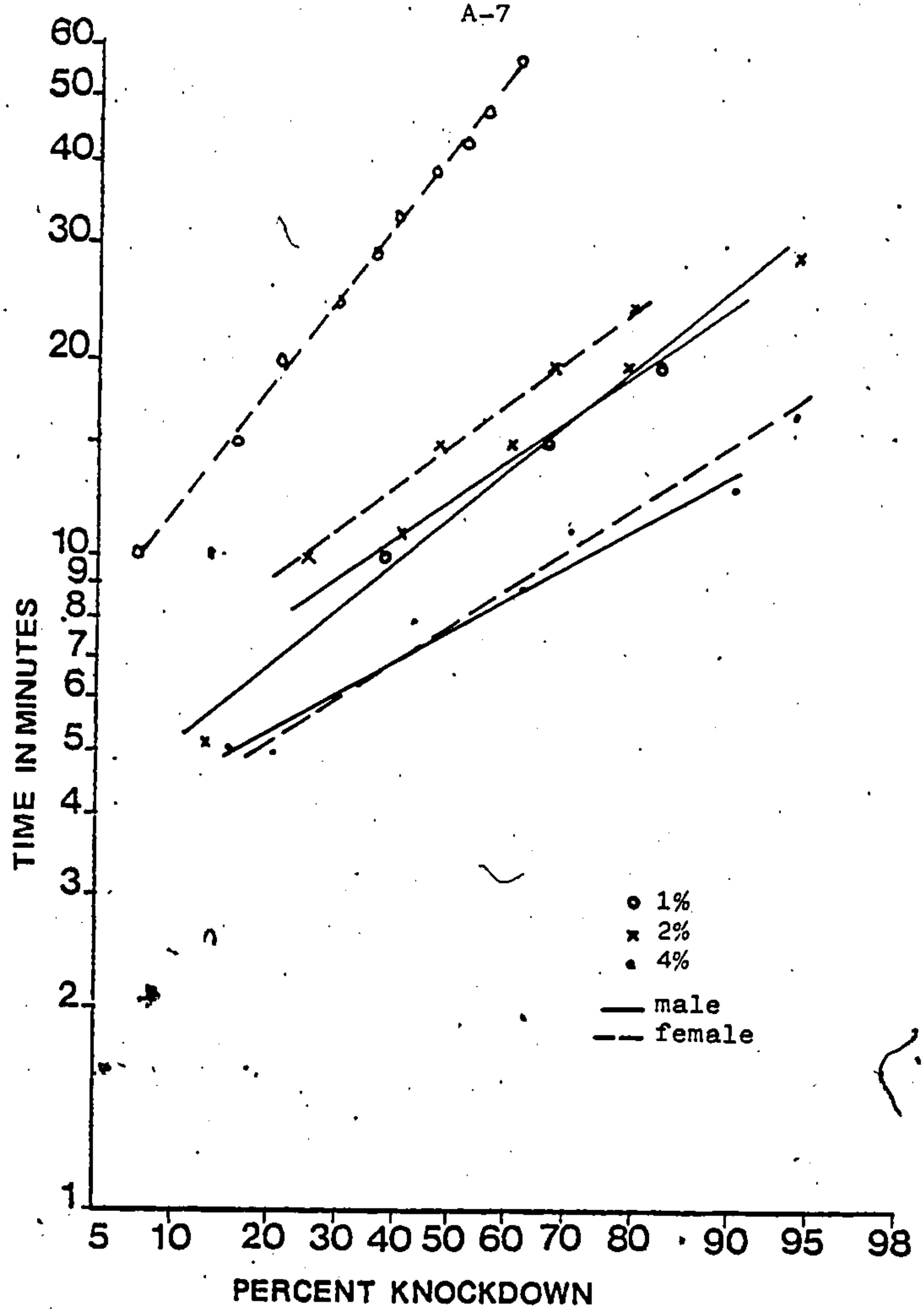
Line			#down Time: (min) 5	10	15	20	25	30	35	40	45	50
A-11 0.5%	f	1.			1	4	5	6		9		13
		2.			5	9	10	12		13		15
		%			18	33	38	45		55		70
	m	1.		3	7	11	11		14		15	
		2.		4	7	8	10		13		14	
		3.		1	6	11	12		14		15	
		4.		4	7	10	11		14		15	
		%		15	34	50	55		69		74	
A15R 10%	f	1.	1	5	10	13		15				
		2.	1	4	10	12		13				
		%	5	23	50	63		70				
	m	1.	1	8	13	18						
		2.	1	7	15	19						
		3.	2	7	13	15						
		%	7	37	68	87						

A-6

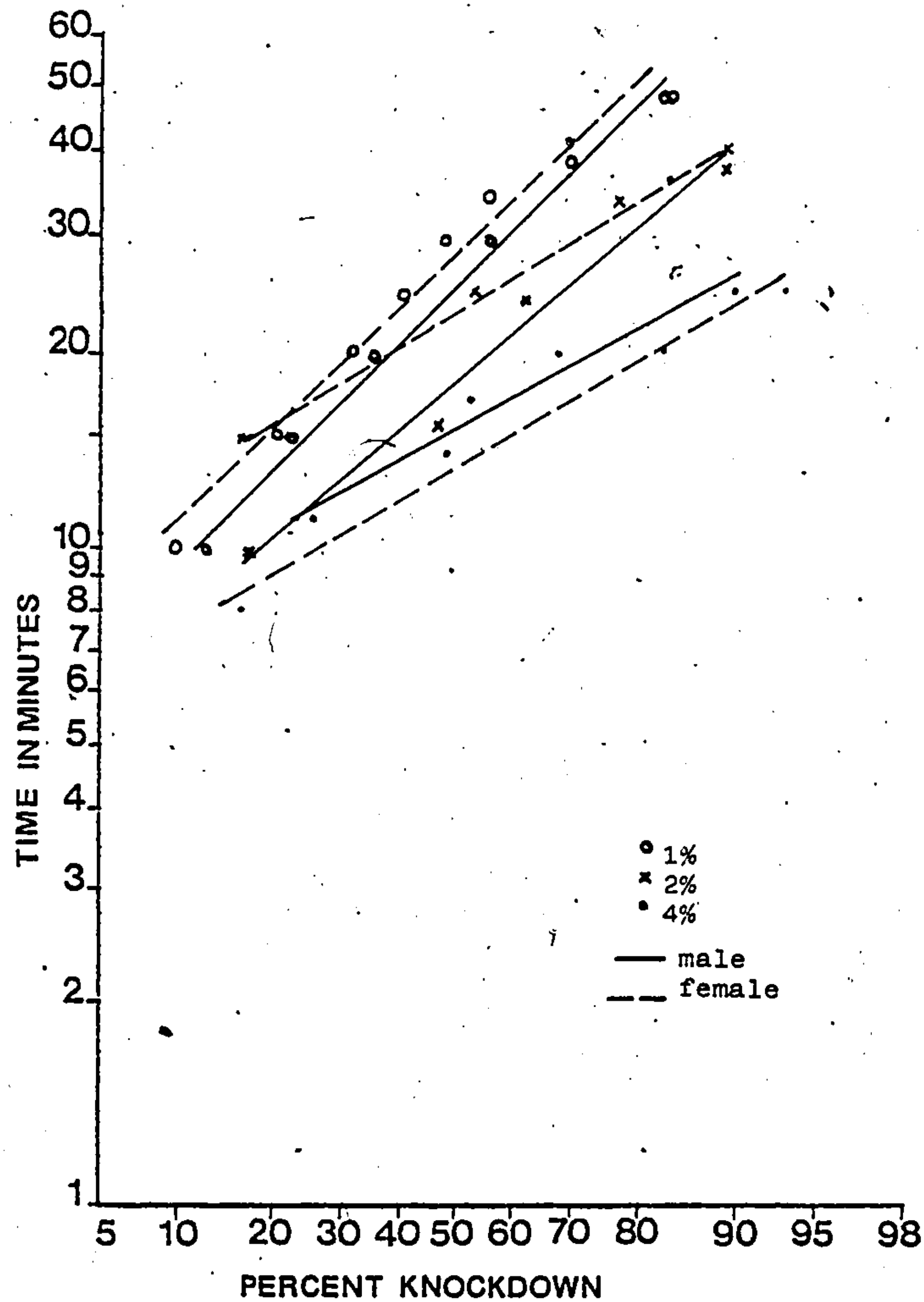


60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
TIME IN MINUTES
5
10
20
30
40
50
60
70
80
90
95
98
PERCENT KNOCKDOWN
1%
2%
4%
male
female

A-7

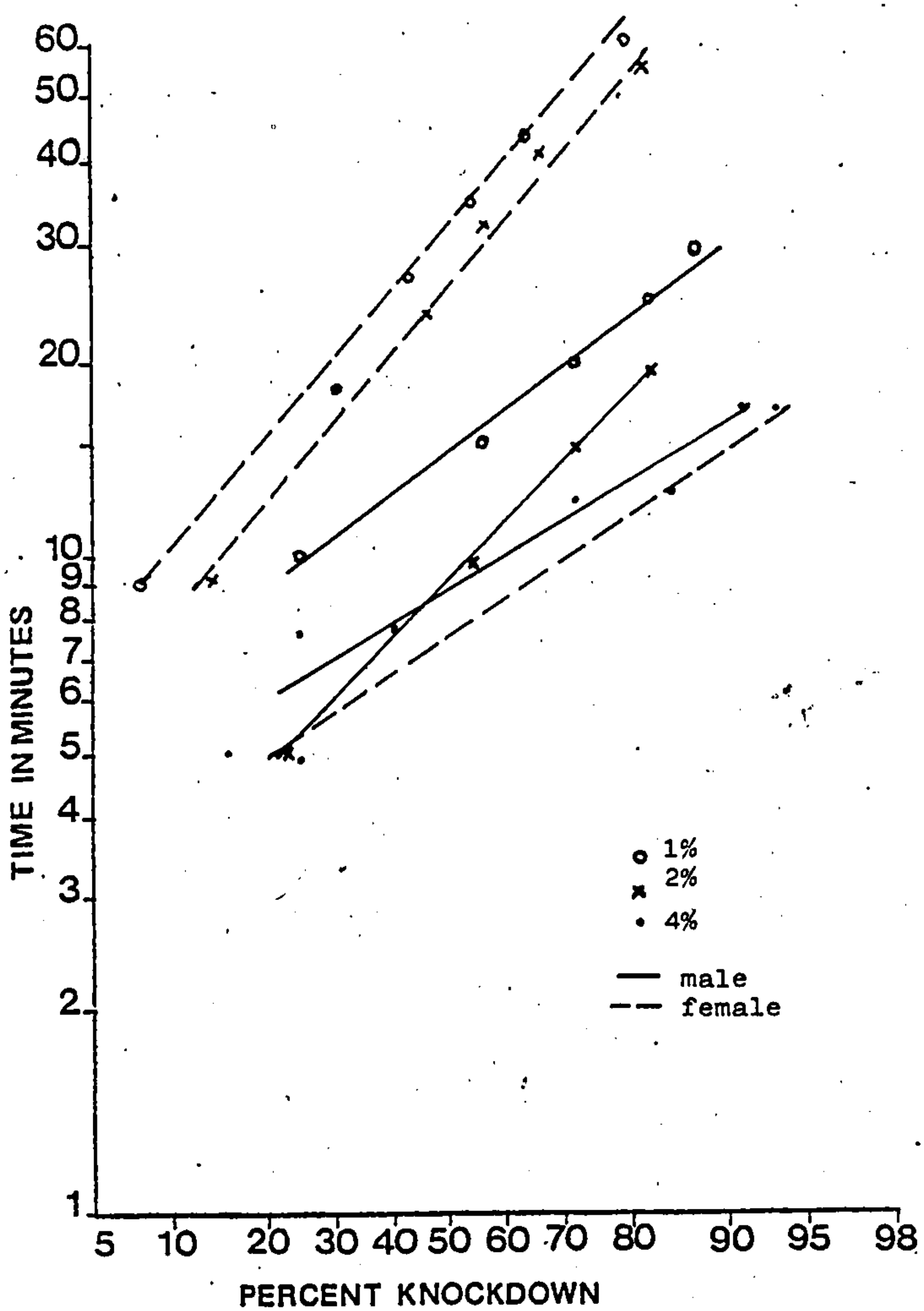

TIME IN MINUTES
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
5
10
20
30
40
50
60
70
80
90
95
98
PERCENT KNOCKDOWN
1%
2%
4%
male
female

A-8

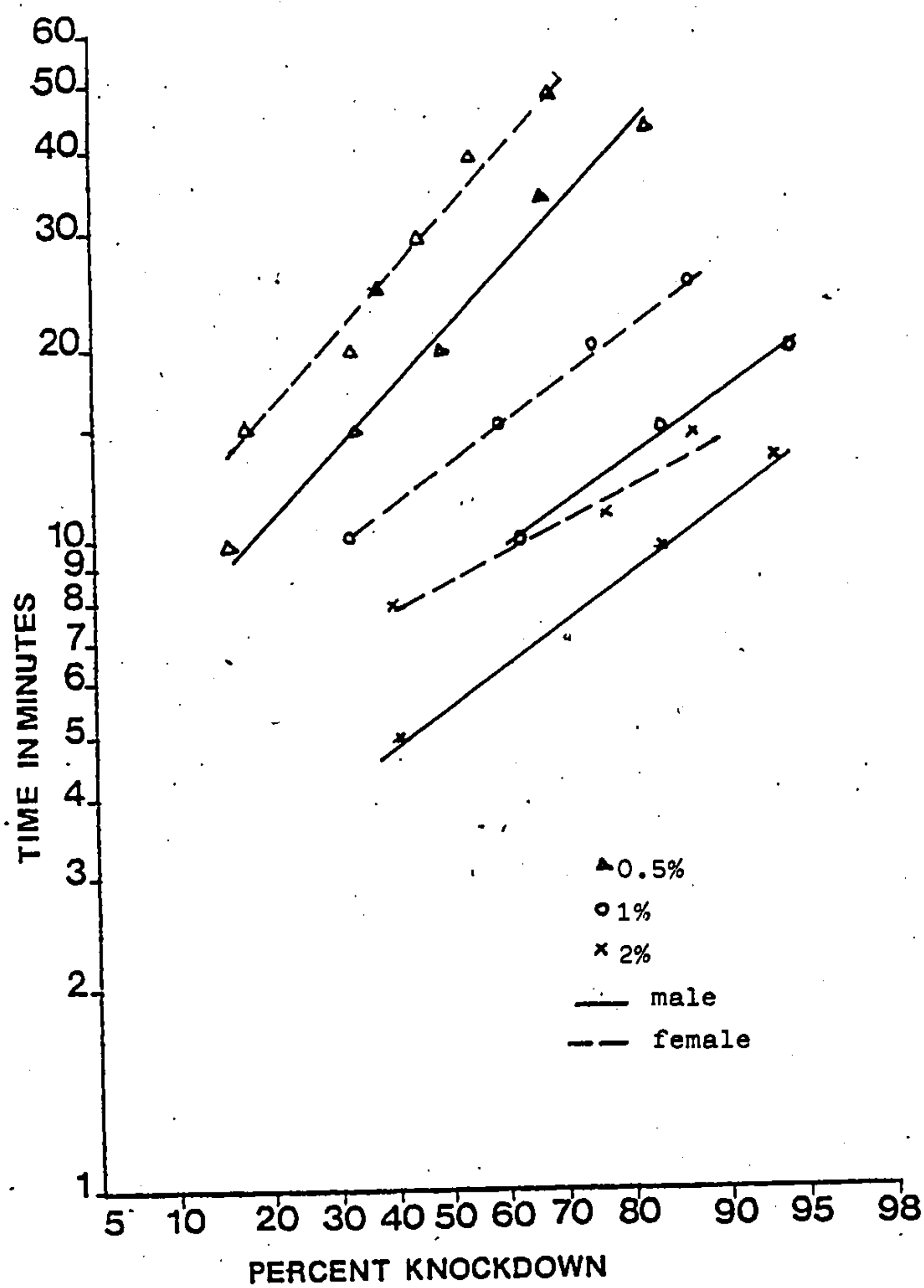


60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
TIME IN MINUTES
5
10
20
30
40
50
60
70
80
90
95
98
PERCENT KNOCKDOWN
1%
2%
4%
male
female

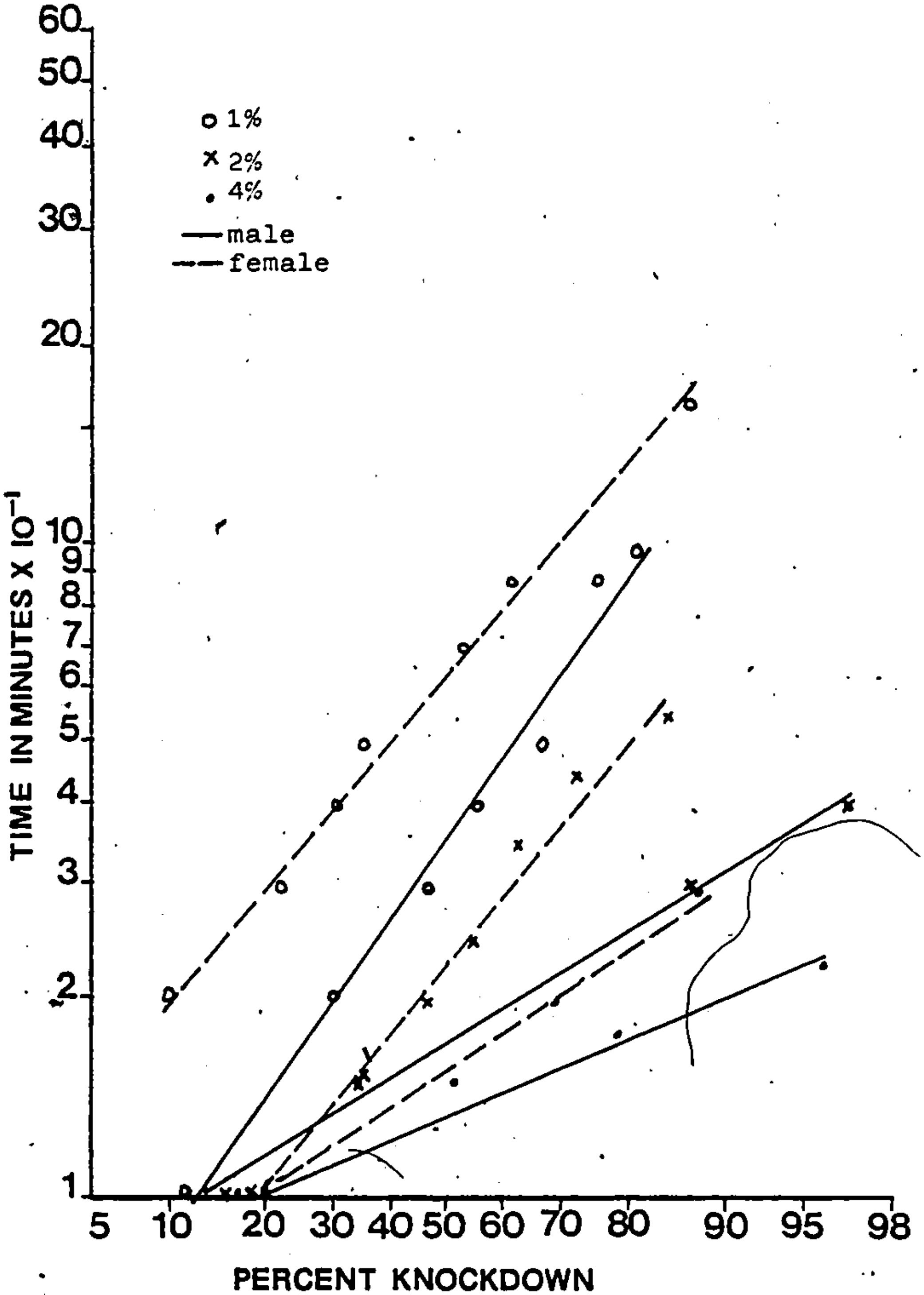
A-10


60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
TIME IN MINUTES
5
10
20
30
40
50
60
70
80
90
95
98
PERCENT KNOCKDOWN
1%
2%
4%
male
female

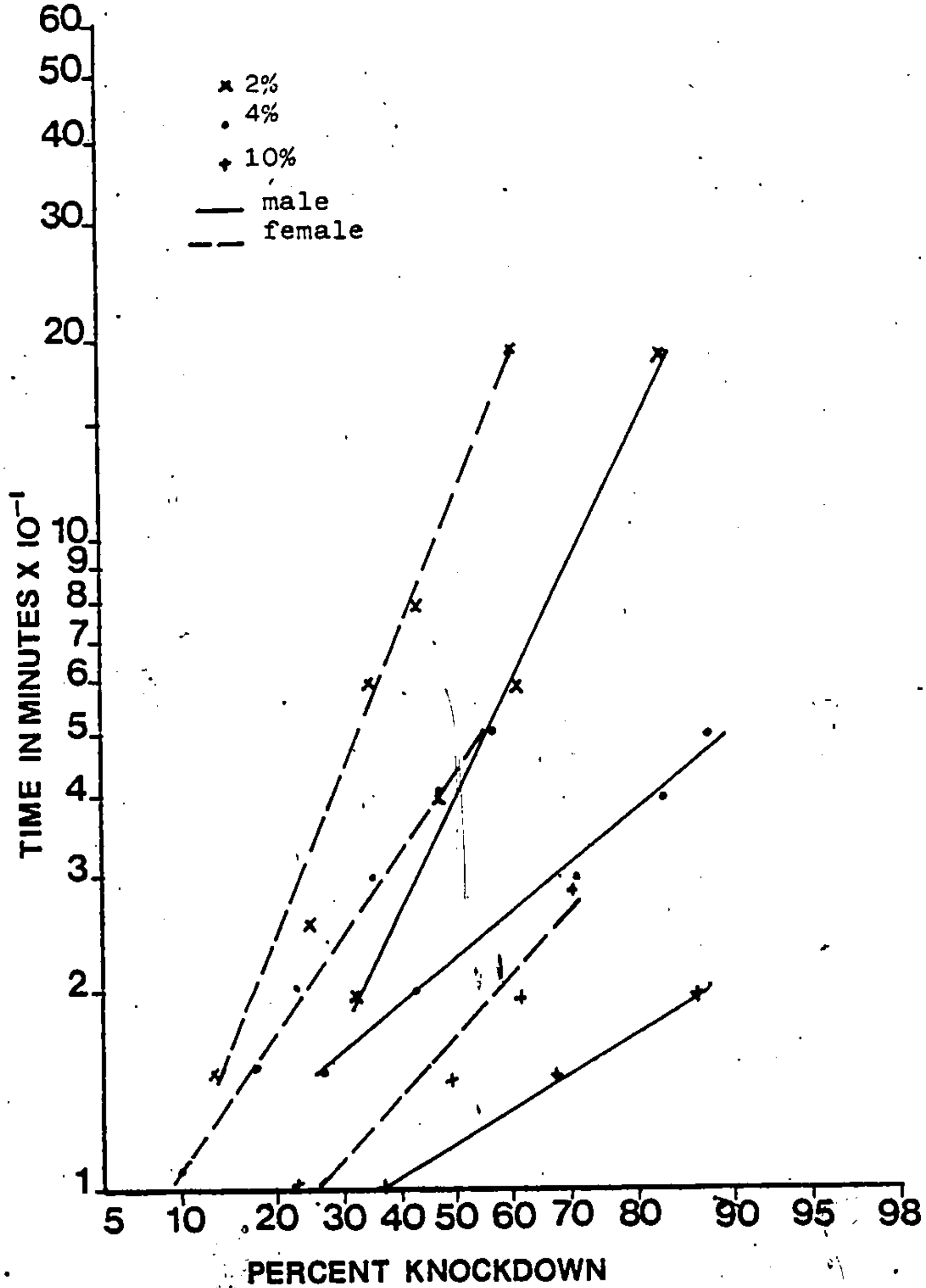
A-11


60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
TIME IN MINUTES
5
10
20
30
40
50
60
70
80
90
95
98
PERCENT KNOCKDOWN
0.5%
1%
2%
male
female

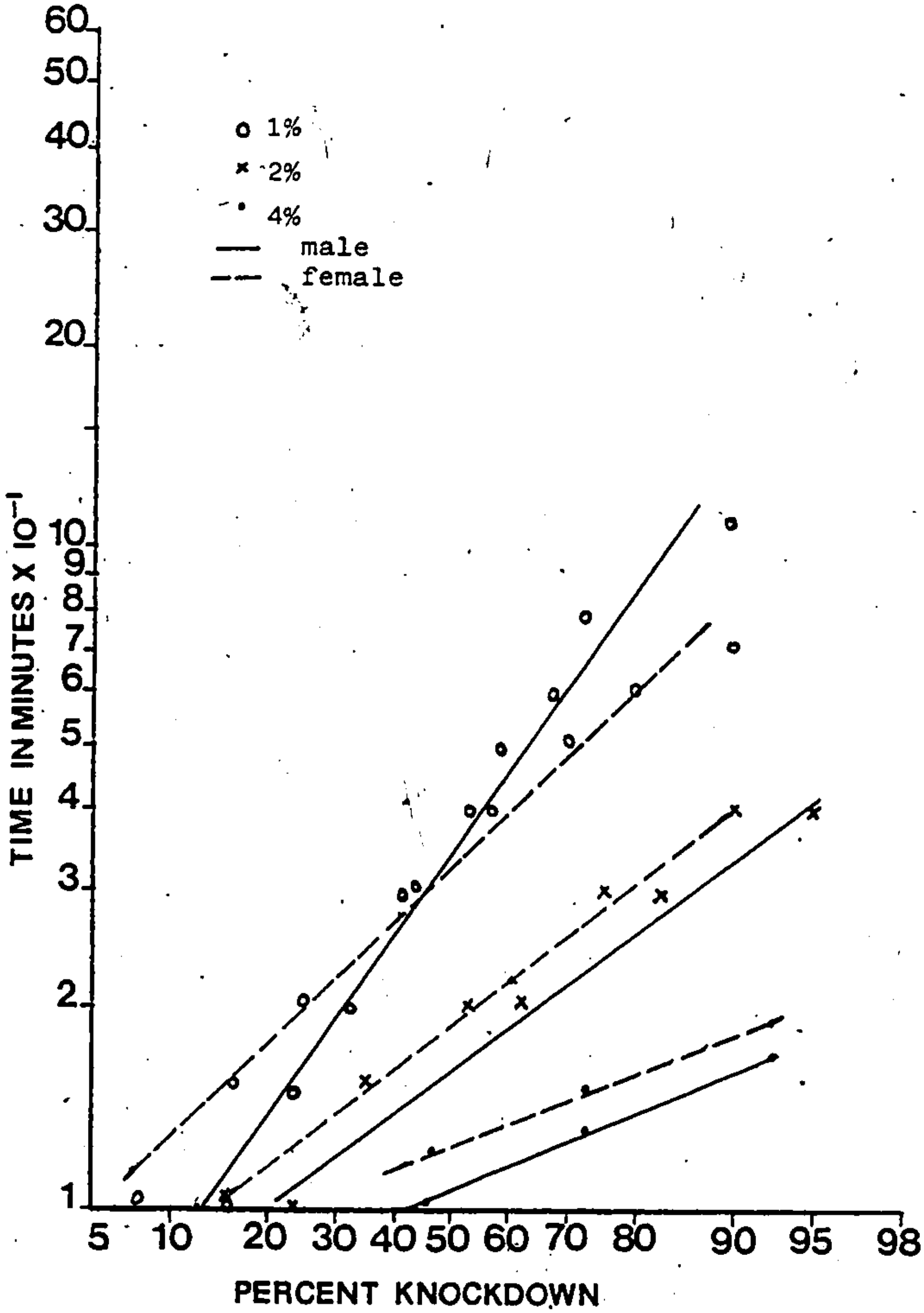
A-12


60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
TIME IN MINUTES X 10⁻¹
1%
2%
4%
male
female
5
10
20
30
40
50
60
70
80
90
95
98
PERCENT KNOCKDOWN

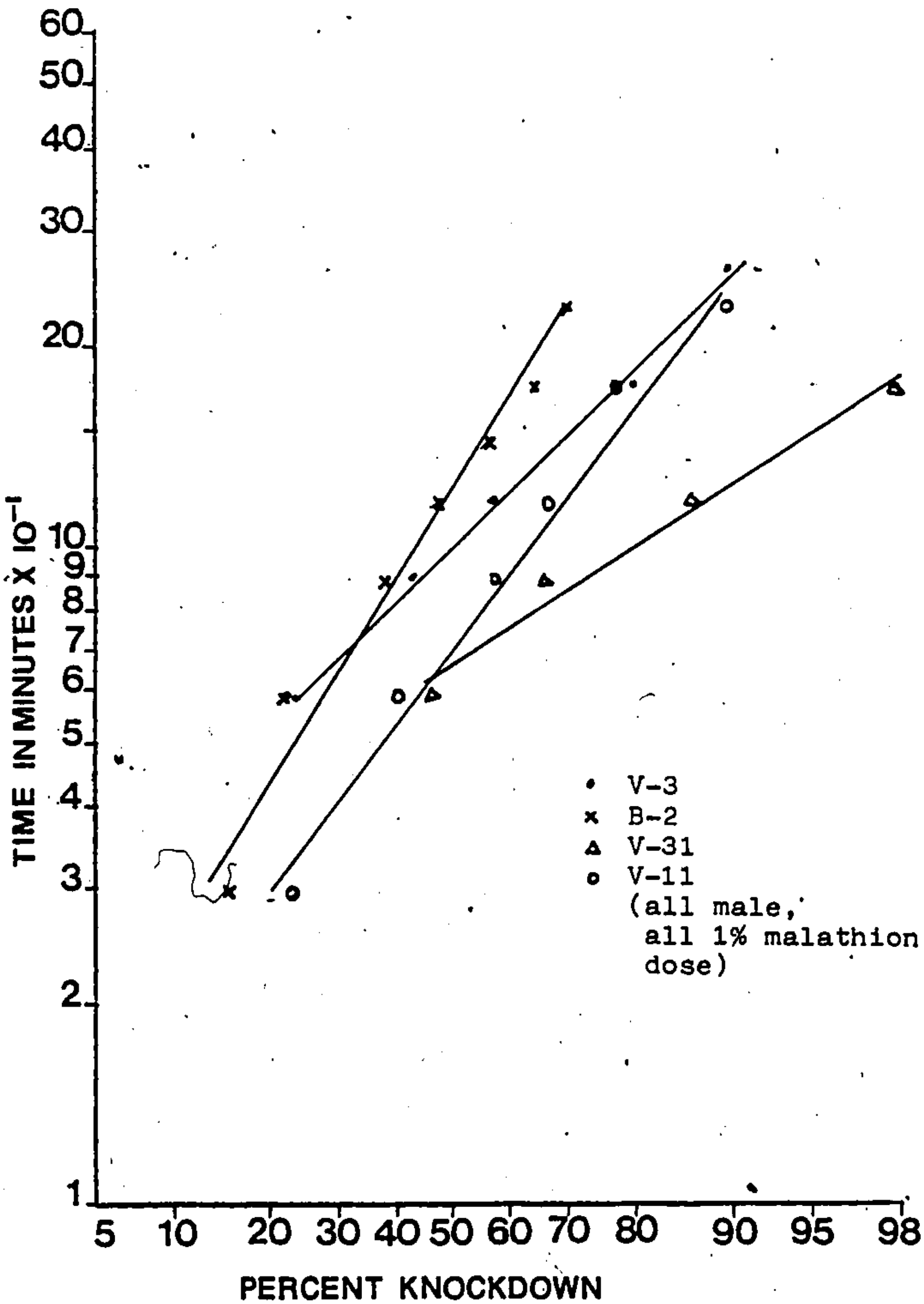
A15R


2%
4%
10%
male
female
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
TIME IN MINUTES X 10⁻¹
5
10
20
30
40
50
60
70
80
90
95
98
PERCENT KNOCKDOWN

J-11



60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
TIME IN MINUTES X 10⁻¹
o 1%
× 2%
• 4%
male
female
5
10
20
30
40
50
60
70
80
90
95
98
PERCENT KNOCKDOWN


60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
TIME IN MINUTES X 10⁻¹
5 10 20 30 40 50 60 70 80 90 95 98
PERCENT KNOCKDOWN
• V-3
× B-2
△ V-31
○ V-11
(all male,
all 1% malathion
dose)

APPENDIX C

Resistance data analysis: tests for significant differences in malathion resistance within and between strains were done using the knockdown data for a dose of 2% malathion applied via vacuum injection (raw data in preceding appendix). This dose was chosen because it is the lowest one at which all lines can be compared (A15R flies give less than 10% total knockdown at a dose of 1% malathion). The results of the resistance test data comparisons are summarized in the table below, which gives the values of the maximum difference, D_{max}, for each comparison. This value was compared to the value of D_{crit} for the Kolmogorov-Smirnov two sample test which was calculated for each comparison (see Methods), and significant differences ($D_{max} > D_{crit}$) are indicated in the table by asterisks (*).

Male vs. Female Compared	D_{max}	Male vs. Male Compared	D_{max}	Female vs. Female Compared	D_{max}
A-6	>50*	A-7,A-8	29*	A-7,A-8	29*
A-7	14	A-7,A-6	19	A-7, A-6	30*
A-8	5	A-7,A-10	12	A-7,A-10	33*
A-10	>40*	A-6,A-11	>40*	A-7,A-11	42*
A-11	10	A-10,J-11	32*	A-6,A-8	19*
A-12	>30*	J-11, A-12	10	A-6,J-11	19*
J-11	10	A15R,A-12	>50*	J-11,A-12	20*
A15R	25*	J-11,A-8	14	A-10,J-11	25*
		A-8,A-12	15	A15R,A-10	48*

APPENDIX D

Wild population line avoidance data and analysis: the raw data from the avoidance runs are listed on the pages following in the form they were stored as raw data files on diskettes. The data groups contain the results from individual runs, which are named by line, sex and run order (eg. A4-M-A is the first run for A-4 line males), and the observations are given in sequence for each surface in the avoidance chamber (i.e. 0% or 1%).

Following the raw data, tabulations of various parameters used in the discussion are given: the mean numbers of flies on each surface (M0% and M1%), the mean avoidance index, and the ratio between the mean surface numbers. The run designations are the same as for the raw data file listings.

Following the tables, the avoidance index histograms are given for each sex and strain, and as with the above information, these were generated via computer.

Homogeneity testing: the results of Bartlett's test for homogeneity of variances done with each run within each class are given in Table D.1 (along with the results for comparisons of all runs within a line). Since variances were found to be homogeneous within each class, the runs were compared for homogeneity via analysis of variance, with the results shown in Table D.2. None of the F values listed are significant, except for the B-17 female comparison.

Inter-sex comparisons: the results of these are given in Table D.3. In most cases the comparison was done by anova, however in those cases where the variances between males and females were inhomogeneous, the means test was used.

A4-F-A
0% 4, 4, 3, 3, 4, 5, 2, 4, 4, 2, 4, 4, 3, 1, 4, 9,
7, 4, 3, 2, 3, 7, 5, 4, 3, 2, 3, 5, 5, 3, 2,
1% 3, 3, 3, 3, 4, 4, 3, 4, 3, 0, 0, 2, 2, 2, 3, 4,
4, 3, 5, 4, 2, 1, 0, 1, 3, 4, 3, 3, 4, 4, 3,

A4-F-B
0% 4, 4, 3, 4, 4, 5, 4, 2, 4, 2, 3, 5, 5, 4, 6, 6,
4, 6, 6, 6, 3, 4, 4, 3, 4, 4, 2, 5, 4, 4, 4,
1% 4, 3, 4, 2, 0, 2, 4, 4, 4, 4, 6, 5, 2, 2, 4, 1,
0, 3, 0, 0, 4, 4, 6, 0, 1, 2, 2, 2, 3, 2, 3,

A4-H-A
0% 8, 7, 7, 8, 9, 8, 10, 9, 8, 10, 7, 8, 7, 7, 7, 5,
6, 4, 7, 8, 7, 7, 8, 8, 8, 9, 9, 9, 6, 6, 6,
1% 2, 1, 1, 2, 2, 2, 1, 3, 1, 1, 2, 1, 0, 1, 1, 4,
0, 1, 1, 0, 2, 1, 0, 4, 1, 2, 2, 3, 3, 2, 2,

A4-H-B
0% 3, 3, 4, 3, 4, 4, 6, 5, 6, 2, 4, 5, 5, 6, 5, 3,
5, 4, 7, 6, 6, 6, 4, 8, 7, 7, 7, 7, 7, 7, 8,
1% 1, 3, 2, 0, 1, 2, 2, 2, 2, 0, 2, 0, 1, 3, 3, 0,
0, 1, 2, 2, 2, 1, 6, 1, 2, 1, 4, 1, 0, 0, 1,

A4-H-C
0% 5, 6, 4, 5, 4, 5, 3, 6, 3, 5, 6, 4, 5, 4, 8, 7,
4, 6, 5, 3, 2, 5, 3, 4, 8, 6, 6, 5, 7, 6, 7,
1% 1, 1, 1, 2, 2, 2, 2, 3, 1, 2, 3, 0, 0, 0, 0, 2,
2, 2, 2, 1, 1, 0, 1, 0, 1, 1, 4, 2, 2, 2, 3,

A5-F-A
0% 3, 3, 6, 6, 6, 6, 2, 4, 7, 2, 5, 4, 4, 4, 3, 1,
5, 4, 3, 4, 3, 5, 2, 4, 4, 3, 3, 4, 4, 3, 5,
1% 2, 2, 5, 3, 3, 4, 2, 4, 2, 6, 3, 3, 4, 6, 3, 4,
2, 2, 1, 2, 3, 1, 5, 4, 4, 6, 1, 2, 3, 2, 1,

A5-F-B
0% 6, 6, 1, 5, 2, 5, 5, 5, 4, 5, 2, 4, 5, 1, 2, 1,
5, 2, 2, 5, 2, 3, 3, 3, 4, 3, 4, 2, 4, 4, 2,
1% 3, 2, 3, 2, 4, 4, 6, 2, 0, 3, 1, 0, 0, 2, 1, 1,
1, 1, 0, 2, 2, 1, 1, 3, 2, 2, 2, 1, 1, 2, 1,

A5-H-A
0% 5, 8, 4, 5, 6, 5, 4, 5, 8, 4, 3, 6, 5, 3, 3, 5,
5, 8, 6, 4, 3, 6, 8, 5, 5, 6, 6, 7, 6, 5, 5,
1% 2, 1, 1, 2, 2, 4, 1, 1, 3, 2, 2, 2, 1, 2, 2, 3,
1, 1, 1, 3, 1, 0, 1, 0, 3, 1, 1, 3, 2, 3, 2,

A5-H-B
0% 6, 2, 5, 1, 3, 3, 8, 6, 3, 3, 2, 6, 4, 3, 5, 3,
5, 3, 4, 5, 2, 2, 2, 3, 4, 5, 5, 3, 5, 5, 5,
1% 3, 3, 2, 3, 2, 3, 2, 2, 2, 2, 1, 2, 3, 0, 0, 2,
0, 1, 2, 2, 2, 2, 2, 4, 3, 1, 1, 1, 1, 0, 1,

A5-H-C
0% 5, 5, 5, 5, 5, 4, 7, 6, 4, 5, 3, 6, 3, 3, 5, 4,
3, 3, 4, 6, 5, 3, 3, 2, 2, 3, 3, 0, 1, 1, 1,
1% 2, 2, 4, 3, 2, 4, 3, 3, 2, 2, 1, 1, 4, 2, 0, 3,
4, 1, 0, 2, 3, 0, 1, 1, 2, 1, 2, 1, 1, 1, 1,

A6-F-A
0% 4, 4, 2, 2, 3, 6, 4, 5, 5, 3, 3, 4, 6, 5, 7, 6,
6, 6, 6, 5, 3, 4, 4, 3, 7, 7, 7, 7, 7, 6, 6,
1% 0, 0, 3, 2, 2, 1, 2, 3, 2, 2, 0, 1, 0, 4, 0, 0,
1, 0, 1, 2, 3, 4, 2, 3, 2, 1, 1, 2, 2, 4, 1,
A6-F-B
0% 2, 5, 3, 7, 6, 4, 5, 4, 3, 5, 5, 5, 7, 5, 6, 8,
4, 8, 7, 6, 4, 7, 8, 8, 7, 7, 8, 8, 9, 9, 7, 10,
6, 5, 5, 5, 5, 7, 3, 6, 6,
1% 0, 5, 2, 2, 4, 2, 4, 6, 3, 6, 2, 2, 5, 3, 4, 0,
0, 2, 1, 2, 1, 2, 0, 3, 4, 2, 0, 1, 3, 3, 2, 0,
1, 0, 1, 1, 2, 3, 2, 3, 1,
A6-F-C
0% 2, 1, 4, 3, 2, 2, 3, 1, 1, 2, 3, 3, 6, 3, 3, 2,
3, 4, 3, 5, 4, 6, 7, 6, 5, 5, 6, 6, 7, 5, 5, 5,
5, 5, 3, 4, 5, 3, 4, 4, 4,
1% 3, 4, 1, 1, 2, 1, 1, 3, 1, 1, 3, 1, 4, 1, 2, 2,
2, 0, 2, 2, 2, 2, 2, 1, 2, 4, 3, 0, 1, 2, 0, 1,
0, 0, 1, 1, 1, 0, 0, 3, 2,
A6-M-A
0% 4, 6, 5, 3, 6, 5, 5, 5, 5, 6, 6, 4, 3, 6, 4, 2,
5, 1, 2, 4, 4, 4, 4, 4, 3, 5, 4,
1% 1, 1, 2, 2, 4, 3, 3, 4, 2, 1, 1, 0, 1, 1, 1, 3,
2, 1, 1, 2, 2, 3, 2, 2, 3, 2, 2,
A6-M-B
0% 2, 5, 3, 6, 5, 3, 4, 5, 5, 5, 8, 7, 7, 5, 6, 4,
1, 3, 3,
1% 2, 3, 3, 3, 2, 5, 2, 4, 2, 5, 3, 0, 4, 2, 2, 2,
4, 2, 4,
A6-M-C
0% 4, 7, 1, 4, 5, 1, 4, 3, 2, 5, 7, 7, 6, 5, 6, 7,
5, 7, 8, 7, 6, 10, 9, 8, 8, 6, 5, 5, 7, 6, 7, 6,
6, 5, 7, 8, 7, 8, 9, 8, 8,
1% 1, 0, 4, 3, 6, 2, 2, 0, 1, 0, 4, 2, 2, 2, 1, 2,
1, 1, 3, 2, 2, 1, 1, 0, 2, 1, 1, 2, 0, 1, 1, 0,
1, 2, 2, 1, 3, 3, 1, 1, 4,
A7-F-A
0% 3, 4, 5, 4, 5, 4, 4, 4, 3, 4, 5, 5, 5, 8, 5, 8,
5, 6, 2, 4, 3, 3, 3, 5, 6, 4, 5, 6, 5, 2, 2,
1% 1, 0, 0, 1, 2, 1, 2, 0, 0, 1, 2, 0, 2, 1, 1, 1,
2, 2, 3, 3, 5, 3, 3, 5, 3, 3, 4, 2, 3, 2, 1,
A7-F-B
0% 2, 5, 3, 3, 7, 5, 3, 6, 3, 2, 4, 4, 3, 7, 7, 9,
9, 10, 9, 7, 5, 4, 4, 7, 5, 6, 4, 4, 2, 3, 2,
1% 2, 2, 1, 1, 0, 1, 1, 1, 2, 1, 1, 0, 1, 1, 4, 4,
1, 4, 2, 2, 3, 4, 3, 4, 3, 5, 2, 0, 4, 2, 1,

A7-M-A
0% 3, 3, 4, 7, 6, 6, 3, 6, 5, 7, 8, 6, 6, 5, 7, 6,
7, 3, 4, 2, 4, 5, 3, 3, 6, 2, 3, 4, 5, 4,
1% 2, 2, 3, 0, 1, 0, 2, 1, 1, 1, 2, 1, 2, 1, 3, 0,
4, 0, 1, 1, 1, 3, 2, 2, 2, 1, 1, 2, 2, 2, 1,
A7-M-B
0% 3, 2, 3, 2, 3, 5, 4, 6, 2, 3, 3, 4, 5, 6, 2, 4,
3, 4, 3, 3, 4, 3, 5, 4, 3, 4, 5, 2, 5, 1, 2,
1% 1, 1, 2, 2, 0, 1, 2, 0, 3, 0, 1, 0, 2, 2, 1, 3,
1, 1, 4, 1, 1, 3, 2, 0, 2, 2, 1, 1, 2, 1, 2,
A7-M-C
0% 4, 2, 4, 6, 3, 3, 3, 5, 3, 4, 6, 1, 3, 3, 7, 4,
3, 3, 4, 5, 3, 3, 3, 3, 5, 4, 5, 4, 6, 6, 4,
1% 2, 2, 1, 0, 0, 1, 1, 2, 1, 1, 0, 1, 0, 1, 1, 0,
2, 1, 1, 1, 0, 1, 1, 1, 0, 1, 1, 1, 32, 0, 1,

A8-F-A
0% 6, 2, 3, 5, 6, 5, 7, 4, 4, 3, 6, 3, 4, 2, 4, 2,
4, 3, 4, 2, 4, 6, 6, 6,
1% 3, 2, 1, 2, 2, 2, 2, 1, 0, 5, 3, 4, 2, 2, 3, 3,
4, 4, 3, 3, 4, 2, 3, 0,
A8-F-B
0% 3, 4, 4, 5, 5, 3, 4, 2, 3, 5, 4, 3, 2, 4, 4, 7,
5, 4, 5, 5, 4, 4, 0, 5, 4,
1% 2, 3, 3, 3, 3, 0, 3, 3, 1, 1, 3, 5, 5, 5, 3, 5,
4, 2, 2, 2, 0, 0, 3, 1, 0,
A8-F-C
0% 4, 5, 8, 6, 5, 3, 5, 5, 5, 6, 6, 3, 4, 6, 4, 6,
4, 2, 7, 6, 7, 6, 5, 4, 3, 1, 3, 5, 2, 5, 4,
1% 2, 3, 4, 3, 2, 2, 2, 3, 2, 2, 3, 1, 1, 0, 4, 2,
3, 3, 3, 4, 2, 2, 3, 1, 3, 3, 5, 2, 1, 2, 3,
A8-M-A
0% 3, 2, 3, 6, 7, 5, 5, 5, 4, 3, 0, 2, 5, 3, 4, 4,
5, 5, 4, 1,
1% 1, 3, 2, 4, 3, 2, 0, 1, 1, 3, 1, 0, 3, 1, 2, 4,
3, 0, 2, 0,
A8-M-B
0% 3, 4, 5, 4, 6, 7, 7, 4, 3, 3, 4, 3, 4, 3, 3,
1% 3, 3, 3, 4, 5, 5, 4, 0, 6, 3, 2, 0, 1, 2, 2,
A8-M-C
0% 6, 3, 6, 6, 4, 5, 6, 6, 0, 3, 3, 5, 5, 1, 3, 1,
2, 0, 4, 2, 1,
1% 2, 3, 1, 5, 3, 1, 2, 3, 4, 2, 2, 3, 2, 5, 1, 3,
2, 1, 1, 1, 0,

A9-F-A
0% 4, 6, 3, 9, 3, 7, 7, 5, 5, 7, 9, 7, 10, 3, 4, 7,
4, 2, 7, 4, 2, 9, 6, 5, 8, 9, 10, 10, 10, 10,
1% 2, 3, 8, 3, 5, 5, 4, 4, 6, 4, 1, 3, 4, 8, 2, 3,
2, 5, 4, 3, 3, 3, 4, 5, 5, 3, 2, 3, 2, 4,
A9-F-B
0% 6, 6, 6, 6, 4, 4, 7, 3, 5, 6, 9, 3, 4, 5, 6, 3,
7, 4, 6, 6, 4, 2, 3, 2, 7, 6, 5, 4, 7, 6, 7,
1% 4, 5, 5, 6, 5, 4, 6, 5, 4, 1, 4, 4, 0, 2, 4, 3,
1, 3, 1, 3, 4, 3, 2, 4, 4, 3, 3, 3, 7, 7, 5,

A9-M-A

0% 2, 2, 2, 3, 6, 3, 3, 4, 1, 5, 5, 3, 5, 6, 4, 6,
8, 6, 7, 9, 7, 8, 10, 9, 7, 6, 11, 10, 10, 9, 8,

1% 3, 1, 4, 0, 3, 2, 2, 3, 2, 7, 3, 3, 4, 4, 3, 5,
5, 3, 5, 4, 5, 1, 6, 1, 0, 4, 1, 4, 3, 5, 3,

A9-M-B

0% 7, 4, 5, 11, 5, 6, 8, 9, 7, 6, 7, 5, 7, 6, 7, 5,
7, 3, 4, 4, 7, 2, 3, 5, 8, 7, 5, 7, 4, 5, 4,

1% 4, 6, 1, 2, 4, 3, 6, 8, 5, 3, 4, 4, 2, 5, 4, 3,
4, 3, 5, 2, 1, 2, 5, 4, 6, 4, 4, 4, 1, 2, 2,

A9-M-C

0% 6, 7, 4, 9, 8, 6, 11, 7, 4, 6, 5, 7, 10, 9, 4, 3,
8, 6, 5, 5, 4, 4, 5, 6, 1, 2, 4, 4, 6, 7, 5,

1% 7, 2, 5, 4, 4, 2, 2, 2, 1, 0, 3, 1, 3, 4, 4, 5,
4, 0, 4, 2, 4, 3, 3, 3, 6, 4, 3, 3, 2, 3, 5,

A10-F-A

0% 9, 5, 9, 7, 6, 7, 6, 5, 5, 7, 6, 7, 3, 6, 5, 5,
11, 9, 10, 10, 10, 13, 11, 10, 8, 12, 15, 9, 11, 12, 14, 13,

1% 0, 1, 2, 2, 3, 1, 0, 0, 0, 2, 2, 2, 1, 2, 0, 1,
3, 3, 3, 2, 3, 3, 4, 7, 4, 4, 2, 3, 5, 5, 6, 1,

A10-F-B

0% 6, 7, 5, 9, 8, 8, 6, 9, 7, 9, 10, 7, 13, 9, 10, 11,
8, 5, 7, 6, 7, 8, 10, 6, 6, 5, 9, 7, 9, 7, 5,

1% 2, 3, 1, 2, 3, 3, 3, 4, 3, 2, 7, 2, 3, 4, 3, 2,
2, 2, 3, 2, 3, 4, 1, 2, 0, 1, 1, 0, 1, 2,

A10-F-C

0% 8, 5, 6, 7, 3, 6, 6, 8, 4, 4, 4, 6, 7, 5, 8, 6,
7, 5, 10, 7, 8, 10, 7, 6, 5, 6, 5, 7, 7, 10, 9, 7,
3, 8, 6, 5, 9, 9, 7, 8, 12,

1% 0, 1, 1, 2, 3, 1, 1, 1, 2, 4, 2, 1, 2, 2, 0, 0,
3, 1, 0, 1, 0, 2, 1, 2, 4, 2, 1, 1, 2, 4, 4, 3,
1, 1, 1, 1, 3, 4, 2, 3, 2,

A10-M-A

0% 4, 3, 4, 3, 9, 7, 4, 2, 1, 3, 1, 2, 5, 4, 3, 3,
2, 5, 5, 5, 2, 4, 6, 8, 2, 4, 0, 5, 4, 3, 3,

1% 0, 2, 2, 2, 4, 2, 4, 3, 1, 0, 3, 2, 1, 0, 2, 2,
0, 1, 3, 1, 2, 0, 1, 3, 4, 6, 3, 4, 2, 2, 5,

A10-M-B

0% 6, 2, 3, 5, 4, 3, 3, 2, 2, 2, 1, 2, 1, 5, 4, 7,
5, 6, 6, 2, 3, 0, 3, 1, 1, 1, 7, 2, 0, 2, 2, 5,
4, 3, 5, 3, 1, 0, 4, 4, 6,

1% 0, 6, 0, 0, 0, 1, 0, 0, 0, 2, 2, 2, 0, 2, 3, 2,
3, 1, 2, 2, 2, 2, 1, 2, 1, 4, 3, 3, 1, 3, 2, 2,
1, 2, 2, 2, 2, 3, 2, 1, 1,

A10-M-C

0% 5, 5, 4, 3, 3, 4, 2, 1, 4, 5, 2, 0, 1, 4, 2, 2,
0, 1, 6, 4, 5, 2, 4, 5, 1, 4, 3, 1, 0, 2, 5, 2,
6, 6, 2, 2, 4, 3, 6, 5,

1% 1, 0, 1, 2, 0, 2, 3, 1, 3, 4, 1, 3, 2, 3, 2, 5,
6, 2, 1, 3, 1, 1, 3, 1, 2, 0, 2, 1, 1, 2, 3, 3,
5, 1, 3, 3, 1, 0, 1, 2,

A10-M-D

0% 6, 5, 5, 6, 5, 5, 8, 5, 7, 7, 6, 8, 9, 10, 10, 7,
8, 7, 10, 12, 10, 8, 3, 8,

1% 1, 2, 2, 1, 2, 2, 3, 3, 3, 4, 5, 2, 6, 2, 5, 6,
4, 4, 7, 3, 5, 2, 3, 2,

A11-F-A
0% 1, 3, 7, 5, 5, 5, 6, 7, 6, 5, 5, 8, 6, 6, 5, 6,
3, 5, 2, 6, 3, 7,
1% 3, 4, 5, 2, 1, 3, 3, 4, 4, 5, 5, 2, 4, 2, 3, 4,
4, 3, 4, 3, 0, 2,
A11-F-B
0% 3, 5, 5, 5, 4, 5, 4, 5, 5, 5, 5, 5, 6, 7, 4, 7,
4, 5, 3, 2,
1% 0, 3, 1, 2, 3, 2, 0, 2, 1, 3, 4, 3, 2, 3, 2, 2,
0, 3, 4, 1,
A11-F-C
0% 5, 4, 4, 3, 4, 5, 4, 4, 3, 0, 4, 4, 5, 4, 4, 4,
3, 5, 3, 2, 2, 4, 1, 1, 1, 0, 1, 3, 2, 3, 3,
1% 2, 3, 3, 4, 4, 4, 4, 2, 1, 1, 1, 2, 3, 4, 2, 3,
3, 1, 3, 1, 1, 2, 1, 2, 0, 1, 1, 1, 0, 1, 0,
A11-F-D
0% 3, 3, 4, 3, 3, 0, 3, 3, 5, 4, 5, 6, 6, 5, 3, 5,
4, 4, 5, 4, 1, 2, 4, 5, 2, 2, 1, 2, 1,
1% 3, 2, 1, 0, 1, 5, 2, 1, 0, 2, 1, 2, 1, 1, 3, 2,
1, 1, 1, 1, 2, 2, 1, 0, 0, 0, 2, 1, 1,
A11-M-A
0% 6, 4, 3, 5, 3, 4, 3, 4, 5, 6, 4, 4, 3,
1% 4, 4, 0, 2, 1, 2, 2, 2, 0, 3, 1, 2, 3,
A11-M-B
0% 4, 6, 3, 2, 3, 2, 3, 6, 3, 5, 2, 2, 4,
1% 2, 3, 2, 3, 0, 1, 2, 0, 1, 3, 4, 2, 0,
A11-M-C
0% 5, 4, 6, 5, 2, 2, 5, 3, 2, 3, 3, 3, 3, 4, 3, 4,
0, 1, 4, 4, 2, 3, 4, 2, 5, 4, 2, 2, 2,
1% 3, 2, 2, 2, 0, 1, 1, 2, 1, 2, 1, 0, 1, 1, 0, 2,
2, 0, 2, 0, 2, 1, 1, 4, 1, 0, 2, 1, 0,
A12-F-A
0% 5, 5, 9, 8, 7, 8, 4, 5, 10, 8, 4, 7, 7, 5, 4, 4,
6, 7, 7, 6, 5, 6, 5, 6, 4, 6,
1% 0, 5, 3, 3, 1, 3, 2, 6, 2, 2, 6, 3, 2, 3, 4, 4,
3, 2, 3, 1, 3, 2, 8, 4, 0, 4,
A12-F-B
0% 6, 6, 2, 7, 6, 7, 3, 1, 2, 2, 6, 4, 3, 4, 3, 7,
3, 5, 6, 5, 3, 2, 4, 5, 7, 5, 1, 4, 1, 4, 3, 3,
5, 6, 5, 5, 4, 6, 4, 5, 5,
1% 4, 1, 3, 2, 2, 2, 2, 1, 2, 1, 3, 1, 2, 3, 4, 2,
1, 1, 4, 3, 3, 2, 2, 4, 3, 3, 3, 2, 3, 0, 1, 3,
2, 3, 2, 3, 2, 3, 2, 3, 3,
A12-F-C
0% 3, 4, 5, 4, 10, 7, 4, 6, 5, 6, 8, 6, 3, 4, 5, 3,
4, 3, 4, 4, 4, 6, 4, 4, 6, 4, 3, 1, 4, 4, 4, 5,
7, 7, 6, 6, 5, 6, 7, 6, 5,
1% 5, 2, 0, 1, 2, 2, 6, 3, 4, 3, 4, 0, 2, 1, 2, 4,
3, 1, 2, 1, 3, 3, 2, 1, 2, 2, 1, 4, 2, 3, 5, 1,
1, 0, 1, 3, 3, 2, 3, 3, 3,

A12-M-A
0% 4, 0, 2, 4, 4, 5, 6, 5, 2, 4, 7, 3, 5, 4, 2, 5,
3, 5, 4, 6,
1% 3, 3, 5, 5, 2, 4, 3, 3, 3, 3, 2, 4, 1, 1, 2, 3,
4, 3, 1, 0,

A12-M-B
0% 4, 0, 1, 3, 3, 1, 4, 0, 1, 6, 6, 5, 4, 5, 4, 4,
2, 4, 7, 2, 3, 3, 0, 2, 5, 4, 4, 4, 7, 2, 2, 2,
3, 1, 4, 3, 2, 4, 3, 5,
1% 1, 3, 2, 1, 0, 1, 1, 3, 3, 3, 1, 1, 1, 1, 2, 2,
2, 3, 1, 2, 2, 2, 1, 3, 1, 1, 2, 4, 2, 1, 2, 1,
2, 2, 3, 2, 3, 3, 4, 1,

A15-F-A
0% 6, 5, 2, 4, 7, 3, 7, 6, 8, 3, 5, 7, 8, 7, 8, 7,
8, 9, 7, 6, 8, 8, 6, 7, 4, 6, 8, 5, 2, 3, 4, 5,
7, 6, 7, 7, 6, 4, 9, 8, 9,
1% 4, 2, 3, 2, 2, 4, 1, 3, 3, 4, 0, 2, 1, 4, 0, 1,
2, 1, 3, 3, 3, 5, 3, 0, 1, 4, 2, 1, 4, 3, 4, 0,
5, 2, 2, 2, 5, 4, 1, 2, 1,

A15-F-B
0% 5, 6, 3, 6, 9, 5, 9, 9, 10, 16, 9, 8, 11, 9, 7, 7,
9, 10, 11, 7, 5, 7, 10, 8, 7, 8, 9, 7, 8, 4, 7, 7,
7, 6, 8, 8, 7, 9, 8, 8, 8,
1% 2, 5, 3, 2, 1, 6, 0, 2, 2, 1, 7, 4, 1, 3, 3, 6,
4, 4, 5, 1, 2, 3, 2, 3, 1, 3, 2, 0, 5, 5, 1, 4,
2, 3, 4, 2, 4, 5, 3, 4, 4,

A15-F-C
0% 5, 1, 6, 4, 4, 5, 3, 4, 5, 2, 5, 6, 7, 6, 7, 10,
8, 8, 8, 9, 7, 8, 9, 8, 6, 10, 11, 5, 7, 9, 9,
1% 2, 1, 3, 5, 3, 1, 3, 5, 4, 3, 2, 3, 2, 5, 2, 2,
2, 2, 1, 2, 5, 3, 1, 2, 5, 1, 2, 2, 4, 5, 3,

A15-M-A
0% 7, 10, 11, 13, 14, 15, 16, 15, 17, 18, 13, 18, 21, 20, 21, 19
,
24, 17, 19, 19, 16, 16, 15, 14, 19, 18, 13, 17, 16, 15, 17, 1
6,
19, 15, 14, 13, 17, 17, 21, 20, 19,
1% 1, 0, 2, 0, 2, 1, 1, 3, 0, 1, 0, 1, 1, 0, 1, 0,
0, 1, 2, 1, 1, 1, 1, 1, 0, 1, 1, 1, 3, 3, 0, 1,
1, [illegible], 0, 1, 1, 1, 1, 1,

A15-M-B
0% 8, 6, 11, 12, 13, 13, 8, 10, 11, 12, 10, 11, 15, 13, 14, 14,
12, 15, 16, 18, 15, 16, 13, 16, 18, 14, 15, 16, 19, 9, 17, 16
,
17, 19, 22, 18, 20, 19, 18, 18, 18,
1% 2, 1, 5, 3, 4, 6, 1, 1, 4, 1, 0, 1, 1, 1, 1, 1,
1, 1, 1, 0, 2, 1, 1, 3, 0, 1, 2, 0, 0, 1, 2, 2,
1, 1, 1, 1, 1, 1, 1, 1, 1,

A15-M-C
0% 9, 10, 8, 9, 11, 11, 14, 12, 12, 11, 11, 9, 12, 12, 13, 14,
11, 14, 10, 11, 11, 11, 10, 11, 8, 11, 11, 11, 13, 10, 9, 8,

11, 12, 11, 11,
1% 1, 0, 1, 1, 2, 1, 1, 0, 1, 1, 0, 1, 1, 0, 0, 0,
1, 1, 1, 0, 0, 1, 1, 2, 1, 1, 1, 1, 1, 2, 1, 1,
2, 0, 4, 2, 1,

A15-M-D
0% 15, 18, 14, 16, 16, 16, 15, 11, 12, 13, 15, 17, 15, 15, 13, 1
5,
14, 15, 13, 14, 16, 17, 12, 13, 12, 11, 10, 13, 12, 15,
1% 1, 0, 1, 0, 0, 1, 1, 1, 1, 2, 0, 0, 2, 2, 0, 2,
3, 0, 2, 1, 1, 1, 4, 2, 1, 4, 3, 2, 3, 0,

A16-F-A
0% 2, 4, 4, 2, 2, 2, 2, 4, 4, 3, 5, 3, 5, 3, 2, 5,
5, 3, 2, 4, 1, 5, 1, 5, 6, 2, 1, 3, 1, 1, 3,
1% 4, 1, 3, 1, 3, 2, 2, 3, 2, 2, 1, 3, 2, 0, 1, 2,
1, 1, 1, 2, 4, 0, 1, 2, 2, 2, 1, 2, 1, 1, 0,

A16-F-B
0% 6, 6, 4, 5, 5, 3, 5, 3, 6, 7, 3, 6, 6, 5, 6, 3,
5, 7, 7, 1, 2, 3, 4, 6, 3, 4, 2, 4, 4, 3, 3,
1% 0, 2, 1, 4, 0, 1, 1, 4, 2, 2, 1, 3, 1, 1, 0, 2,
1, 3, 0, 2, 2, 2, 1, 2, 3, 2, 1, 0, 4, 0, 4,

A16-F-C
0% 4, 5, 3, 5, 0, 1, 3, 1, 3, 4, 1, 2, 3, 3, 4, 5,
6, 7, 5, 2, 3, 2, 3, 3, 2, 2, 3, 3, 3, 4, 4,
1% 1, 1, 1, 2, 1, 2, 2, 2, 0, 1, 1, 2, 2, 3, 3, 5,
2, 2, 2, 0, 1, 3, 2, 0, 1, 0, 0, 1, 2, 0, 1,

A16-F-D
0% 3, 5, 3, 5, 5, 5, 2, 4, 3, 4, 3, 5, 5, 5, 4, 2,
2, 2, 2, 2, 3, 3, 5, 3, 4, 3, 3, 2, 2, 3, 4,
1% 0, 3, 0, 1, 1, 1, 1, 2, 4, 1, 2, 1, 2, 3, 0, 1,
1, 1, 0, 2, 1, 3, 2, 1, 2, 0, 0, 1, 2, 1, 2,

A16-M-A
0% 2, 5, 4, 3, 2, 5, 5, 5, 6, 5, 1, 5, 2, 1, 5, 3,
4, 5, 1, 4, 4, 5, 4, 8, 7, 6, 8, 7, 5, 5, 4,
1% 1, 1, 1, 2, 2, 3, 3, 2, 2, 4, 2, 4, 1, 1, 1, 2,
3, 4, 1, 2, 3, 4, 0, 3, 5, 4, 2, 4, 4, 2, 3,

A16-M-B
0% 3, 1, 3, 3, 4, 6, 3, 5, 4, 4, 4, 3, 2, 6, 4, 4,
3, 2, 3, 5, 2, 3, 3, 3, 2, 3, 2, 2, 3, 3, 3,
1% 2, 3, 2, 2, 1, 4, 0, 0, 2, 2, 1, 0, 1, 0, 3, 2,
3, 0, 1, 1, 0, 1, 0, 1, 3, 3, 2, 2, 1, 2, 1,

A17-F-A
0% 0, 5, 1, 2, 5, 7, 3, 2, 2, 5, 1, 4, 4, 2, 5, 5,
4, 6, 3, 5, 6, 3, 6, 6, 4, 4, 4, 5, 3, 3, 2,
1% 2, 3, 1, 0, 1, 1, 4, 1, 1, 1, 0, 2, 1, 1, 1, 3,
2, 2, 2, 3, 4, 2, 1, 1, 4, 2, 2, 2, 4, 2, 0,

A17-F-B
0% 4, 6, 3, 2, 3, 3, 6, 5, 3, 5, 1, 5, 2, 2, 3, 5,
3, 3, 4, 3, 7, 5, 6, 5, 5, 8, 5, 3, 4, 4, 5,
1% 1, 2, 2, 0, 4, 3, 2, 3, 1, 2, 3, 0, 1, 3, 0, 2,
1, 1, 1, 2, 2, 2, 2, 2, 1, 5, 2, 2, 5, 3, 1,

A17-F-C
0% 2, 3, 3, 3, 3, 3, 4, 3, 4, 4, 2, 4, 1, 1, 3, 4,
4, 3, 2, 5, 5, 2, 1, 5, 4, 5, 3, 2, 5, 3, 2,
1% 2, 0, 0, 2, 0, 1, 1, 1, 0, 1, 0, 2, 0, 2, 0, 2,
3, 3, 4, 3, 1, 1, 1, 4, 1, 1, 4, 0, 1, 0, 2,

A17-M-A
0% 4, 6, 4, 4, 5, 3, 5, 2, 1, 2, 9, 4, 4, 5, 5, 6,
6, 6, 6, 7, 5, 4, 8, 6, 5, 9, 7, 6, 6, 6, 6,
1% 2, 3, 5, 1, 3, 1, 3, 3, 3, 2, 1, 1, 2, 4, 2, 2,
4, 5, 4, 1, 5, 3, 1, 2, 3, 2, 4, 5, 1, 2, 1,

A17-M-B
0% 10, 9, 6, 8, 4, 5, 2, 6, 5, 10, 9, 8, 10, 13, 11, 12,
11, 13, 11, 8, 9, 9, 7, 7, 9, 7, 11, 7, 7, 9, 7,
1% 3, 4, 6, 4, 2, 4, 6, 2, 5, 1, 1, 0, 2, 3, 1, 3,
6, 7, 4, 1, 4, 2, 4, 7, 5, 3, 3, 1, 3, 5, 7,

A17-M-C
0% 5, 1, 4, 7, 6, 6, 8, 9, 8, 9, 9, 9, 8, 8, 4, 4,
6, 7, 7, 9, 4, 6, 3, 7, 4, 4, 5, 6, 7, 2, 3,
1% 1, 3, 2, 1, 1, 3, 3, 1, 3, 3, 1, 3, 2, 1, 1, 2,
4, 1, 2, 4, 3, 4, 2, 2, 5, 3, 2, 3, 3, 2, 2,

B2-F-A
0% 10, 13, 13, 15, 13, 18, 16, 16, 21, 21, 20, 18, 22, 24, 24, 23;
25, 16, 21, 18, 21, 19, 20, 23, 24, 24, 26, 25, 28, 30, 30,
1% 1, 2, 2, 1, 4, 3, 2, 2, 1, 2, 2, 1, 2, 2, 3, 2,
2, 3, 3, 2, 3, 2, 1, 0, 0, 0, 1, 0, 1, 0, 3,

B2-F-B
0% 7, 5, 9, 11, 9, 9, 8, 13, 14, 11, 13, 12, 15, 15, 13, 17,
14, 14, 16, 19, 20, 19, 22, 20, 22, 22, 21, 24, 24, 25, 23,
1% 0, 1, 3, 3, 2, 0, 3, 3, 1, 2, 4, 3, 0, 1, 2, 2,
2, 0, 1, 2, 0, 1, 3, 2, 0, 1, 0, 0, 0, 1, 0,

B2-M-A
0% 12, 12, 11, 10, 12, 13, 17, 16, 18, 22, 25, 26, 22, 25, 24, 21,
22, 23, 22, 22, 21, 23, 19, 25, 22, 25, 24, 25, 22, 22, 22,
1% 1, 3, 4, 1, 6, 4, 2, 3, 1, 1, 2, 0, 2, 0, 0, 2,
0, 0, 1, 0, 0, 1, 1, 2, 2, 0, 2, 1, 0, 1, 1,

B2-M-B
0% 9, 15, 16, 13, 15, 14, 20, 22, 21, 25, 26, 27, 29, 25, 26, 22,
23, 21, 22, 23, 23, 22, 23, 23, 21, 22, 23, 22, 24, 24, 23,
1% 0, 3, 4, 5, 4, 6, 2, 4, 2, 1, 1, 1, 2, 2, 2, 3,
2, 1, 1, 1, 2, 1, 3, 1, 1, 1, 0, 0, 1, 0, 0,

B2-M-C
0% 8, 6, 8, 7, 8, 10, 12, 15, 14, 18, 14, 15, 16, 18, 17, 16,
13, 21, 17, 18, 21, 23, 24, 21, 24, 25, 24, 28, 25, 26, 24,
1% 1, 2, 2, 1, 2, 2, 1, 2, 1, 1, 0, 1, 1, 1, 2, 1,
3, 2, 4, 3, 2, 4, 2, 0, 2, 2, 0, 1, 3, 1, 0,

B4-F-A
0% 5, 6, 6, 4, 5, 7, 5, 7, 7, 2, 10, 9, 8, 9, 10, 4,
7, 5, 5, 5, 6, 4, 4, 6, 6, 11, 9, 11, 13, 12, 11,
1% 2, 3, 2, 3, 1, 4, 4, 3, 3, 0, 0, 2, 1, 1, 2, 0,
1, 1, 0, 2, 0, 0, 0, 1, 1, 1, 1, 1, 1, 0, 0,

B4-F-B
0% 5, 6, 7, 9, 6, 7, 11, 8, 4, 9, 7, 11, 10, 13, 8, 8,
9, 8, 9, 8, 8, 11, 10, 8, 10, 7, 9, 12, 11, 10, 11,
1% 3, 3, 2, 1, 0, 1, 1, 1, 4, 2, 1, 1, 1, 0, 0, 1,
1, 1, 0, 2, 0, 1, 1, 0, 2, 1, 1, 0, 2, 1, 0,

B4-H-A
0% 10, 7, 8, 7, 6, 4, 7, 7, 7, 8, 8, 6, 6, 9, 8, 9,
7, 8, 10, 5, 9, 10, 10, 10, 10, 10, 11, 10, 11, 11, 12,
1% 2, 1, 2, 2, 3, 3, 1, 1, 2, 0, 2, 1, 2, 2, 1, 1,
1, 4, 3, 2, 1, 2, 0, 1, 7, 3, 2, 3, 0, 0, 1,
B4-H-B
0% 8, 10, 9, 6, 9, 8, 9, 8, 6, 6, 4, 6, 8, 8, 10, 8,
4, 5, 6, 6, 8, 9, 9, 6, 7, 6, 10, 7, 10, 10, 12,
1% 6, 5, 5, 1, 4, 4, 2, 4, 4, 2, 1, 0, 2, 3, 2, 2,
3, 5, 1, 1, 1, 3, 0, 0, 2, 1, 1, 2, 0, 0, 0,

B7-F-B
0% 11, 12, 11, 14, 14, 15, 13, 13, 14, 13, 15, 14, 15, 16, 17, 16,
16, 15, 14, 12, 12, 11, 11, 10, 10, 9, 8, 8,
1% 3, 5, 1, 3, 2, 1, 3, 4, 5, 3, 2, 5, 3, 4, 3, 0,
1, 2, 2, 1, 0, 2, 0, 3, 2, 3, 5, 2,
B7-F-C
0% 8, 12, 10, 7, 12, 11, 12, 9, 8, 8, 8, 9, 9, 10, 10, 11,
11, 12, 12, 13, 12, 11, 12, 12, 11, 12, 10, 9, 9, 8, 8,
1% 0, 0, 0, 2, 1, 1, 0, 1, 1, 1, 1, 0, 1, 0, 0, 2,
0, 0, 1, 0, 2, 3, 2, 3, 6, 4, 2, 2, 1, 2, 1,
B7-F-D
0% 7, 6, 7, 9, 9, 9, 7, 8, 8, 7, 10, 12, 10, 11, 11, 10,
10, 13, 12, 9, 11, 10, 9, 9, 8, 10, 8, 7, 7, 9, 7,
1% 3, 2, 1, 1, 0, 0, 1, 2, 0, 2, 4, 2, 2, 0, 2, 1,
0, 3, 2, 4, 3, 3, 1, 2, 3, 2, 1, 1, 0, 0, 0,
B7-H-A
0% 9, 9, 10, 8, 9, 8, 8, 7, 9, 7, 6, 8, 7, 6, 3, 7,
3,
1% 1, 2, 2, 2, 3, 1, 1, 0, 2, 1, 1, 3, 3, 1, 2, 2,
2,
B7-H-B
0% 6, 8, 7, 5, 8, 11, 8, 5, 7, 4, 7, 5, 4, 2, 3, 3,
3,
1% 1, 1, 3, 1, 2, 1, 1, 2, 1, 2, 0, 2, 3, 0, 1, 1,
1,
B7-H-C
0% 4, 5, 3, 5, 5, 9, 12, 14, 11, 12, 8, 9, 6, 9, 7, 5,
6, 8, 8, 4, 10, 10, 9, 12, 11, 9, 9, 7, 7, 7, 7,
1% 1, 2, 0, 0, 1, 2, 2, 0, 0, 0, 1, 2, 0, 0, 0, 2,
4, 2, 1, 2, 2, 1, 0, 0, 2, 1, 1, 1, 2, 2, 2,

B8-F-A
0% 5, 5, 5, 5, 8, 7, 8, 7, 8, 10, 9, 9, 8, 7, 7, 8,
9, 9, 11, 11, 7, 7, 8, 10, 9, 8, 11, 13, 10, 9, 8,
1% 2, 1, 0, 3, 1, 3, 2, 4, 0, 0, 1, 1, 1, 1, 0, 2,
1, 1, 0, 2, 0, 1, 1, 1, 0, 1, 1, 2, 2, 2, 0,
B8-F-B
0% 5, 5, 5, 3, 4, 7, 5, 5, 7, 5, 5, 5, 4, 4, 7, 4,
6, 5, 5, 5, 8, 5, 5, 6, 6, 5, 6, 6, 5, 5, 4,
1% 2, 2, 1, 1, 1, 1, 2, 1, 2, 2, 1, 0, 3, 1, 2, 0,
0, 0, 1, 0, 0, 1, 0, 2, 1, 1, 0, 0, 2, 1, 1,

B3-H-A
0% 5, 7, 3, 5, 4, 6, 5, 9, 8, 7, 8, 8, 7, 3, 5, 6,
4, 5, 4, 6, 6, 6, 6, 8, 9, 7, 9, 7, 8, 8, 9,
1% 0, 4, 1, 1, 1, 1, 2, 1, 1, 1, 0, 1, 2, 2, 0, 0,
0, 1, 0, 0, 0, 1, 0, 0, 0, 1, 1, 1, 0, 1, 0,

B3-H-B
0% 6, 8, 6, 11, 5, 4, 8, 8, 9, 6, 6, 9, 7, 10, 6, 7,
8, 10, 9, 9, 10, 6, 9, 12, 10, 11, 10, 11, 11, 12, 10,
1% 0, 1, 2, 1, 0, 1, 0, 1, 2, 2, 2, 0, 0, 0, 0, 0,
1, 1, 0, 1, 3, 1, 1, 2, 0, 1, 0, 3, 3, 2, 1,

B8-H-C
0% 8, 7, 6, 7, 7, 9, 7, 9, 8, 7, 7, 7, 5, 10, 7, 9,
9, 9, 6, 8, 10, 11, 11, 8, 10, 9, 11, 10, 12, 15, 12,
1% 1, 1, 2, 3, 1, 2, 1, 2, 1, 1, 2, 0, 1, 0, 0, 3,
1, 0, 0, 1, 0, 2, 2, 2, 2, 2, 1, 1, 1, 1, 0,

B10-F-A
0% 11, 10, 8, 8, 6, 6, 11, 11, 9, 8, 8, 11, 11, 13, 9, 9,
8, 8, 9, 12, 11, 10, 10, 7, 8, 8, 10, 8, 8, 10, 9,
1% 3, 2, 3, 0, 2, 3, 2, 2, 3, 1, 2, 3, 0, 1, 0, 1,
2, 1, 2, 1, 2, 0, 1, 1, 2, 1, 2, 2, 1, 2, 1,

B10-F-B
0% 8, 7, 7, 9, 6, 7, 7, 9, 12, 10, 10, 9, 8, 6, 6, 7,
11, 10, 11, 11, 11, 12, 12, 10, 9, 9, 10, 9, 10, 10, 10,
1% 2, 2, 1, 1, 2, 2, 2, 1, 1, 1, 1, 3, 3, 1, 0, 4,
3, 1, 1, 3, 3, 1, 0, 2, 2, 3, 2, 1, 2, 0, 2,

B10-H-A
0% 9, 8, 6, 5, 4, 5, 5, 6, 6, 5, 3, 5, 7, 10, 9, 7,
9, 6, 7, 8, 6, 7, 8, 7, 6, 6, 4, 7, 7, 6, 6,
1% 2, 2, 2, 4, 3, 2, 1, 1, 0, 1, 0, 1, 2, 0, 0, 3,
1, 1, 0, 0, 0, 1, 1, 1, 0, 0, 1, 0, 2, 1, 3,

B10-H-B
0% 6, 6, 8, 12, 8, 7, 8, 10, 7, 6, 7, 8, 8, 7, 9, 8,
5, 5, 6, 6, 7, 8, 9, 4, 7, 6, 8, 7, 7, 7, 7,
1% 5, 4, 2, 0, 0, 2, 1, 2, 1, 2, 0, 1, 0, 1, 2, 1,
2, 2, 1, 2, 2, 0, 0, 0, 0, 0, 1, 1, 1, 2, 1,

B10-H-C
0% 7, 5, 9, 9, 8, 10, 8, 9, 9, 13, 11, 12, 8, 7, 6, 7,
8, 6, 6, 6, 8, 9, 8, 7, 7, 9, 6, 7, 7, 6, 6,
1% 1, 4, 1, 3, 0, 1, 1, 0, 2, 1, 0, 2, 1, 2, 2, 1,
2, 1, 1, 0, 0, 0, 1, 1, 1, 3, 1, 1, 1, 0, 1,

B13-F-A
0% 5, 3, 4, 3, 3, 5, 3, 5, 4, 3, 3, 7, 8, 3, 5, 3,
5, 5, 4, 6, 5, 3, 4, 3, 3, 5, 7, 4, 4, 5, 6,
1% 4, 6, 1, 2, 1, 4, 4, 4, 3, 2, 2, 2, 1, 1, 1, 3,
2, 3, 2, 4, 1, 1, 3, 5, 2, 3, 1, 2, 1, 3, 2,

B13-F-B
0% 5, 3, 3, 4, 1, 4, 4, 4, 6, 3, 2, 5, 5, 3, 4, 3,
2, 5, 1, 5, 4, 2, 4, 3, 1, 5, 4, 6, 3, 6, 4,
1% 2, 0, 3, 3, 4, 3, 1, 2, 0, 4, 3, 3, 3, 0, 4, 2,
4, 1, 2, 2, 2, 2, 3, 2, 2, 1, 3, 2, 3, 2, 4,

B13-M-A
0% 4, 6, 3, 3, 3, 4, 6, 5, 3, 4, 3, 3, 2, 6, 3, 7,
4, 2, 2, 4, 2, 5, 4, 6, 5, 4, 2, 5, 4, 5, 4,
1% 2, 0, 1, 1, 4, 1, 4, 4, 5, 2, 2, 2, 0, 1, 1, 4,
4, 1, 1, 3, 4, 3, 3, 2, 2, 4, 3, 4, 2, 0, 2,
B13-M-B
0% 4, 4, 2, 1, 5, 5, 4, 4, 4, 2, 2, 4, 4, 4, 2, 5,
5, 6, 4, 7, 3, 2, 5, 3, 6, 5, 3, 6, 2, 5, 4,
1% 2, 3, 1, 3, 2, 3, 2, 3, 1, 1, 5, 1, 2, 2, 3, 3,
1, 2, 2, 2, 3, 3, 4, 1, 1, 3, 1, 2, 1, 3, 3,
B13-M-C
0% 8, 3, 2, 5, 1, 3, 3, 4, 2, 2, 4, 5, 2, 2, 3, 5,
2, 3, 3, 2, 4, 3, 3, 5, 4, 2, 5, 2, 3, 3, 3,
1% 2, 5, 3, 2, 4, 2, 2, 2, 3, 3, 0, 1, 2, 1, 1, 1,
3, 2, 4, 2, 3, 2, 4, 1, 1, 1, 2, 3, 1, 1, 2,

B16-F-A
0% 4, 3, 5, 7, 5, 6, 5, 6, 8, 4, 4, 5, 3, 2, 6, 4,
4, 6, 7, 3, 8, 3, 7, 4, 5, 6, 4, 5, 6, 5, 4,
1% 1, 2, 3, 2, 3, 2, 2, 2, 1, 1, 3, 0, 6, 1, 2, 0,
0, 5, 3, 1, 3, 4, 4, 3, 2, 3, 2, 1, 1, 2, 1,
B16-F-B
0% 7, 6, 8, 6, 9, 5, 7, 6, 6, 3, 5, 5, 6, 5, 6, 7,
7, 2, 4, 5, 3, 4, 6, 7, 2, 7, 8, 5, 5, 5, 7,
1% 3, 2, 1, 2, 3, 2, 1, 1, 1, 1, 0, 2, 4, 2, 1, 5,
2, 5, 4, 2, 1, 2, 2, 1, 4, 3, 3, 6, 1, 2, 3,
B16-F-C
0% 4, 5, 8, 9, 6, 7, 3, 5, 4, 3, 2, 3, 3, 5, 7, 8,
5, 4, 6, 6, 6, 6, 5, 6, 7, 5, 4, 5, 4, 3, 3,
1% 2, 1, 1, 2, 2, 2, 2, 1, 2, 2, 2, 1, 0, 1, 1, 2,
2, 2, 2, 3, 1, 0, 3, 2, 0, 1, 1, 0, 1, 1, 0,
B16-M-A
0% 4, 6, 4, 2, 3, 2, 5, 3, 2, 4, 1, 2, 1, 3, 4, 3,
0, 3, 2, 0, 2, 4, 2, 3, 2, 2, 2, 2, 2, 2, 3,
1% 1, 2, 1, 0, 1, 2, 1, 1, 0, 0, 1, 0, 1, 0, 1, 2,
2, 2, 1, 2, 1, 1, 4, 2, 1, 2, 1, 2, 1, 1, 2,
B16-M-B
0% 5, 4, 1, 4, 6, 5, 4, 1, 4, 4, 2, 7, 3, 5, 5, 3,
6, 4, 4, 4, 2, 5, 3, 3, 2, 4, 3, 4, 3, 3, 5,
1% 3, 2, 1, 1, 1, 0, 4, 1, 1, 1, 0, 3, 1, 0, 4, 0,
1, 2, 2, 1, 3, 1, 2, 1, 2, 1, 0, 1, 2, 2, 1,
B16-M-C
0% 6, 5, 4, 4, 5, 3, 3, 5, 3, 6, 4, 3, 6, 6, 4, 4,
5, 2, 4, 4, 4, 4, 4, 4, 4, 2, 3, 0, 2, 3, 3,
1% 3, 1, 1, 0, 2, 3, 3, 2, 0, 2, 0, 1, 3, 0, 1, 1,
0, 1, 1, 0, 0, 1, 1, 1, 1, 1, 0, 3, 1, 0, 1,

B17-F-A
0% 8, 3, 9, 7, 9, 9, 7, 8, 9, 11, 7, 8, 11, 7, 8, 7,
6, 9, 7, 9, 7, 8, 8, 7, 7, 13, 11, 6, 9, 6, 8,
1% 4, 5, 3, 4, 4, 4, 4, 1, 5, 1, 3, 3, 3, 4, 5, 3,
4, 5, 3, 2, 5, 3, 5, 6, 3, 2, 1, 2, 3, 1, 3,
B17-F-B
0% 9, 8, 9, 9, 8, 4, 4, 9, 10, 11, 11, 14, 11, 15, 11, 10,
10, 13, 12, 10, 9, 13, 9, 12, 11, 9, 11, 13, 15, 16, 15,
1% 3, 4, 3, 4, 4, 5, 3, 3, 3, 3, 2, 3, 3, 3, 5, 3,
1, 1, 2, 1, 2, 2, 4, 2, 2, 4, 2, 2, 2, 2, 4,

B17-H-B
0% 13, 11, 10, 13, 11, 13, 13, 14, 18, 13, 13, 10, 12, 12, 14, 14,
13, 11, 11, 16, 15, 13, 15, 14, 15, 12, 10, 10, 10, 11, 10,
1% 3, 4, 0, 5, 5, 4, 3, 1, 1, 3, 2, 2, 2, 4, 1, 4,
2, 8, 1, 4, 4, 0, 5, 6, 4, 3, 7, 6, 4, 5, 5,

B17-H-C
0% 6, 7, 6, 7, 9, 4, 6, 6, 4, 7, 11, 6, 7, 8, 6, 5,
4, 8, 6, 7, 9, 7, 7, 5, 9, 7, 8, 9, 9, 8, 9,
1% 5, 2, 6, 4, 4, 4, 3, 3, 2, 2, 2, 1, 2, 5, 1, 1,
2, 0, 2, 0, 2, 3, 5, 2, 4, 3, 1, 2, 2, 3, 5,

J8-F-A
0% 3, 4, 4, 7, 4, 5, 4, 6, 4, 3, 6, 6, 7, 3, 3, 7,
4, 6, 5, 4, 8, 3, 6, 6, 5, 5, 2, 4, 4, 1, 0,
1% 2, 2, 0, 2, 1, 1, 2, 0, 3, 1, 1, 0, 1, 1, 1, 0,
1, 1, 3, 1, 0, 2, 1, 2, 1, 4, 4, 3, 4, 5, 4,

J8-F-B
0% 2, 0, 4, 3, 2, 1, 2, 4, 2, 1, 1, 3, 2, 3, 0, 1,
3, 2, 3, 1, 5, 4, 6, 3, 5, 4, 5, 3, 4, 3, 4,
1% 1, 2, 2, 2, 3, 2, 2, 2, 2, 0, 3, 2, 0, 1, 2, 1,
2, 2, 0, 3, 1, 1, 0, 1, 1, 0, 2, 0, 1, 0, 0,

J8-H-A
0% 4, 2, 4, 5, 2, 4, 2, 3, 5, 5, 7, 5, 4, 4, 4, 4,
2, 4, 6, 4, 6, 6, 5, 8, 8, 11, 8, 5, 4, 6, 5,
1% 2, 1, 3, 1, 2, 0, 3, 2, 3, 1, 2, 4, 3, 4, 6, 7,
6, 3, 4, 3, 3, 3, 3, 3, 6, 2, 4, 2, 3, 1, 0,

J8-H-B
0% 4, 3, 3, 3, 5, 2, 3, 6, 5, 5, 5, 6, 3, 2, 6, 5,
3, 1, 3, 3, 4, 2, 5, 2, 2, 5, 2, 5, 1, 2, 4,
1% 2, 2, 2, 2, 2, 3, 1, 0, 4, 2, 4, 5, 1, 3, 4, 2,
4, 2, 3, 2, 3, 1, 0, 1, 2, 3, 2, 1, 3, 3, 4,

J11-F-A
0% 5, 5, 3, 6, 6, 7, 6, 8, 7, 10, 11, 9, 12, 8, 11, 13,
9, 7, 10, 8, 6, 7, 6, 6, 8, 11, 6, 4, 5, 6, 3,
1% 1, 1, 1, 2, 2, 0, 1, 2, 0, 2, 1, 0, 2, 1, 2, 1,
1, 3, 4, 3, 2, 3, 3, 1, 3, 2, 5, 1, 1, 1, 1,

J11-F-B
0% 2, 2, 6, 5, 4, 5, 4, 4, 5, 5, 9, 7, 8, 9, 8, 9,
6, 8, 7, 7, 11, 7, 6, 7, 6, 8, 11, 12, 5, 7, 7, 9,
6, 6, 6, 4, 5, 6, 3,
1% 3, 3, 4, 0, 2, 0, 2, 3, 2, 2, 2, 2, 1, 1, 2, 2,
5, 5, 3, 3, 1, 3, 4, 3, 1, 4, 2, 3, 2, 4, 4, 4,
2, 1, 4, 1, 1, 0, 1,

J11-F-C
0% 12, 9, 9, 8, 5, 5, 6, 7, 7, 8, 9, 8, 10, 11, 10, 8,
6, 7, 6, 8, 8, 8, 7, 7, 7, 7, 7, 6, 6, 8, 9, 5,
5, 6, 7, 7, 4, 5, 8, 8, 7,
1% 2, 1, 3, 3, 2, 2, 2, 1, 3, 3, 1, 2, 2, 2, 1, 2,
3, 1, 0, 0, 1, 2, 4, 3, 2, 2, 2, 2, 2, 3, 4, 2,
5, 3, 1, 1, 2, 4, 3, 3, 1,

J11-M-A
0% 5, 5, 9, 7, 6, 7, 10, 9, 9, 8, 8, 7, 9, 12, 9, 15,
12, 10, 13, 12, 10, 8, 9, 7, 8, 5, 8, 7, 9, 8, 8, 8,
7, 9, 8, 9, 7, 10, 7, 7, 8,
1% 1, 2, 1, 0, 2, 0, 0, 1, 2, 1, 0, 0, 2, 1, 1, 1,
0, 2, 1, 2, 2, 2, 3, 0, 2, 3, 2, 1, 3, 0, 3, 5,
4, 3, 2, 2, 1, 4, 3, 3, 4,
J11-M-B
0% 5, 6, 9, 7, 6, 11, 8, 9, 8, 7, 8, 7, 9, 10, 10, 3,
2, 6, 8, 8, 8, 11, 8, 5, 6, 6, 4, 5, 5, 7, 5, 5,
8, 5, 4, 10, 8, 6, 11, 10, 6, 5,
1% 1, 2, 2, 1, 1, 1, 3, 2, 0, 0, 2, 3, 0, 2, 1, 2,
0, 1, 0, 0, 2, 2, 3, 2, 0, 1, 0, 1, 3, 1, 0, 2,
3, 2, 1, 4, 3, 2, 3, 1, 1,
J11-M-C
0% 10, 10, 13, 14, 14, 11, 12, 10, 11, 10, 8, 9, 10, 7, 10, 11,
9, 10, 7, 10, 11, 13, 11, 10, 8, 8, 10, 9, 9, 10, 6, 7,
6, 6, 6, 5, 7, 8, 7, 8, 7,
1% 1, 2, 2, 1, 1, 2, 2, 1, 0, 1, 2, 1, 2, 2, 2, 3,
1, 2, 1, 0, 1, 1, 3, 2, 2, 1, 2, 1, 2, 2, 0, 2,
2, 0, 1, 4, 2, 3, 2, 3, 2,

J19-F-A
0% 5, 6, 3, 4, 5, 7, 10, 6, 4, 5, 5, 6, 5, 7, 9, 9,
8, 3, 11, 3, 8, 5, 6, 7, 8, 7, 7, 8, 7, 8, 7,
1% 3, 1, 4, 4, 5, 1, 5, 3, 4, 4, 1, 4, 2, 2, 4, 4,
5, 6, 4, 7, 2, 3, 3, 4, 4, 3, 6, 5, 3, 4, 3,
J19-F-B
0% 5, 7, 7, 8, 7, 9, 8, 10, 5, 6, 6, 7, 7, 8, 10, 8,
8, 9, 8, 4, 10, 6, 7, 6, 7, 7, 5, 6, 7, 7, 5,
1% 5, 4, 1, 1, 4, 2, 2, 3, 4, 3, 4, 4, 3, 4, 3, 2,
1, 4, 3, 4, 2, 8, 5, 4, 2, 3, 4, 3, 3, 0, 4,
J19-M-A
0% 8, 9, 8, 9, 11, 6, 6, 5, 6, 10, 9, 10, 7, 8, 7, 8,
12, 10, 12, 11, 10, 7, 10, 7, 8, 8, 9, 6, 7, 9, 8,
1% 0, 3, 3, 1, 1, 4, 2, 2, 2, 2, 2, 3, 8, 5, 6, 6,
4, 6, 4, 3, 3, 3, 2, 4, 3, 1, 2, 5, 2, 6, 3,
J19-M-B
0% 6, 5, 8, 7, 9, 8, 7, 6, 9, 8, 6, 8, 7, 9, 8, 8,
10, 9, 6, 8, 10, 10, 6, 5, 6, 7, 3, 7, 7, 11, 9,
1% 5, 2, 5, 5, 3, 3, 1, 2, 1, 6, 7, 5, 2, 2, 3, 2,
5, 2, 6, 1, 3, 3, 7, 2, 4, 4, 7, 4, 1, 7, 4,

```
V3-F-A
  0%  7, 5, 7, 4, 5, 5, 4, 2, 6, 6, 6, 7, 7, 7, 8, 8,
      11, 9, 9, 8, 9, 7, 6, 7, 9, 8, 7, 9, 8, 9, 9,
  1%  4, 1, 2, 2, 2, 2, 2, 2, 1, 2, 1, 1, 1, 1, 2, 1,
      2, 1, 2, 1, 3, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0,
V3-H-A
  0%  7, 6, 4, 6, 6, 4, 8, 7, 6, 6, 7, 7, 11, 7, 11, 8,
      9, 9, 10, 9, 6, 8, 10, 7, 8, 9, 10, 10, 11, 10, 7,
  1%  1, 2, 1, 1, 1, 1, 1, 1, 1, 2, 2, 0, 0, 1, 1, 2,
      1, 2, 1, 2, 1, 0, 2, 1, 2, 0, 0, 1, 0, 1, 2,
B3-H-B
  0%  3, 6, 3, 2, 1, 4, 5, 5, 4, 4, 4, 6, 4, 6, 6, 3,
      2, 4, 5, 7, 4, 3, 6, 3, 4, 3, 4, 6, 6, 5, 6,
  1%  3, 1, 1, 2, 1, 1, 1, 0, 2, 0, 0, 1, 0, 1, 0, 0,
      0, 1, 0, 1, 2, 0, 2, 0, 1, 0, 1, 1, 0, 0, 1,
V3-H-C
  0%  5, 4, 5, 1, 3, 4, 8, 5, 6, 4, 6, 7, 10, 9, 12, 8,
      8, 9, 8, 9, 7, 8, 10, 11, 10, 13, 15, 13, 12, 11, 10,
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      1, 1, 2, 0, 0, 1, 0, 1, 0, 1, 0, 1, 0, 0, 1,
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V11-F-A
0% 6, 8, 6, 6, 10, 7, 10, 7, 8, 7, 9, 6, 9, 6, 10, 10,
14, 13, 13, 15, 16, 15, 14, 17, 15, 18, 17, 15, 15, 15, 18,
1% 0, 0, 1, 1, 1, 1, 1, 3, 1, 0, 1, 0, 0, 2, 1, 0,
1, 1, 4, 4, 1, 1, 1, 1, 1, 2, 2, 1, 1, 2, 1,
V11-F-C
0% 7, 8, 11, 12, 13, 10, 10, 13, 15, 11, 10, 9, 10, 11, 10, 12,
13, 16, 12, 12, 14, 11, 15, 15, 13, 18, 17, 16, 16, 14, 15,
1% 3, 2, 1, 2, 4, 1, 2, 2, 1, 2, 2, 1, 0, 0, 1, 1,
1, 1, 1, 2, 1, 4, 1, 1, 3, 1, 2, 4, 1, 2, 3,
V11-H-A
0% 15, 15, 21, 16, 17, 22, 21, 22, 22, 24, 24, 25, 22, 23, 24,
27, 28, 26, 26, 27, 27, 29, 28, 27, 28, 26, 28, 29, 29, 30,
1% 3, 0, 1, 1, 2, 3, 2, 0, 0, 2, 0, 0, 3, 0, 2, 0,
0, 0, 0, 1, 1, 0, 2, 1, 4, 0, 1, 0, 1, 0, 0,
V11-H-B
0% 20, 18, 19, 20, 21, 21, 22, 21, 23, 20, 25, 25, 23, 25, 26,
29, 30, 32, 32, 30, 30, 31, 32, 29, 31, 27, 30, 32, 30, 31,
1% 2, 4, 3, 2, 1, 2, 2, 2, 1, 2, 1, 0, 1, 2, 1, 1,
2, 2, 1, 2, 2, 1, 1, 1, 1, 1, 2, 1, 0, 1, 1,
V11-H-C
0% 14, 16, 17, 21, 20, 21, 21, 22, 19, 26, 24, 24, 27, 26, 28,
26, 27, 33, 32, 33, 28, 29, 31, 30, 30, 32, 30, 30, 29, 28,
1% 0, 0, 1, 0, 0, 1, 1, 0, 1, 1, 2, 2, 2, 1, 2, 1,
3, 2, 1, 2, 2, 1, 2, 3, 3, 0, 1, 1, 1, 1, 2,

V31-F-A
0% 1, 6, 5, 2, 4, 5, 7, 2, 6, 3, 7, 6, 6, 5, 6, 6,
6, 6, 4, 5, 6, 6, 8, 7, 8, 7, 8, 6, 6, 5, 8,
1% 2, 1, 3, 2, 1, 2, 4, 3, 2, 2, 1, 0, 1, 1, 0, 0,
1, 3, 4, 2, 2, 1, 2, 1, 2, 1, 1, 3, 1, 2, 3,
V31-F-B
0% 9, 8, 7, 5, 5, 5, 6, 5, 3, 8, 6, 5, 6, 8, 8, 5,
7, 5, 6, 6, 6, 6, 8, 5, 6, 6, 7, 8, 8, 7, 6,
1% 2, 3, 1, 2, 1, 1, 0, 1, 0, 0, 1, 1, 1, 1, 1, 2,
1, 1, 2, 3, 2, 3, 3, 3, 3, 4, 3, 3, 3, 2, 2,
V31-F-C
0% 3, 3, 5, 6, 6, 9, 7, 6, 5, 6, 7, 7, 8, 8, 8, 7,
3, 9, 9, 11, 11, 12, 8, 8, 9, 8, 9, 9, 7, 9, 10,
1% 4, 3, 3, 3, 3, 3, 3, 3, 2, 2, 1, 1, 2, 2, 2, 1,
2, 2, 2, 1, 3, 3, 4, 6, 4, 3, 3, 3, 3, 3, 4,
V31-H-A
0% 11, 13, 16, 17, 16, 15, 12, 13, 12, 14, 17, 17, 18, 19, 19,
16, 18, 16, 14, 16, 17, 12, 13, 14, 13, 16, 16, 17, 16, 15,
1% 6, 5, 7, 5, 5, 4, 3, 2, 5, 4, 3, 3, 3, 5, 6, 5,
6, 6, 7, 6, 4, 5, 3, 4, 4, 3, 3, 3, 3, 4, 3,
V31-H-B
0% 5, 2, 2, 3, 4, 3, 8, 6, 6, 9, 9, 7, 7, 7, 7, 6,
8, 7, 6, 10, 10, 11, 11, 11, 11, 13, 12, 11, 12, 11, 9,
1% 1, 2, 2, 2, 0, 2, 2, 2, 0, 1, 3, 2, 1, 2, 1, 0,
0, 1, 1, 1, 1, 2, 2, 1, 1, 1, 1, 2, 3, 3, 2,

R-A4

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A4-F-A	3.806	1.661	2.806	1.301	.5828	.1864	1.356
A4-F-B	4.129	1.147	2.677	1.720	.6356	.2083	1.542
A4-M-A	7.516	1.363	1.580	1.057	.8333	.1023	4.755
A4-M-B	5.290	1.636	1.548	1.337	.7956	.1538	3.416
A4-M-C	5.064	1.504	1.483	1.028	.7853	.1262	3.413

R-A5

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A5-F	5.677	1.620	2.290	1.295	.7116	.1679	2.478
A5-F-C	3.451	1.524	1.806	1.327	.6670	.1880	1.910
A5-M-A	5.290	1.465	1.741	.9649	.7540	.1211	3.037
A5-M-B	3.903	1.578	1.774	1.023	.6833	.1866	2.199
A5-M-C	3.709	1.696	1.903	1.193	.6483	.1944	1.949
A6-F-A	4.935	1.590	1.645	1.252	.7565	.1851	2.999
A6-F-B	5.975	1.836	2.195	1.631	.7479	.1625	2.722
A6-F-C	3.902	1.609	1.585	1.139	.7104	.1964	2.461
A6-M-A	4.259	1.318	1.925	.9971	.6893	.1321	2.211
A6-M-B	4.578	1.804	2.842	1.258	.6074	.1706	1.611
A6-M-C	6.097	2.034	1.682	1.293	.7813	.1716	3.623
A7-F-A	4.419	1.478	1.903	1.374	.7166	.1814	2.322
A7-F-B	4.967	2.316	2.032	1.401	.7146	.1543	2.444
A7-M-A	4.612	1.856	1.516	.9616	.7573	.1325	3.042
A7-M-B	3.483	1.261	1.451	.9946	.7126	.1712	2.399
A7-M-C	3.935	1.340	1.870	5.625	.7896	.1827	2.103

R-A8&10

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A8-F-A	4.208	1.531	2.500	1.251	.6290	.1761	1.683
A8-F-B	3.920	1.351	2.480	1.610	.6345	.2374	1.580
A8-F-C	4.677	1.599	2.451	1.059	.6524	.1426	1.907
A8-M-A	3.800	1.704	1.800	1.321	.6865	.2387	2.111
A8-M-B	4.200	1.424	2.866	1.767	.6307	.1807	1.465
A8-M-C	3.428	2.087	2.238	1.338	.5784	.2697	1.531
A9-F-A	6.400	2.620	3.766	1.633	.6158	.1654	1.699
A9-F-B	5.129	1.688	3.709	1.696	.5885	.1521	1.382
A9-M-A	5.967	2.798	3.193	1.701	.6422	.1687	1.868
A9-M-B	5.806	1.922	3.645	1.664	.6193	.1214	1.592
A9-M-C	5.741	2.250	3.161	1.593	.6426	.1861	1.816
A10-F-A	8.625	3.066	2.406	1.775	.8038	.1088	3.584
A10-F-B	7.966	1.325	2.366	1.376	.7773	.0969	3.366
A10-F-C	6.731	1.987	1.756	1.199	.7964	.1232	3.833
A10-M-A	3.741	1.982	2.161	1.529	.6395	.2431	1.731
A10-M-B	3.121	1.951	1.560	1.073	.6426	.2878	1.999
A10-M-C	3.150	1.805	2.025	1.404	.5870	.2632	1.555
A10-M-D	7.291	2.156	3.291	1.654	.6959	.1016	2.215

R-A11&12

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A11-F-A	5.090	1.743	3.181	1.296	.6156	.1691	1.6
A11-F-B	4.700	1.218	2.050	1.234	.7213	.1524	2.292
A11-F-C	3.064	1.459	1.967	1.277	.6049	.2292	1.557
A11-F-D	3.379	1.567	1.379	1.082	.7076	.2333	2.449
A11-M-A	4.153	1.068	2.000	1.290	.7023	.1573	2.076
A11-M-B	3.461	1.450	1.769	1.300	.6775	.2159	1.956
A11-M-C	3.172	1.364	1.275	.9962	.7289	.2242	2.486
A12-F-A	6.076	1.647	3.038	1.821	.6845	.1647	2
A12-F-B	4.268	1.732	2.341	.9646	.6345	.1479	1.822
A12-F-C	4.926	1.649	2.341	1.389	.6851	.1655	2.104
A12-M-A	4.000	1.654	2.750	1.332	.5802	.2205	1.454
A12-M-B	3.225	1.790	1.900	.9554	.5888	.2408	1.697

R-A15&J11

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A15-F-A	6.146	1.917	2.414	1.448	.7180	.1742	2.545
A15-F-B	7.853	2.185	3.024	1.680	.7262	.1355	2.596
A15-F-C	6.516	2.420	2.774	1.359	.6873	.1460	2.348
A15-M-A	16.85	1.981	1.000	.8164	.9414	.0484	16.84
A15-M-B	14.51	3.668	1.463	1.305	.9057	.0785	9.916
A15-M-C	10.62	2.372	.9729	.7988	.9201	.0596	10.91
A15-M-D	14.1	1.971	1.366	1.188	.9107	.0781	10.31
A16-F-A	3.064	1.481	1.709	1.039	.6360	.1868	1.792
A16-F-B	4.419	1.628	1.677	1.275	.7300	.1892	2.634
A16-F-C	3.193	1.536	1.483	1.121	.6820	.2352	2.152
A16-F-D	3.419	1.148	1.354	1.018	.7394	.1637	2.523
A16-M-A	4.387	1.873	2.451	1.260	.6376	.1290	1.789
A16-M-B	3.258	1.153	1.483	1.121	.7119	.1999	2.195

R-A17

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A17-F-A	3.774	1.726	1.806	1.166	.6823	.1990	2.089
A17-F-B	4.129	1.586	1.967	1.251	.6908	.1709	2.098
A17-F-C	3.161	1.213	1.387	1.282	.7370	.2171	2.279
A17-M-A	5.225	1.838	2.612	1.358	.6623	.1552	2
A17-M-B	8.387	2.577	3.516	1.981	.7075	.1588	2.385
A17-M-C	5.967	2.243	2.354	1.081	.7016	.1464	2.534

R-B2

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
B2-F-A	20.54	5.045	1.709	1.070	.9182	.0538	12.01
B2-F-B	15.67	5.653	1.387	1.229	.9080	.0874	11.30
B2-M-A	20.16	4.831	1.419	1.432	.9257	.0839	14.20
B2-M-B	21.41	4.410	1.838	1.529	.9178	.0782	11.64
B2-M-C	17.22	6.200	1.612	1.054	.9046	.0654	10.67

R-B4

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
B4-F-A	7.064	2.768	1.322	1.221	.8505	.1368	5.341
B4-F-B	8.709	2.068	1.129	.9913	.8818	.1157	7.714
B4-M-A	8.419	1.962	1.806	1.400	.8267	.1109	4.660
B4-M-B	7.677	1.938	2.161	1.753	.7948	.1424	3.552

R-B7

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
B7-F-B	12.82	2.480	2.500	1.503	.8396	.0926	5.128
B7-F-C	10.19	1.701	1.290	1.370	.8964	.0919	7.899
B7-F-D	9.032	1.760	1.548	1.233	.8644	.0996	5.833
B7-M-A	7.294	1.961	1.705	.8488	.8054	.1053	4.275
B7-M-B	5.647	2.396	1.352	.8617	.8045	.1176	4.173
B7-M-C	8.000	2.720	1.161	1.003	.8708	.1125	6.888

R-B8

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
B8-F-A	8.258	1.914	1.193	1.013	.8775	.1041	6.918
B8-F-B	5.225	1.055	1.032	.8360	.8439	.1171	5.062
B8-M-A	6.387	1.782	.7741	.8835	.8951	.1133	8.249
B8-M-B	8.516	2.158	1.032	.9826	.8999	.0901	8.249
B8-M-C	8.741	2.144	1.193	.8725	.8820	.0836	7.324

R-B10

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
B10-F-A	9.193	1.681	1.580	.9228	.8552	.0825	5.816
B10-F-B	9.129	1.821	1.709	1.006	.8444	.0863	5.339
B10-M-A	6.451	1.609	1.161	1.098	.8561	.1284	5.555
B10-M-B	7.225	1.542	1.258	1.182	.8595	.1211	5.743
B10-M-C	7.870	1.857	1.161	.9694	.8755	.0971	6.777

R-B13

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
B13-F-A	4.387	1.382	2.451	1.337	.6508	.1392	1.789
B13-F-B	3.677	1.423	2.322	1.165	.6126	.2000	1.583
B13-M-A	3.967	1.378	2.322	1.423	.6523	.1682	1.708
B13-M-B	3.935	1.459	2.225	1.023	.6361	.1470	1.768
B13-M-C	3.258	1.413	2.129	1.147	.6058	.1825	1.530

R-B16

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
B16-F-A	4.967	1.516	2.129	1.408	.7181	.1504	2.333
B16-F-B	5.612	1.686	2.322	1.423	.7143	.1555	2.416
B16-F-C	5.064	1.730	1.387	.8436	.7872	.1302	3.651
B16-M-A	2.580	1.310	1.258	.8550	.6618	.2470	2.051
B16-M-B	3.806	1.400	1.451	1.090	.7342	.1706	2.622
B16-M-C	3.838	1.344	1.129	1.024	.7821	.2067	3.399

R-B17

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
B17-F-A	8.032	1.870	3.354	1.355	.7050	.1157	2.394
B17-F-B	11.67	2.737	1.903	1.106	.8562	.0873	6.135
B17-F-C	10.67	2.832	2.806	1.077	.7820	.1065	3.804
B17-M-A	4.838	1.319	3.451	1.859	.5984	.1633	1.401
B17-M-B	12.58	2.012	3.483	1.981	.7908	.1101	3.611
B17-M-C	7.000	1.693	2.677	1.535	.7334	.1251	2.614
J8-F-A	4.483	1.805	1.741	1.389	.7144	.2352	2.574
J8-F-B	2.774	1.521	1.322	.9793	.6617	.2908	2.097
J8-M-A	4.903	2.005	2.903	1.700	.6377	.1713	1.688
J8-M-B	3.548	1.501	2.354	1.226	.5980	.1696	1.506
J11-F-A	7.387	2.551	1.709	1.160	.8135	.1028	4.320
J11-F-B	6.461	2.292	2.358	1.347	.7347	.1426	2.739
J11-F-C	7.341	1.697	2.146	1.085	.7757	.1062	3.420
J11-M-A	8.512	2.099	1.756	1.299	.8350	.1157	4.847
J11-M-B	7.195	1.952	1.487	1.098	.8359	.1130	4.836
J11-M-C	9.219	2.241	1.634	.8875	.8488	.0871	5.641
J19-F-A	6.419	2.012	3.645	1.450	.6381	.1356	1.761
J19-F-B	7.096	1.535	3.193	1.514	.6944	.1322	2.222
J19-M-A	8.419	1.821	3.258	1.825	.7308	.1214	2.584
J19-M-B	7.516	1.748	3.677	1.955	.6829	.1411	2.043

R-V3

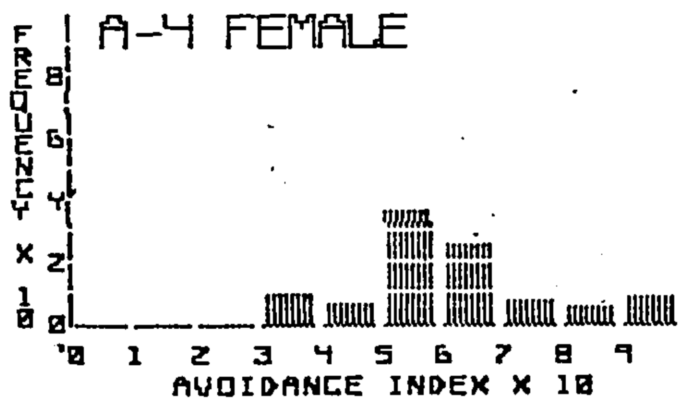
RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
V3-F-A	9.129	3.575	1.096	1.164	.8889	.1218	8.323
V3-F-B	7.064	1.896	1.193	1.013	.8505	.1306	5.918
V3-M-A	4.322	1.469	.7741	.8045	.8557	.1588	5.583
V3-M-B	8.096	3.300	.6774	.8321	.9025	.1317	11.95

R-V11

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
V11-F-A	11.45	4.137	1.193	1.013	.9085	.0753	9.594
V11-F-B	12.54	2.718	1.709	1.070	.8804	.0722	7.339
V11-M-A	24.35	4.239	.9677	1.168	.9603	.0486	25.16
V11-M-B	26.29	4.705	1.483	.8112	.9432	.0376	17.71
V11-M-C	25.83	5.164	1.290	.9016	.9552	.0293	20.02

R-V31

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
V31-F-A	5.580	1.765	1.741	1.063	.7526	.1648	3.203
V31-F-B	6.322	1.351	1.806	1.077	.7908	.1056	3.499
V31-F-C	7.516	2.249	2.709	1.070	.7275	.1087	2.773
V31-M-A	15.35	2.199	4.354	1.355	.7806	.0558	3.525
V31-M-B	7.870	3.106	1.451	.8500	.8295	.1316	5.422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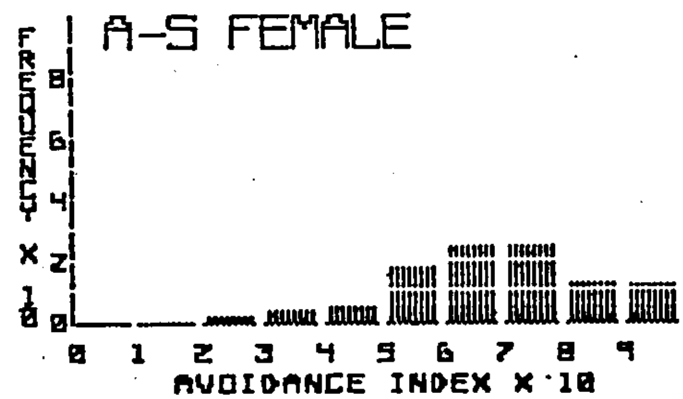
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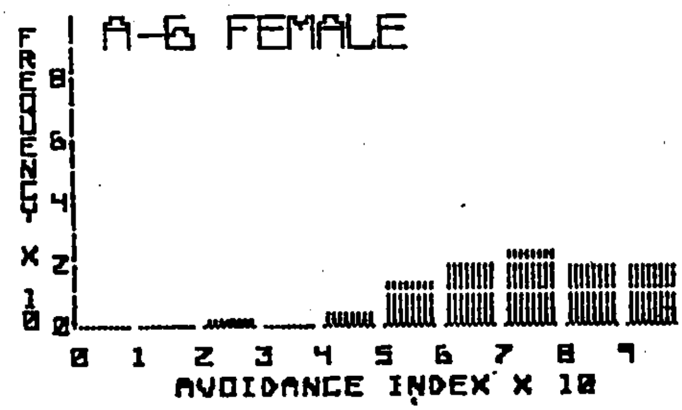
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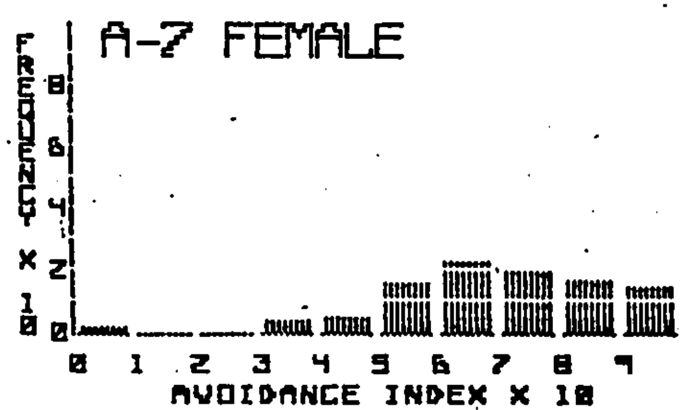
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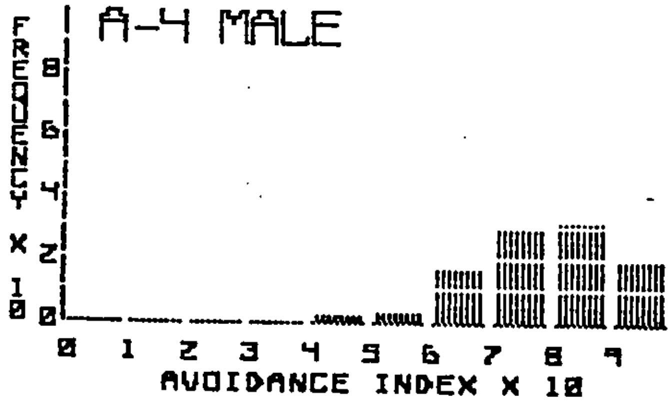
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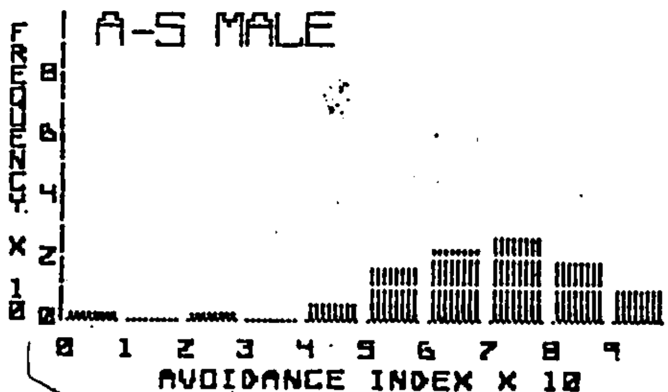
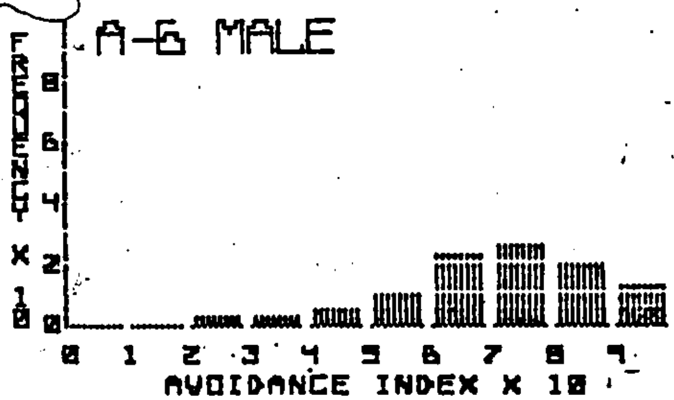
AVOIDANCE INDEX X 10


A-5 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-6 MALE
FREQUENCY X 10
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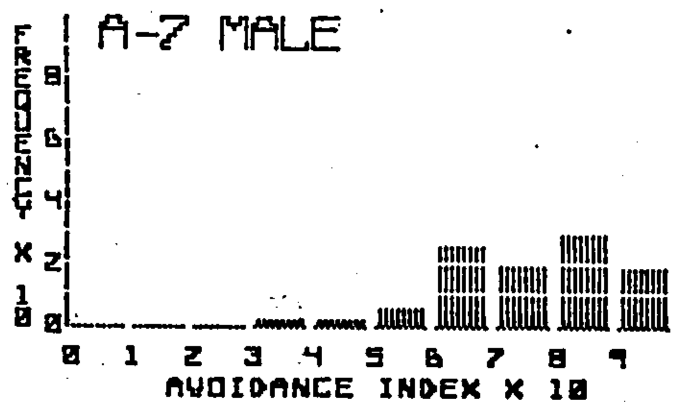
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-7 MALE
FREQUENCY X 10
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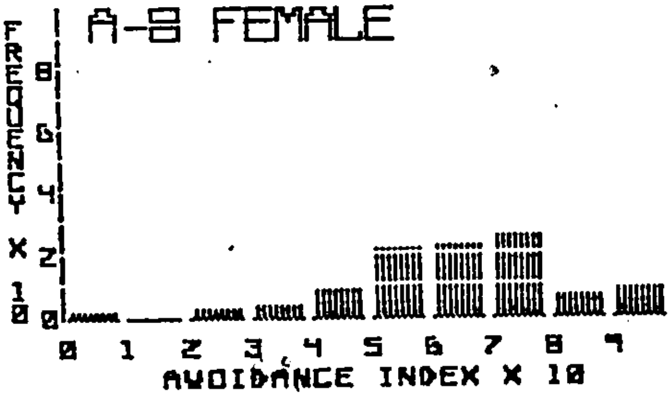
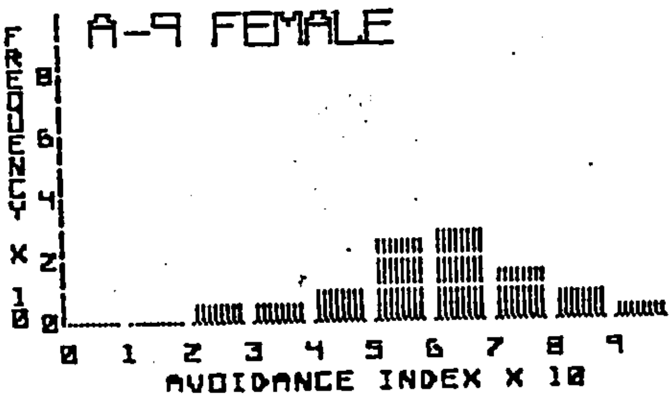
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-8 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-9 FEMALE
FREQUENCY X 10
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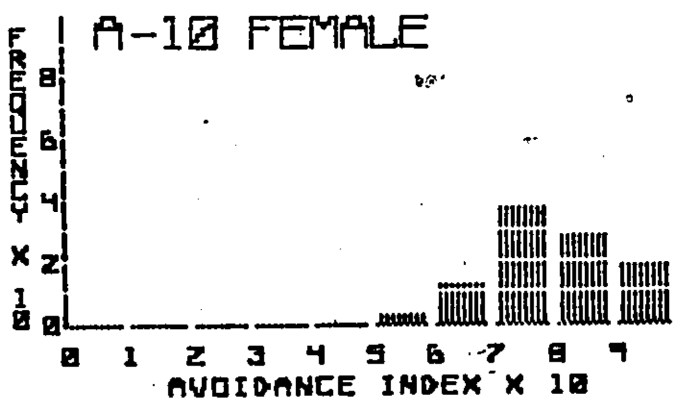
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10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FREQUENCY X 10
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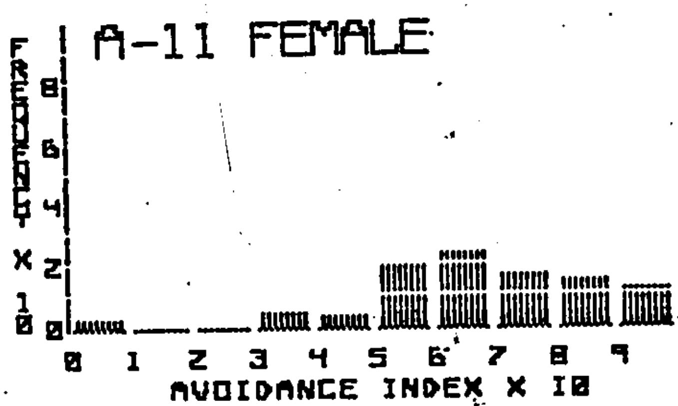
AVOIDANCE INDEX X 10


A-11 FEMALE
FREQUENCY X 10
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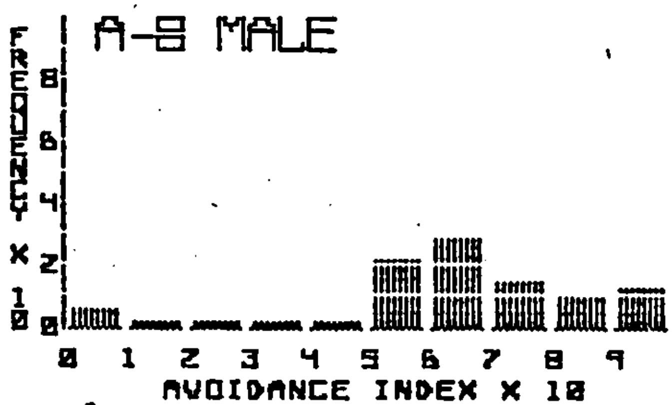
A-8 MALE
FREQUENCY X 10
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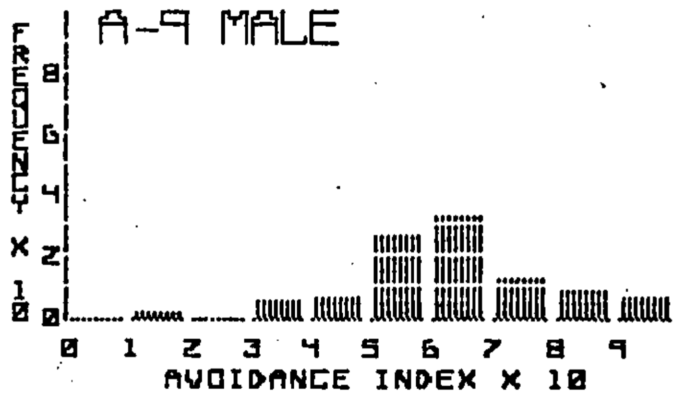
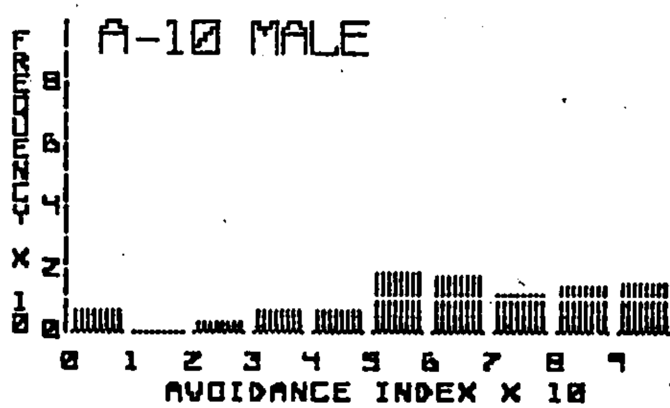
AVOIDANCE INDEX X 10


A-9 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-10 MALE
FREQUENCY X 10
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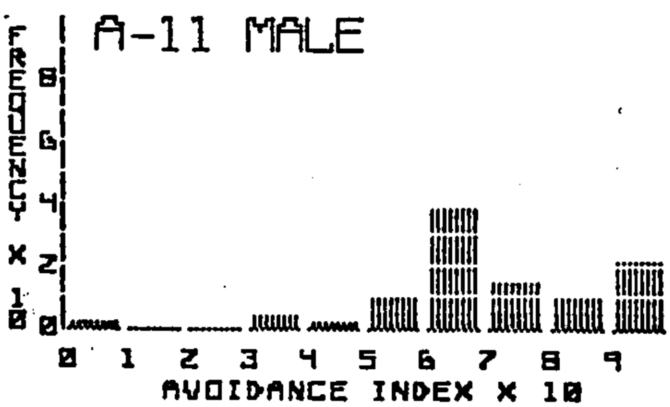
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-11 MALE
FREQUENCY X 10
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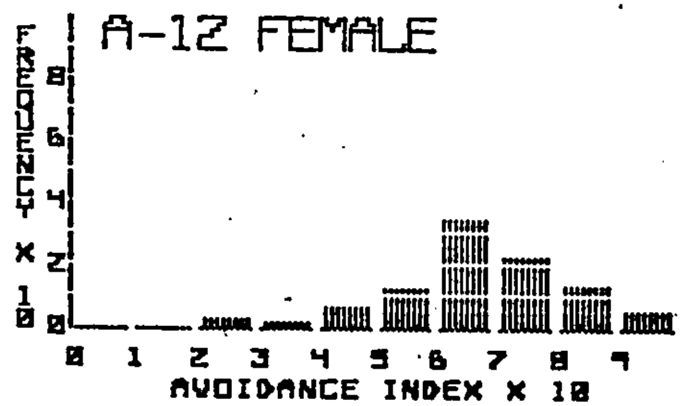
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-12 FEMALE
FREQUENCY X 10
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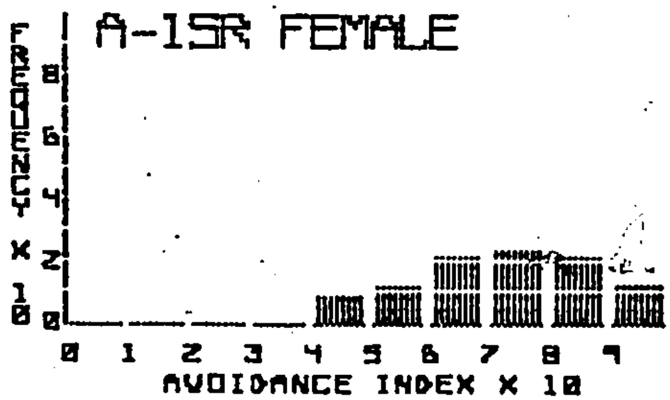
AVOIDANCE INDEX X 10


A-15R FEMALE
FREQUENCY X 10
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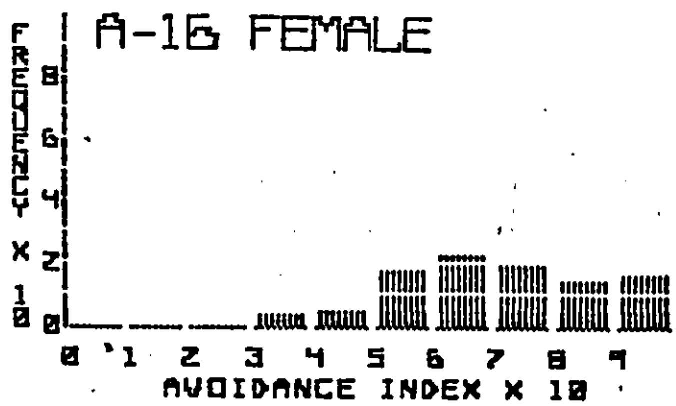
AVOIDANCE INDEX X 10


A-16 FEMALE
FREQUENCY X 10
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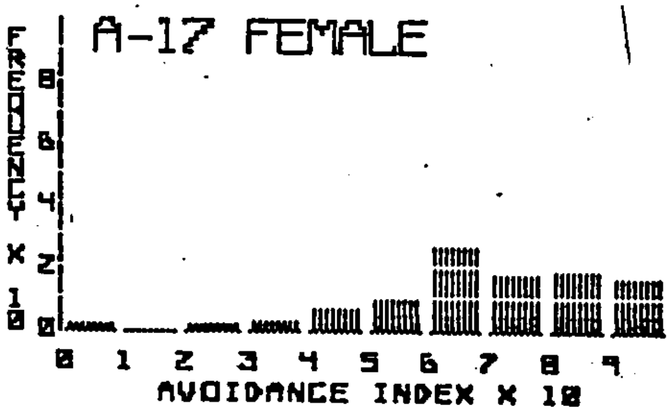
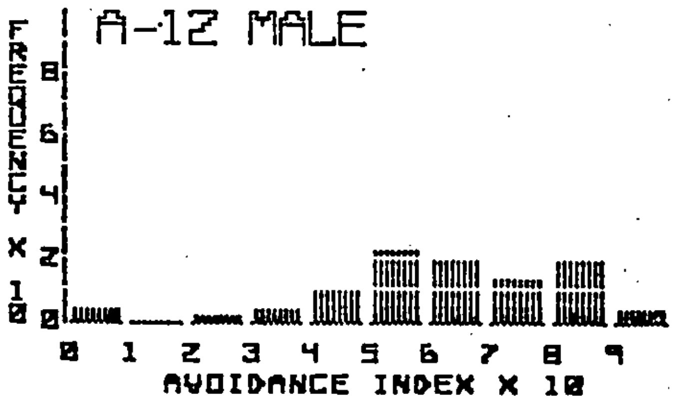
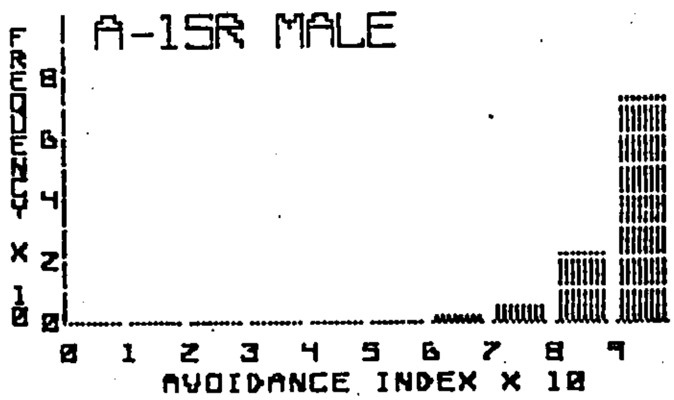
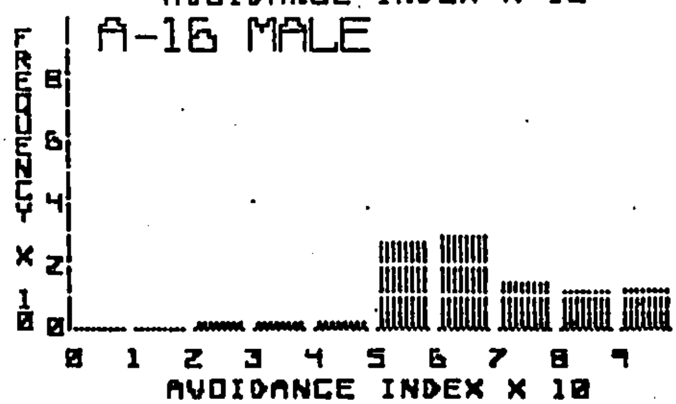
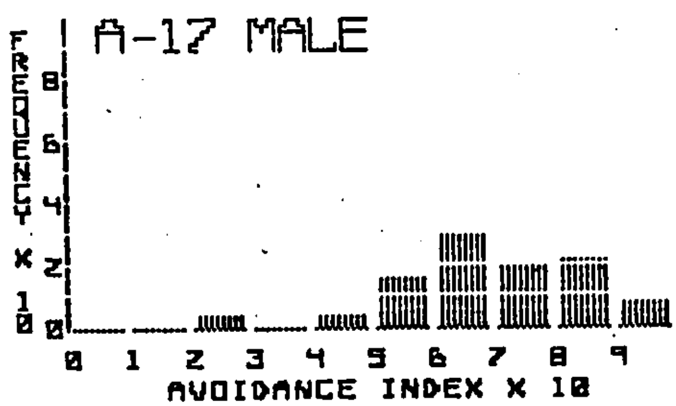
AVOIDANCE INDEX X 10

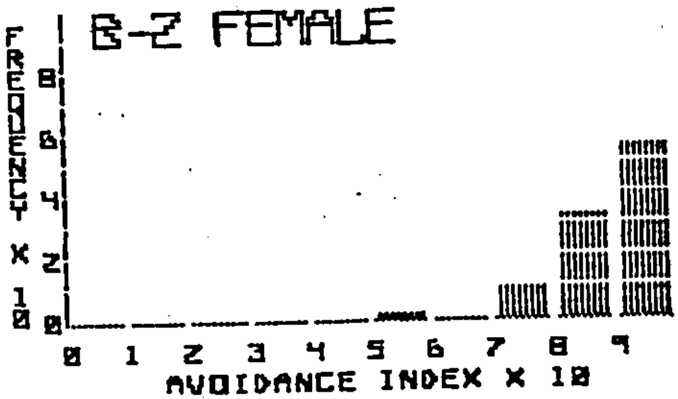

A-17 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

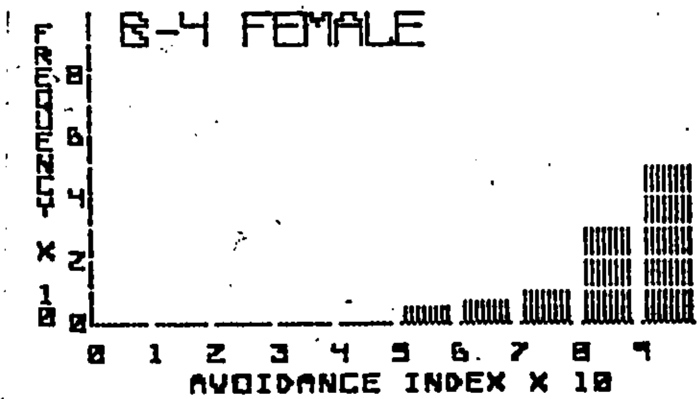

A-12 MALE
FREQUENCY X 10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

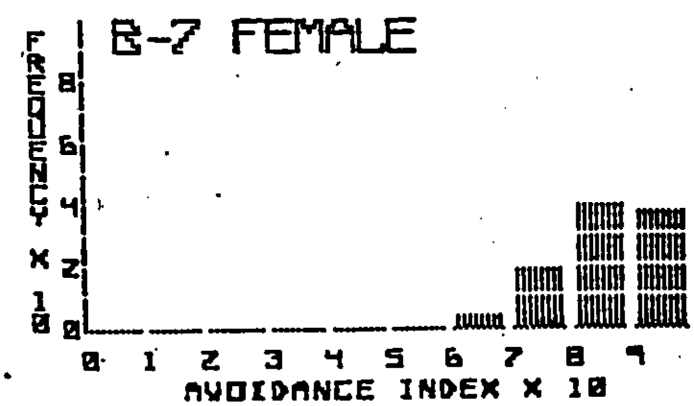

A-15R MALE
FREQUENCY X 10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

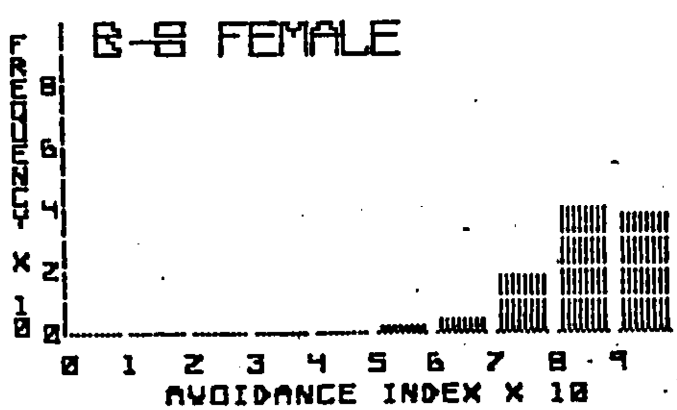

A-16 MALE
FREQUENCY X 10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-17 MALE
FREQUENCY X 10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


B-2 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


B-4 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


B-7 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


B-8 FEMALE
FREQUENCY X 10
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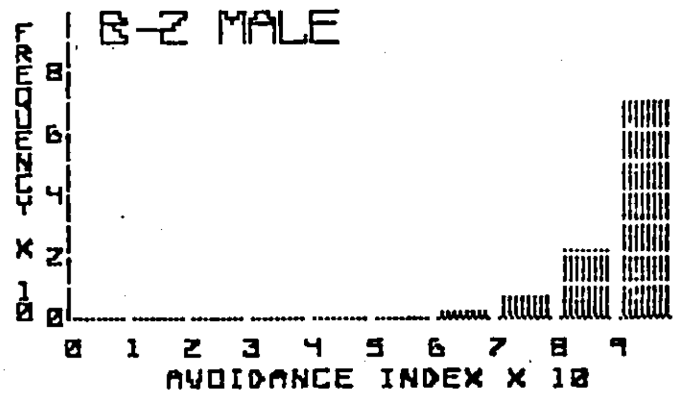
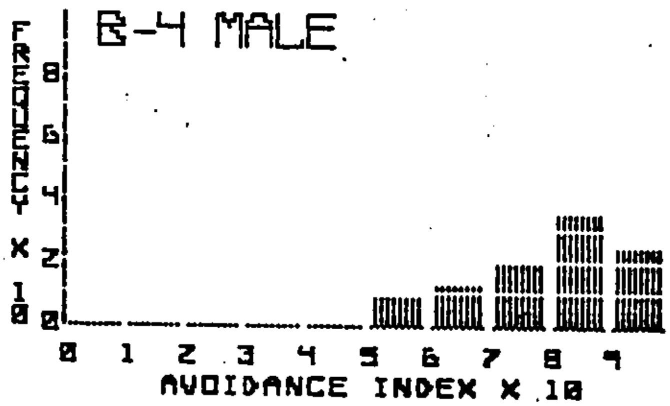
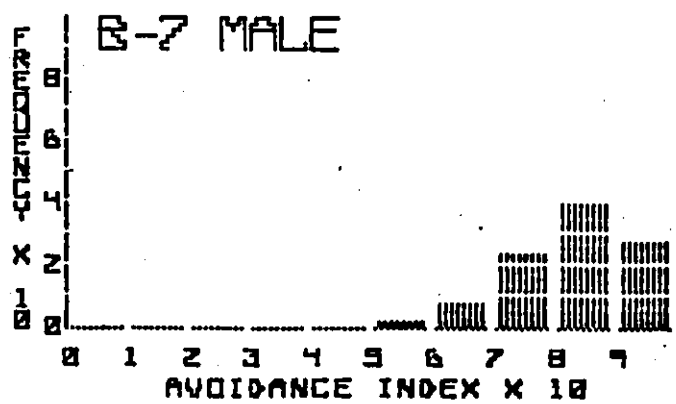
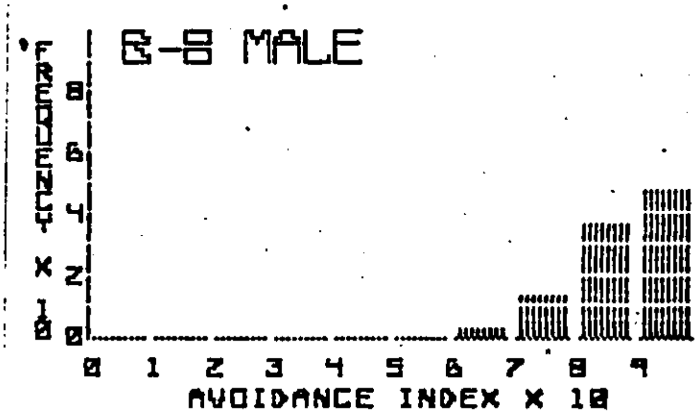
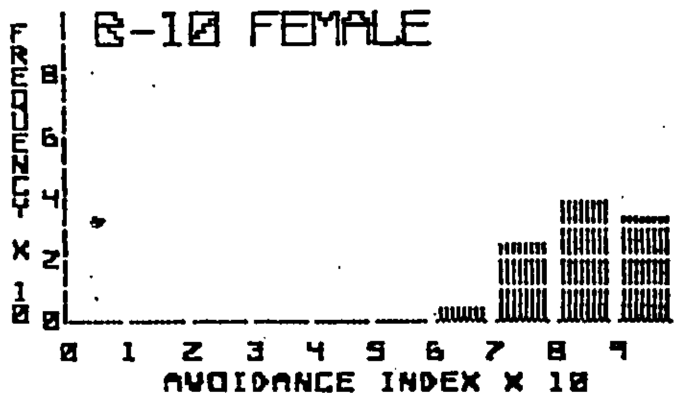
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

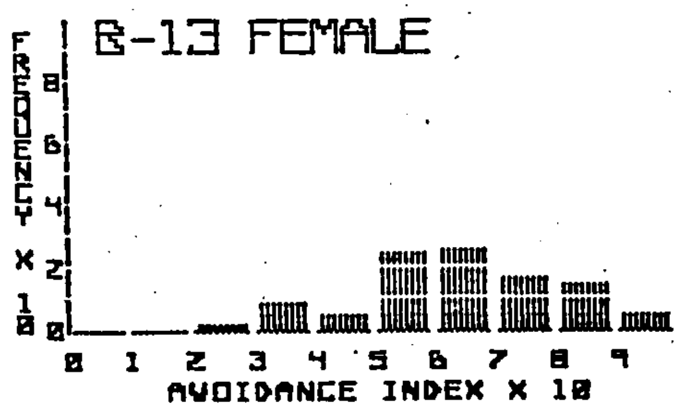

B-2 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

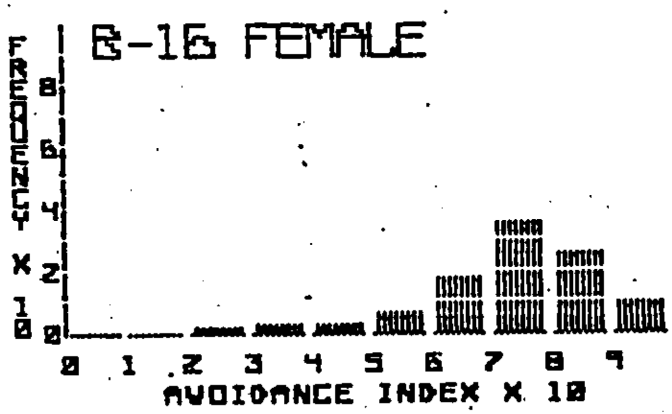

B-4 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

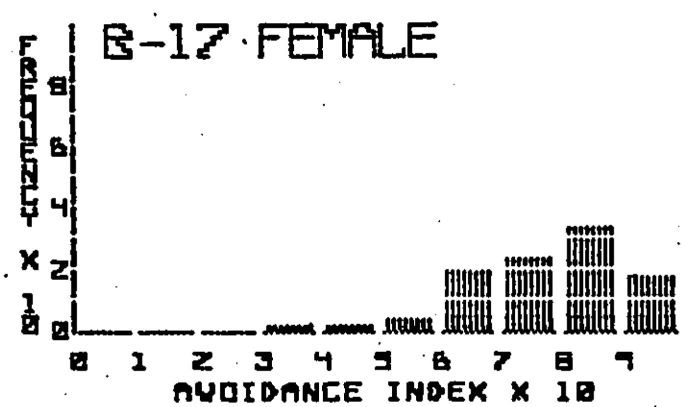

B-7 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

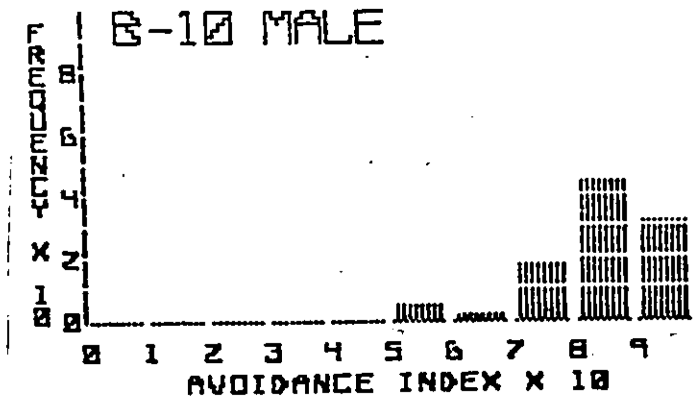

B-8 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

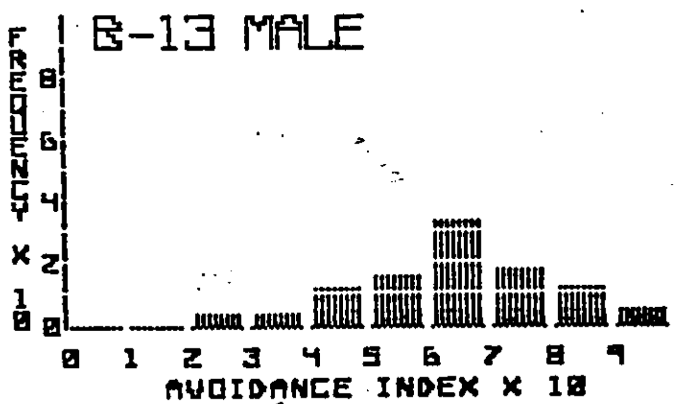

B-10 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

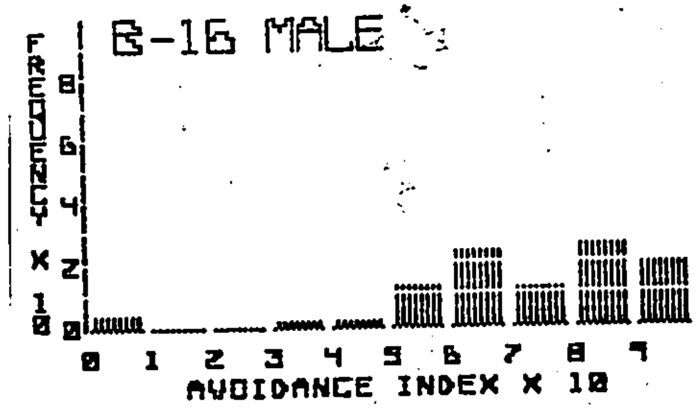

B-13 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

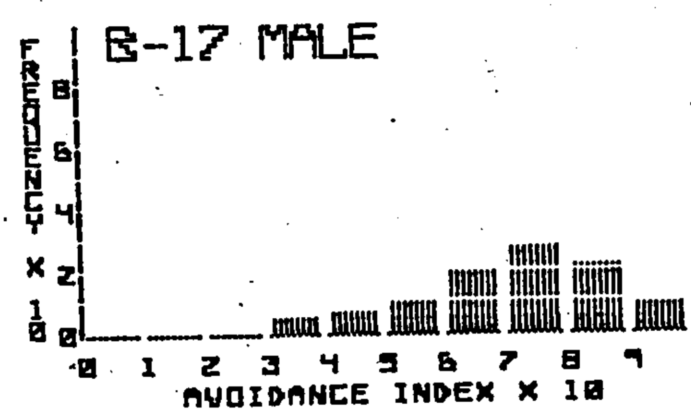

B-16 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

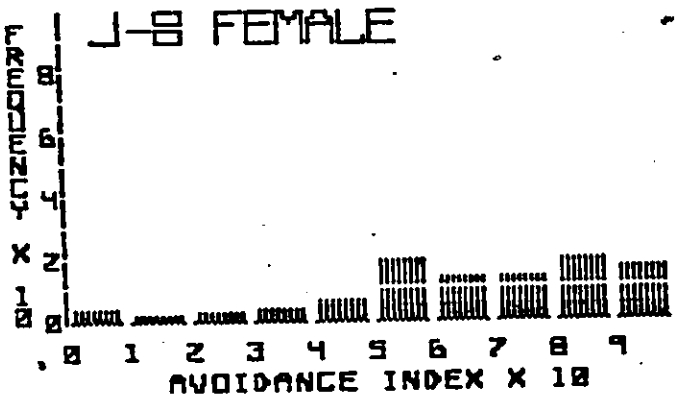

B-17 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

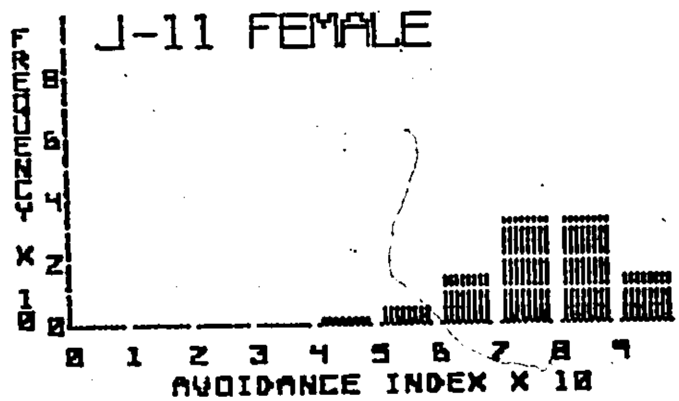

B-10 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

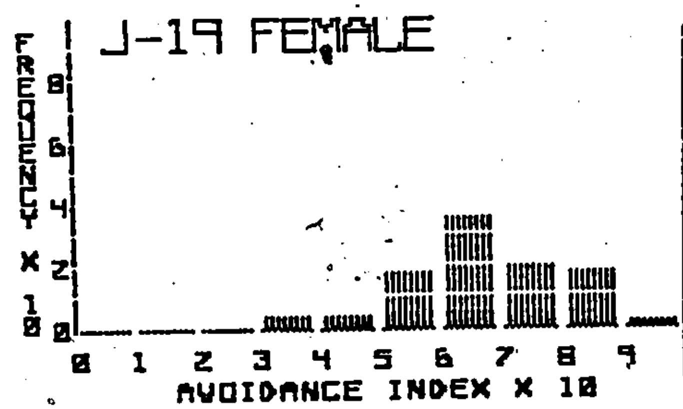

B-13 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

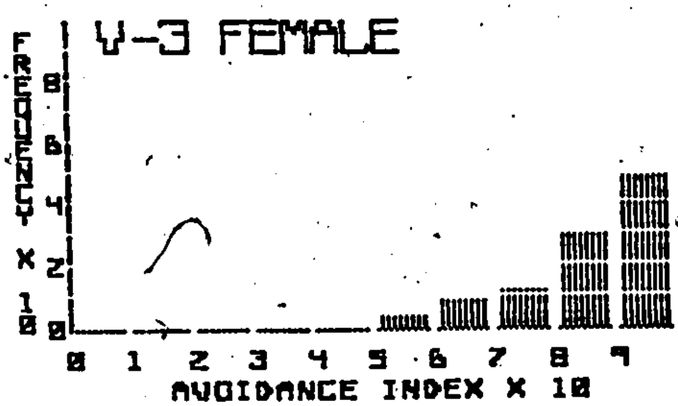

B-16 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

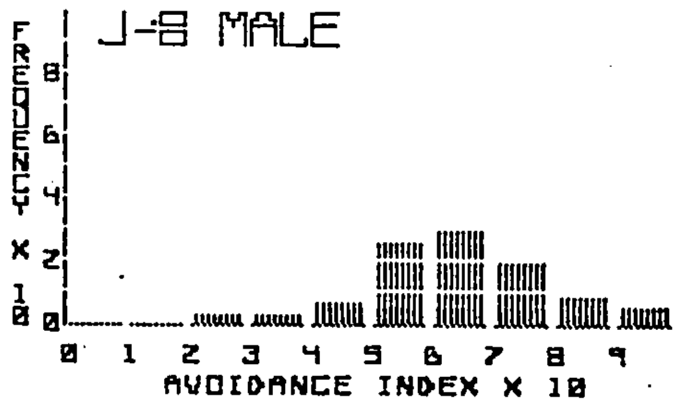

B-17 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

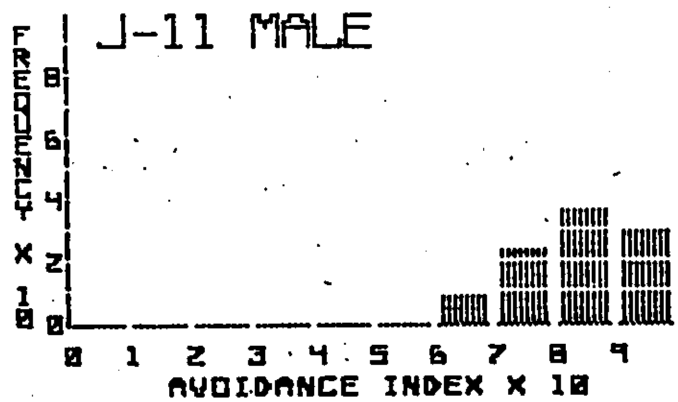

J-8 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

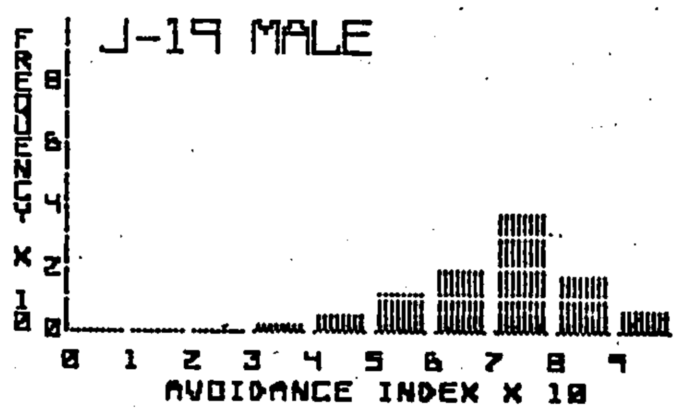

J-11 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

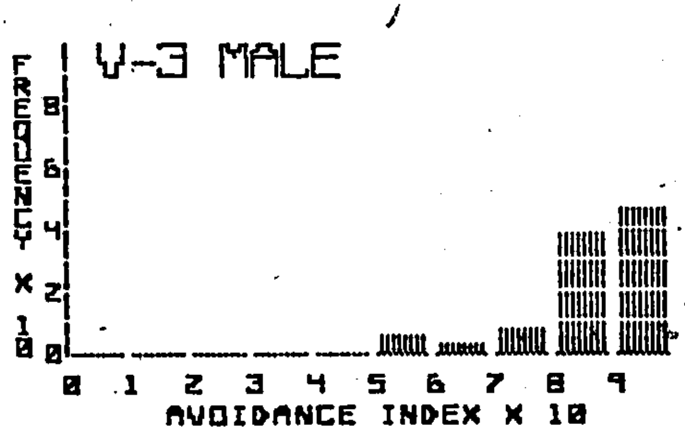

J-19 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


V-3 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


J-8 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


J-11 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


J-19 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


V-3 MALE
FREQUENCY X 10
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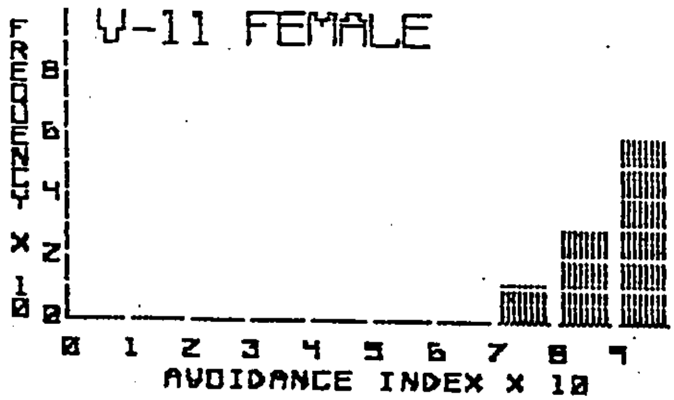
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


V-11 FEMALE
FREQUENCY X 10
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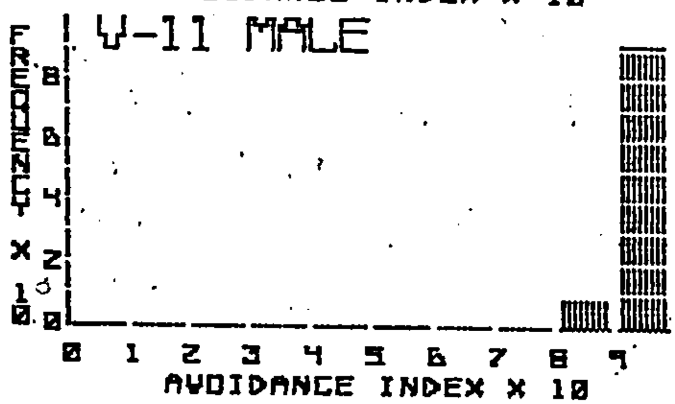
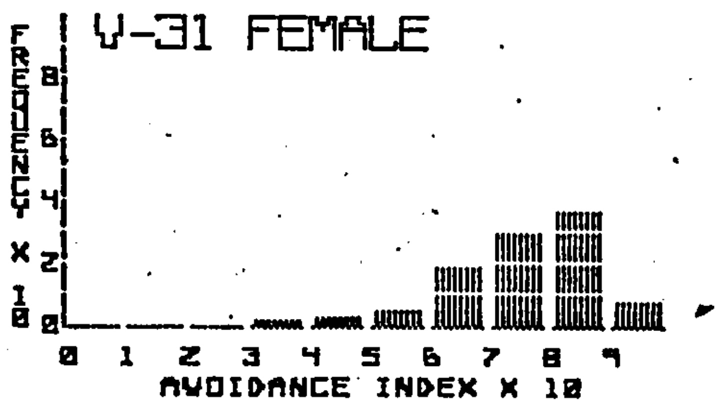
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


V-11 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


V-31 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

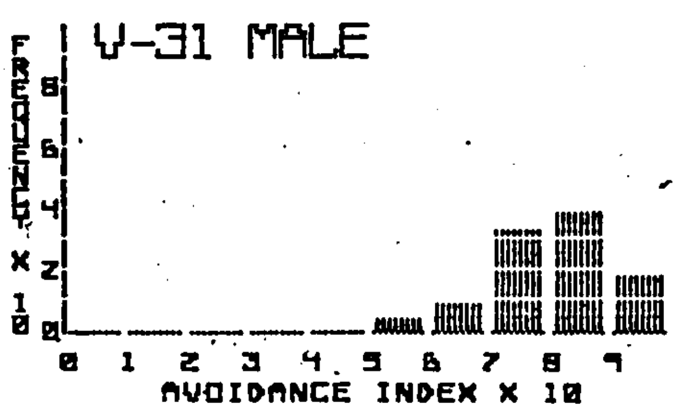

V-31 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

Table D.1 results of Bartlett's test for homogeneity of variances (d.f.=#runs compared - 1 for each case).

Line	FEMALE Runs Compared	FEMALE χ^2	MALE Runs Compared	MALE χ^2	MALE VS. FEMALE χ^2
A-4	AB	0.37	ABC	4.9	4.4
A-5	AB	0.4	ABC	4.3	8.0
A-6	ABC	1.5	ABC	2.3	8.8
A-7	AB	0.8	ABC	3.3	3.6
A-8	AB	2.1	ABC	2.5	18*
A-9	AB	0.2	ABC	5.5	3.4
A-10	ABC	1.9	ABCD	1.0	53*
A-11	ABCD	6.0	ABC	1.9	9.6
A-12	ABC	0.6	AB	0.2	17*
A15R	ABC	2.7	AB	1.3	18*
A-16	ABCD	4.2	AB	5.5	0.9
A-17	ABC	1.7	ABC	0.2	11
B-2	AB	0.6	ABC	1.9	8.1
B-4	AB	0.8	AB	1.9	2.6
B-7	ABC	0.2	ABC	0.2	4.0
B-8	AB	0.4	ABC	3.1	2.5
B-10	AB	0.1	ABC	2.5	9.5*
B-13	AB	3.8	ABC	1.4	8.9
B-16	ABC	1.1	ABC	4.0	12*
B-17	ABC	2.4	ABC	5.0	5.9
J-8	AB	1.3	AB	0.0	19*
J-11	ABC	4.9	ABC	3.7	7.1
J-19	AB	0.0	AB	0.7	0.5
V-3	AB	0.1	AB	1.0	6.4
V-11	AB	1.6	ABC	0.5	9.7*
V-31	AB	0.0	AB	1.1	1.9

*$p < 0.05$

Table D.2 results of homogeneity anova testing for runs within each sex.

Line	FEMALE d.f.	FEMALE F	MALE d.f.	MALE F
A-4	1,60	1.11	2,90	1.18
A-5	1,60	0.97	2,90	3.09
A-6	2,77	0.35	2,94	1.23
A-7	1,60	0.0	2,65	2.87
A-8	2,58	0.15	2,45	1.57
A-9	1,59	0.45	2,90	0.21
A-10	2,110	0.71	3,153	0.86
A-11	3,98	2.10	2,53	0.49
A-12	2,125	1.69	1,58	0.02
A15R	2,150	0.01	3,168	1.53
A-16	3,120	1.85	1,60	3.02
A-17	2,90	0.69	2,90	0.79
B-2	1,60	0.31	2,90	0.61
B-4	1,60	0.94	1,60	0.97
B-7	2,87	2.72	2,62	2.81
B-8	1,60	1.42	2,90	0.29
B-10	1,60	0.25	2,90	0.24
B-13	1,60	0.76	2,90	0.62
B-16	2,90	2.46	2,90	2.57
B-17	2,90	9.00*	2,90	3.68*
J-8	1,60	0.62	1,60	0.84
J-11	2,110	3.22	2,120	0.22
J-19	1,60	2.74	1,60	2.05
V-3	1,60	1.43	1,60	1.59
V-11	1,60	2.25	2,90	1.57
V-31	2,90	1.89	1,60	3.63

*$p < 0.05$

Table D.3 results of inter-sex comparisons - d.f. less than 10 indicates that the means test was used, all others were compared via anova.

Line	d.f.	F
A-4	1,184	78.7*
A-5	1,153	0.04
A-6	1,198	0.81
A-7	1,194	8.48*
A-8	1,4.5	0.09
A-9	1,151	1.51
A-10	1,4.5	35.4*
A-11	1,155	2.52
A-12	1,4.5	54.9*
A15R	1,4.5	54.5*
A-16	1,184	0.56
A-17	1,184	0.25
B-2	1,184	1.76
B-4	1,122	5.88*
B-7	1,153	3.38
B-8	1,153	3.56
B-10	1,4.5	0.21
B-13	1,153	1.19
B-16	1,4.5	0.26
B-17	1,184	13.0*
J-8	1,4.5	6.06
J-11	1,250	13.67*
J-19	1,122	2.83
V-3	1,153	0.13
V-11	1,4.5	4.2
V-31	1,153	5.93*

*$p<0.05$

APPENDIX E

Wild population line avoidance control run data: the raw data and parameter listings for the (1%-1%) control runs (males and females) and the (0%-0%) control runs (males) are shown on the pages following. Comparison of all control runs by anova gave an $F_{(23,1347)}$ value of 1.154 ($p>0.1$), and the average avoidance index value over all runs was 0.50 (s. dev. = 0.03).

A15R-F-1/1

1% 3, 2, 2, 2, 0, 0, 1, 2, 7, 3, 5, 3, 2, 2, 3, 2,
3, 3, 2, 3, 1, 2, 2, 3, 3, 5, 3, 4, 3, 4, 5, 2,
2, 2, 3, 3, 3, 4, 3, 3, 2, 4, 2, 3, 2, 3, 4, 3,
3, 2, 2, 2, 1, 1, 1, 4, 1, 1, 1, 2, 3, 4,

1% 2, 1, 5, 2, 4, 6, 2, 5, 1, 1, 1, 4, 5, 3, 3, 2,
2, 4, 1, 4, 4, 3, 5, 1, 4, 1, 2, 3, 1, 1, 2, 2,
3, 1, 2, 3, 2, 2, 0, 2, 2, 3, 1, 2, 2, 3, 2, 2,
1, 1, 4, 2, 0, 0, 1, 2, 1, 2, 2, 2, 1, 3,

A15R-H-1/1

1% 2, 3, 3, 1, 7, 5, 4, 2, 5, 5, 4, 4, 6, 5, 5, 3,
5, 3, 4, 5, 5, 2, 2, 6, 4, 5, 2, 2, 6, 4, 6, 1,
2, 2, 1, 1, 1, 2, 5, 4, 3, 3, 3, 5, 2, 4, 3, 4,
2, 4, 4, 4, 3, 3, 4, 4, 3, 6, 4, 4, 1, 6,

1% 3, 3, 2, 2, 0, 2, 0, 6, 3, 5, 5, 2, 4, 3, 4, 8,
7, 5, 4, 3, 2, 3, 5, 5, 3, 2, 4, 3, 6, 3, 2, 3,
3, 4, 2, 0, 3, 3, 3, 4, 2, 2, 4, 2, 5, 6, 5, 3,
6, 3, 3, 2, 3, 4, 2, 1, 1, 4, 3, 4, 4, 2,

A6-F-1/1

1% 0, 1, 0, 0, 1, 1, 1, 2, 1, 0, 4, 1, 3, 2, 0, 3,
7, 1, 3, 2, 1, 6, 5, 2, 2, 2, 8, 4, 4, 6, 5, 1,
1, 2, 0, 2, 1, 1, 2, 0, 4, 0, 2, 2, 3, 1, 0, 2,
2, 3, 2, 3, 1, 1, 3, 2, 1, 1, 3, 1, 3, 3,

1% 3, 2, 4, 1, 1, 2, 0, 4, 4, 2, 6, 5, 2, 2, 3, 6,
0, 3, 2, 6, 2, 2, 0, 2, 5, 1, 0, 4, 7, 1, 0, 3,
0, 0, 2, 1, 2, 2, 0, 1, 2, 1, 1, 3, 1, 3, 2, 2,
1, 4, 1, 2, 2, 4, 1, 3, 3, 3, 3, 4, 1, 2,

A6-H-1/1

1% 5, 4, 1, 2, 3, 2, 1, 5, 3, 2, 2, 5, 3, 5, 2, 3,
5, 3, 5, 7, 4, 5, 5, 4, 7, 7, 8, 4, 9, 7, 7, 3,
1, 2, 1, 1, 1, 2, 2, 1, 3, 1, 3, 1, 0, 1, 2, 1,
3, 2, 2, 2, 2, 1, 2, 3, 2, 2, 2, 5, 3, 3,

1% 1, 1, 3, 2, 4, 1, 0, 0, 6, 3, 4, 5, 6, 4, 5, 4,
5, 1, 4, 5, 5, 3, 4, 5, 8, 6, 6, 7, 6, 6, 7, 0,
2, 1, 0, 1, 1, 1, 2, 3, 1, 1, 4, 1, 1, 0, 1, 4,
3, 2, 3, 1, 0, 3, 3, 2, 3, 2, 3, 2, 2, 3,

A7-F 1-1

1% 0, 1, 2, 5, 0, 2, 1, 1, 3, 3, 1, 4, 2, 2, 2, 5,
3, 2, 2, 3, 1, 1, 1, 3, 1, 2, 0, 3, 2, 3, 3, 0,
1, 1, 1, 2, 2, 1, 1, 2, 1, 1, 1, 2, 2, 1, 0, 0,
4, 3, 0, 2, 3, 4, 2, 2, 1, 2, 4, 2, 5, 0,

1% 5, 2, 2, 5, 2, 2, 1, 2, 0, 4, 4, 2, 1, 1, 2, 4,
3, 2, 2, 4, 1, 2, 4, 4, 1, 1, 3, 3, 2, 1, 2, 2,
1, 1, 5, 2, 1, 0, 2, 1, 2, 1, 3, 0, 0, 2, 1, 2,
5, 2, 4, 3, 5, 1, 5, 0, 1, 3, 3, 4, 3, 4,

A7-H 1-1

1% 0, 0, 1, 3, 1, 1, 1, 2, 2, 3, 2, 2, 0, 0, 1, 3,
1, 2, 2, 2, 1, 0, 3, 3, 4, 3, 2, 3, 5, 4, 4, 1,
1, 1, 0, 3, 3, 1, 1, 1, 2, 1, 2, 0, 3, 2, 2, 1,
3, 6, 2, 2, 4, 2, 1, 4, 4, 4, 3, 2, 3, 3,

1% 2, 1, 1, 3, 0, 2, 1, 0, 0, 2, 1, 1, 4, 2, 2, 1,
2, 1, 2, 2, 2, 2, 4, 1, 1, 4, 2, 2, 3, 3, 2, 0,
1, 2, 1, 2, 1, 0, 1, 2, 2, 2, 0, 2, 0, 1, 3, 0,
4, 3, 4, 1, 3, 2, 3, 1, 5, 5, 7, 4, 6, 5,

A10-H-1/1

1% 2, 5, 3, 3, 5, 4, 1, 6, 4, 2, 5, 4, 3, 3, 5, 5,
6, 4, 3, 2, 4, 3, 3, 2, 5, 3, 2, 2, 4, 5, 6, 5,
3, 2, 3, 3,

1% 1, 4, 4, 2, 4, 6, 5, 4, 5, 3, 5, 4, 4, 4, 5, 3,
2, 1, 3, 5, 4, 4, 5, 6, 1, 1, 2, 4, 2, 6, 3, 3,
3, 3, 2, 1,

A11-F-1/1

1% 1, 3, 0, 2, 3, 2, 2, 1, 0, 0, 1, 1, 3, 1, 1, 2,
4, 3, 4, 4, 2, 5, 2, 3, 1, 3, 2, 2, 5, 5, 2, 5,
3, 2, 2, 1, 4, 3, 2,

1% 3, 2, 1, 4, 1, 2, 1, 0, 1, 1, 0, 1, 2, 5, 3, 2,
3, 0, 4, 2, 6, 1, 5, 4, 2, 4, 3, 5, 3, 2, 5, 4,
5, 2, 3, 1, 0, 2, 6,

A11-H-1/1

1% 1, 1, 2, 1, 3, 4, 1, 2, 2, 3, 3, 1, 3, 2, 4, 3,
4, 3, 4, 4, 7, 2, 6, 5, 5, 5, 6, 4, 6, 4, 4, 3,
3, 6, 1, 1,

1% 1, 2, 4, 3, 3, 2, 2, 1, 2, 2, 4, 2, 5, 3, 1, 5,
4, 5, 4, 2, 4, 2, 5, 5, 6, 4, 5, 9, 5, 5, 6, 6,
2, 2, 2, 2,

A12-F-1/1

1% 3, 2, 5, 4, 2, 4, 5, 2, 2, 4, 4, 4, 5, 5, 6, 2,
3, 2, 4, 6, 2, 3, 4, 4, 4, 6, 3, 4, 4, 4, 4, 4,
4, 4, 3, 4, 6, 4, 6, 4, 5, 5, 5, 7, 3, 5, 3, 7,
5, 4, 5, 4, 4, 4, 4, 5, 3, 4, 3, 3, 3, 0,

1% 3, 6, 3, 1, 4, 3, 2, 7, 3, 3, 3, 4, 4, 3, 2, 2,
3, 1, 2, 2, 4, 6, 6, 4, 4, 2, 5, 6, 6, 5, 2, 3,
5, 5, 5, 5, 5, 2, 5, 2, 5, 2, 5, 2, 5, 3, 3, 5,
4, 6, 6, 5, 2, 5, 7, 6, 3, 3, 3, 2, 3, 2,

A12-H-1/1

1% 1, 2, 2, 2, 2, 1, 2, 4, 1, 5, 5, 3, 3, 3, 3, 2,
2, 0, 4, 1, 3, 1, 2, 3, 5, 5, 5, 4, 3, 4, 4, 3,
2, 2, 5, 3, 3, 2, 2, 3, 3, 5, 3, 6, 3, 4, 2, 2,

1% 3, 5, 2, 3, 4, 1, 1, 2, 2, 2, 1, 2, 3, 2, 3, 6,
0, 2, 2, 2, 1, 3, 3, 5, 3, 2, 5, 6, 3, 2, 3, 2,
5, 5, 3, 2, 2, 2, 3, 2, 4, 3, 2, 4, 2, 5, 4, 2,

J11-F-1/1

1% 1, 0, 1, 4, 2, 0, 1, 3, 3, 1, 4, 5, 3, 1, 4, 4,
1, 1, 1, 2, 3, 3, 3, 4, 3, 5, 6, 4, 5, 4, 3, 0,
1, 3, 3, 0, 2, 1, 3, 1, 4, 1, 3, 2, 6, 3, 4, 3,
1, 1, 3, 1, 4, 4, 5, 5, 4, 4, 4, 4, 5, 4,

1% 4, 1, 2, 1, 4, 2, 3, 4, 4, 2, 1, 4, 6, 4, 3, 2,
3, 2, 3, 3, 2, 2, 5, 4, 3, 6, 5, 2, 2, 3, 3, 2,
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5, 6, 4, 5, 5, 5, 3, 4, 5, 4, 3, 3, 2, 4,

J11-H-1/1

1% 2, 5, 5, 5, 6, 4, 5, 3, 5, 3, 4, 5, 4, 5, 3, 1,
6, 3, 5, 2, 4, 2, 3, 4, 6, 4, 6, 1, 3, 2, 3, 1,
2, 1, 2, 2, 1, 2, 4, 3, 3, 3, 1, 3, 2, 2, 5, 3,
4, 1, 3, 4, 6, 4, 1, 4, 7, 6, 4, 4, 7, 1,

1% 1, 1, 0, 3, 1, 2, 1, 6, 1, 4, 3, 3, 2, 3, 4, 4,
2, 8, 6, 6, 4, 4, 5, 2, 5, 5, 4, 6, 7, 5, 6, 1,
0, 1, 1, 0, 2, 1, 2, 4, 3, 1, 4, 3, 3, 2, 3, 5,
3, 6, 6, 5, 5, 5, 5, 3, 2, 6, 6, 2, 3, 2,

A8-F-1/1

1% 4, 3, 5, 5, 6, 5, 6, 2, 5, 5, 4, 4, 6, 4, 7, 4,
4, 6, 5, 5, 3, 5, 3, 5, 6, 3, 6, 6, 3, 2, 0, 2,
2, 4, 6, 4, 2, 3, 0, 2, 2, 3, 2, 5, 6, 5, 5, 4,
3, 2,

1% 6, 6, 4, 6, 3, 4, 4, 5, 5, 5, 3, 7, 6, 5, 5, 3,
5, 4, 5, 5, 2, 4, 4, 2, 3, 1, 3, 3, 5, 5, 3, 4,
3, 1, 7, 1, 2, 1, 5, 2, 4, 2, 4, 2, 5, 7, 4, 4,
3, 3,

A10-F-1/1

1% 3, 3, 3, 3, 3, 6, 3, 2, 1, 0, 3, 3, 2, 5, 4, 3,
2, 2, 3, 5, 6, 4, 3, 3, 3, 4, 2, 2, 2, 3, 3, 4,
1, 7, 3, 4, 1, 2, 2, 2, 1, 3, 3, 3, 2, 3, 5, 5,
6, 5, 1, 2, 3,

1% 3, 1, 4, 1, 4, 1, 1, 1, 2, 4, 2, 4, 2, 3, 4, 5,
4, 5, 3, 4, 4, 4, 5, 3, 5, 3, 1, 5, 1, 2, 2, 2,
3, 4, 4, 5, 3, 4, 3, 3, 3, 2, 1, 3, 7, 6, 6, 3,
4, 1, 3, 2, 2,

A10-F-1/1

1% 2, 2, 3, 2, 2, 4, 3, 2, 3, 3, 0, 3, 2, 4, 2, 1,
3, 4, 4, 4, 2, 3, 3, 5, 4, 3, 4, 1, 5, 4, 1, 0,
2, 3, 1, 0, 1, 2, 2, 2,

1% 3, 4, 2, 3, 4, 1, 0, 1, 3, 1, 4, 2, 2, 2, 4, 3,
3, 0, 2, 2, 4, 2, 3, 3, 2, 2, 2, 3, 2, 5, 4, 5,
1, 1, 2, 2, 2, 1, 1, 2,

A6

0% 6, 2, 3, 4, 2, 3, 3, 3, 2, 5, 4, 2, 6, 7, 8, 1,
7, 6, 7, 7, 5, 5, 5, 8, 9, 7, 4, 5, 6, 6, 8,

0% 2, 4, 3, 6, 2, 2, 3, 4, 5, 7, 5, 6, 5, 4, 3, 5,
4, 4, 1, 3, 4, 4, 6, 7, 6, 4, 4, 6, 7, 6, 3,

A7

0% 6, 5, 6, 4, 4, 4, 2, 5, 6, 5, 2, 3, 4, 2, 1, 2,
3, 4, 1, 1, 4, 3, 1, 4, 1, 3, 4, 1, 5, 0, 1, 2,
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5, 3, 5, 6, 4, 4, 5, 3, 3, 4, 4, 5, 3, 1, 4, 4,
5, 3, 7, 5, 5, 8, 4, 4, 3, 1, 3,

0% 5, 4, 5, 2, 3, 3, 7, 4, 2, 2, 0, 0, 1, 3, 0, 3,
1, 1, 3, 2, 2, 2, 3, 3, 5, 2, 3, 5, 4, 2, 4, 3,
3, 2, 3, 5, 4, 3, 2, 3, 4, 5, 1, 2, 3, 2, 0, 1,
4, 4, 2, 3, 4, 3, 5, 4, 3, 4, 4, 3, 2, 4, 3, 0,
2, 3, 3, 4, 5, 1, 3, 3, 8, 5, 2, 4, 4, 5, 2, 4,
5, 4, 8, 2, 6, 4, 3, 3, 0, 5, 3,

A8

0% 5, 2, 5, 4, 4, 3, 4, 2, 1, 1, 4, 3, 3, 5, 5, 6,
4, 2, 5, 3, 5, 5, 5, 4, 2, 4, 7, 5, 7, 5, 5, 3,
3, 5, 1, 1, 3, 3, 3, 1, 2,

0% 2, 5, 4, 5, 4, 4, 2, 5, 5, 5, 5, 5, 3, 3, 4, 4,
2, 3, 1, 2, 3, 4, 5, 4, 2, 3, 3, 4, 6, 8, 2, 3,
2, 4, 5, 4, 4, 4, 3, 4, 3,

A10

0% 4, 5, 4, 3, 4, 2, 2, 8, 6, 2, 1, 2, 0, 2, 1, 4,
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4, 4, 3, 3, 6, 3, 4, 5, 5, 5, 4, 3, 2, 2, 5, 6,
6, 3, 4, 3, 2, 3, 5, 2, 3, 1, 2, 2,

∅1% 4, 3, 4, 3, 3, 4, 4, 2, 3, 7, 5, 2, 3, 5, 5, 3,
2, 3, 5, 3, 2, 0, 0, 1, 4, 2, 5, 3, 3, 3, 3, 5,
3, 6, 4, 2, 2, 4, 4, 4, 3, 4, 5, 1, 1, 2, 2, 2,
1, 1, 3, 2, 3, 6, 6, 3, 3, 5, 2, 6, 5, 6, 2, 4,
4, 5, 4, 1, 3, 3, 3, 2, 3, 1, 2, 1, 3, 1, 3, 1,
5, 7, 6, 5, 6, 3, 1, 3, 6, 4, 4, 2,

A11

0% 6, 8, 9, 3, 6, 6, 6, 2, 5, 2, 0, 2, 1, 0, 1, 5,
4, 4, 3, 3, 4, 6, 2, 4, 3, 5, 5, 5, 5, 7, 3, 5,
3, 2, 6, 3, 4, 5, 2, 5, 5, 3, 4, 6, 2, 4, 5, 3,
6, 3, 6,

∅1% 7, 5, 4, 7, 4, 7, 7, 9, 7, 11, 2, 4, 5, 4, 4, 3,
3, 6, 4, 7, 5, 3, 6, 6, 7, 3, 4, 4, 4, 1, 6, 4,
5, 3, 3, 5, 5, 4, 4, 3, 3, 3, 5, 4, 0, 2, 5, 2,
2, 3, 5,

A12

0% 5, 3, 4, 4, 5, 7, 4, 6, 5, 6, 3, 6, 7, 5, 3, 4,
4, 5, 4, 4, 4, 4, 3, 6, 1, 6, 5, 2, 2, 5, 4, 5,
7, 5, 7, 8, 3, 9, 7, 4, 6,

∅1% 4, 5, 6, 3, 2, 4, 6, 4, 2, 4, 7, 3, 6, 5, 4, 5,
8, 6, 2, 6, 3, 7, 5, 8, 5, 3, 3, 8, 4, 7, 7, 5,
0, 5, 2, 4, 5, 6, 2, 1, 2,

A15R

0% 4, 0, 4, 3, 2, 4, 4, 3, 4, 3, 4, 2, 4, 1, 3, 5,
2, 4, 2, 2, 7, 4, 6, 2, 4, 3, 8, 4, 4, 6, 5, 5,
2, 6, 4, 7, 7, 6, 7, 8, 7, 4, 6, 4, 4, 4, 6, 3,
6, 3, 3, 2, 4, 6, 2, 4, 7, 5, 3, 6, 6, 5, 6, 5,
9, 10, 8, 4, 6, 6, 6, 6,

∅1% 5, 6, 3, 3, 5, 1, 5, 4, 3, 4, 3, 4, 1, 6, 6, 1,
2, 6, 6, 7, 1, 5, 7, 5, 6, 8, 2, 6, 6, 2, 1, 4,
4, 5, 5, 3, 5, 4, 5, 6, 5, 6, 5, 6, 3, 6, 2, 4,
5, 6, 4, 3, 5, 7, 7, 7, 6, 6, 6, 9, 7, 8, 10, 6,
9, 11, 8, 7, 9, 7, 6, 7,

J11

0% 5, 7, 4, 7, 7, 4, 6, 7, 5, 4, 5, 3, 4, 5, 5, 5,
3, 3, 4, 2, 2, 2, 2, 2, 2, 4, 3, 3, 2, 3, 0, 2,
4, 3, 6, 5, 3, 3, 3, 4, 2, 3, 5, 1, 0, 5, 4, 4,
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4, 6, 7, 5, 5, 6, 5, 5, 5, 5, 4, 5, 4, 4, 5, 5,
1, 2, 6, 4, 6, 7, 0, 4, 4, 4, 4, 4,

∅1% 6, 4, 5, 5, 5, 6, 3, 2, 1, 6, 3, 2, 2, 2, 4, 4,
3, 4, 6, 6, 6, 7, 1, 2, 4, 4, 4, 3, 5, 3, 3, 3,
4, 5, 4, 2, 5, 3, 3, 4, 3, 4, 4, 6, 5, 2, 3, 2,
4, 1, 4, 2, 1, 2, 6, 3, 5, 9, 5, 4, 5, 2, 5, 4,
2, 0, 5, 5, 4, 5, 9, 6, 7, 6, 4, 5, 3, 4, 6, 5,
5, 5, 7, 5, 4, 2, 5, 3, 6, 7, 5, 8,

R-A8-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A8-F-1/1	3.980	1.647	3.900	1.593	.5016	.1671	1.020
A10-F-1/1	3.056	1.446	3.150	1.485	.4950	.1753	.9700

R-A10-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A10-F-1/1	2.525	1.300	2.375	1.233	.5120	.2383	1.063
A10-M-1/1	3.611	1.336	3.444	1.501	.5213	.1550	1.048

R-A11-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A11-F-1/1	2.358	1.404	2.589	1.727	.4847	.2650	.9108
A11-M-1/1	3.305	1.704	3.527	1.812	.4783	.1350	.9370

R-A12-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A12-F-1/1	3.967	1.305	3.790	1.569	.5146	.1465	1.046
A12-M-1/1	2.916	1.350	2.833	1.373	.5065	.1796	1.029

R-J11-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
J11-F-1/1	2.806	1.597	3.048	1.335	.4457	.2006	.9206
J11-M-1/1	3.467	1.656	3.370	1.960	.5267	.2109	1.028

R-A15R-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A15R-F-1/1	2.596	1.247	2.274	1.357	.5523	.2082	1.141
A15R-M-1/1	3.596	1.530	3.306	1.615	.5271	.1856	1.087

R-A6-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A6-F-1/1	2.112	1.765	2.290	1.663	.4591	.3085	.9225
A6-M-1/1	3.145	2.039	2.951	2.067	.5415	.2122	1.065

R-A7-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A7-F	1.887	1.294	2.306	1.443	.4571	.2607	.8181
A7-M	2.080	1.346	2.080	1.550	.5147	.2799	1

R-A6-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A6	5.032	2.152	4.354	1.603	.5249	.1527	1.155

R-A7-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A7	3.384	1.665	3.131	1.620	.5269	.2209	1.080

R-A8-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A8	3.658	1.606	3.731	1.342	.4863	.1619	.9803

R-A10-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A10	2.880	1.510	3.293	1.614	.4724	.2075	.8745

R-A11-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A11	4.058	1.922	4.490	2.003	.4704	.1965	.9039

R-A12-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A12	4.804	1.691	4.487	2.001	.5257	.1763	1.070

R-J11-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
J11	4.130	1.658	4.163	1.774	.4978	.1760	.9921

R-A15R-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A15R	4.597	1.962	5.194	2.185	.4728	.1636	.8850

APPENDIX F

Results for wild line cross progeny: i) Avoidance - the raw data from avoidance testing of the F_1 progeny are listed on the pages following. Individual runs are separated by brackets (I) and are listed under the cross name followed by the sex of the flies tested e.g. the female progeny of cross A-11 male x J-11 female have their results listed under the designation A11MXJ11F-F.

Homogeneity testing - the results of anova comparisons (variances homogeneous) of individual run results within each group are shown in Table F.1 below. (in each case $p>0.05$).

Table F.1 results of homogeneity testing.

Male Parent	Female Parent	df	F maleF_1	df	F femaleF_1
A-11	J-11	2,112	0.45	2,83	0.43
J-11	A-11	3,122	1.8	2,120	1.8
A-11	A-10	3,135	2.3	2,89	1.4
A-10	A-11	2,94	0.18	2,120	1.1
A15R	A-11	2,120	1.4	2,120	1.1
A-11	A15R	1,80	0.07	1,80	0.04

Index distributions - the avoidance index distributions for F_1 flies are given following the raw data and parameter table. The avoidance testing results for the F_3 flies from crosses 5 and 6 are given following these.

Comparisons - the results of anova comparisons of the avoidance results for the F_1 flies are shown in Tables F.2 (intersex) and F.3 (interstrain). Note that the crosses are numbered in Table F.2 and the parents are given for reference, along with their $\bar{A}$ values.

Table F.2 results of intersex comparisons (all cases have $p>0.05$).

	Parents					
Cross #	Male	$\bar{A}$	Female	$\bar{A}$	df	F
1	A-11	0.66	J-11	0.83	1,199	2.0
2	J-11	0.77	A-11	0.71	1,247	2.3
3	A-11		A-10	0.63	1,227	2.6
4	A-10	0.80	A-11		1,218	0.3
5	A15R	0.92	A-11		1,244	1.5
6	A-11		A15R	0.71	1,162	0.3

Table F.3 results of intersex comparisons.

Compared	df	F
1,2	3,446	11*
3,4,5,6	7,851	2.1
vs. male parent:		
1	2,253	2.6
2	2,387	44*
3	2,281	5.2*
vs. female parent:		
1	2,309	1.4
2	2,348	1.3
3	2,319	3.9*
5	2,345	6.4*

* $p<0.05$

A11MXA10F-H
0% 3, 2, 5, 3, 2, 2, 2, 0, 4, 3, 0, 3, 3, 3, 1, 2,
2, 4, 2, 5, 5, 3, 5, 5, 4, 3, 6, 5, 5, 3, 5, 5,
4, 6, 9, 6, 5, 6, 5, 5, 5, 2, 1, 5, 4, 4, 2, 3,
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3, 3, 1, 2, 3, 3, 4, 4, 4,
A11MXA10F-F
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A10MXA11F-H
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6,

A10HXA11F-F

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A11H-J11F-H

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16, 11, 10, 11, 12, 11, 5, 7, 11, 7, 4, 6, 7, 6, 7, 8,
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4, 4, 4, 2, 6, 3, 3, 4, 5, 4, 5, 4, 3, 5, 6, 5,
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A11MXJ11F-F

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10, 9, 13, 8, 8, 8,

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A15RMXA11F-F

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A11MXA15RF-H

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A11MXA15RF-F

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1, 2,

R-A11XJ11

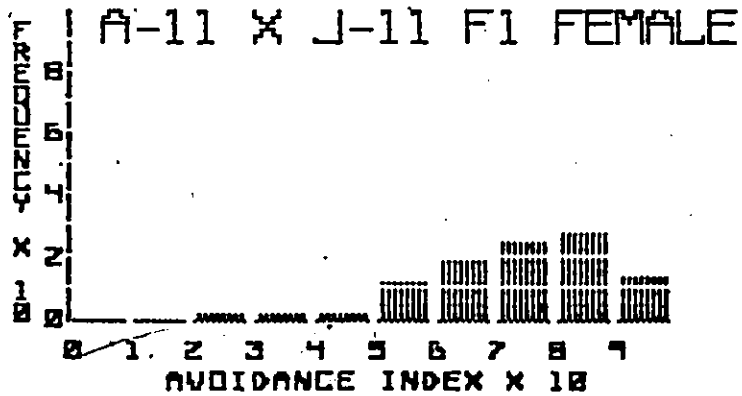
RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A11M-J11F-M	6.591	3.324	1.782	1.240	.7751	.1754	3.697
A11MXJ11F-F	5.093	2.095	1.918	1.399	.7410	.1627	2.654
J11MXA11F-M	3.849	1.859	1.825	1.271	.6878	.1780	2.108
J11MXA11F-F	3.951	1.999	2.065	1.239	.6522	.1899	1.913

R-A11XA10

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A11MXA10F-M	4.379	2.040	2.372	1.283	.6389	.1879	1.846
A11MXA10F-F	2.902	2.000	1.934	1.299	.5909	.2598	1.5
A10MXA11F-M	3.546	2.254	2.577	1.824	.5754	.2216	1.376
A10MXA11F-F	2.617	1.2[illegible]8	1.772	1.022	.5920	.2156	1.477

R-A11XA15R

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
A15RMXA11F-M	2.308	1.242	1.878	1.120	.5505	.2334	1.229
A15RMXA11F-F	3.203	1.824	2.211	1.313	.5865	.2119	1.448
A11MXA15RF-M	3.243	1.852	2.439	1.228	.5686	.2015	1.33
A11MXA15RF-F	2.817	1.352	2.317	1.265	.5517	.2007	1.215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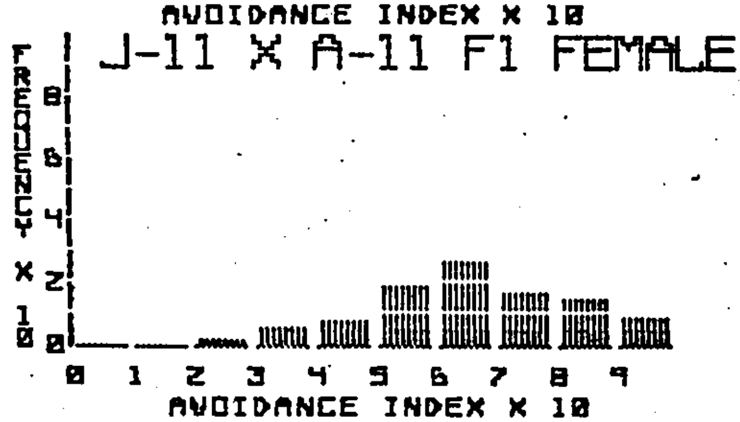
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


J-11 X A-11 F1 FEMALE
FREQUENCY X 10
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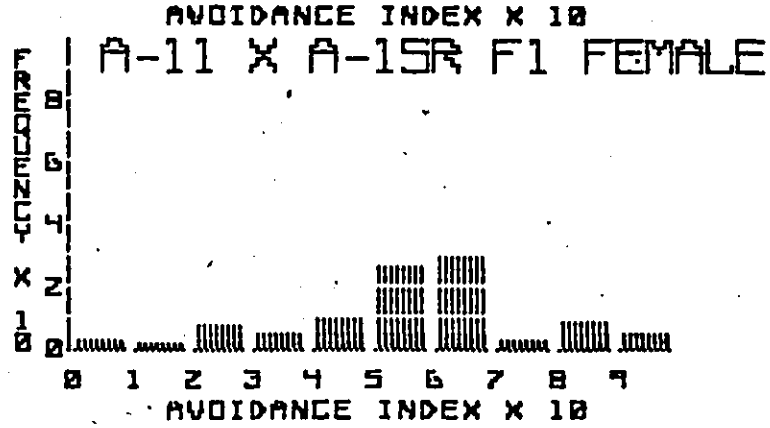
AVOIDANCE INDEX X 10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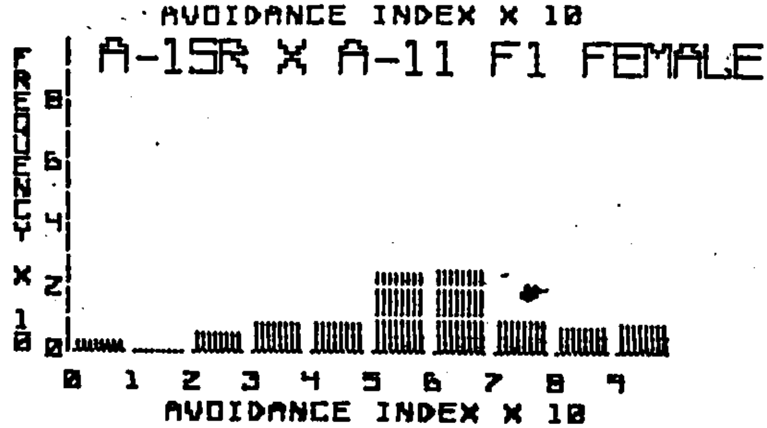
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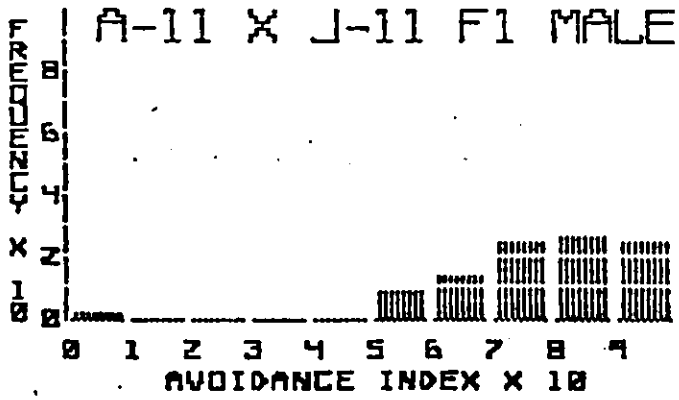
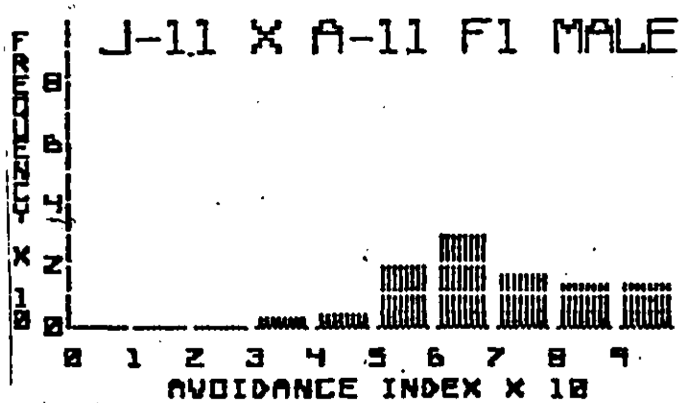
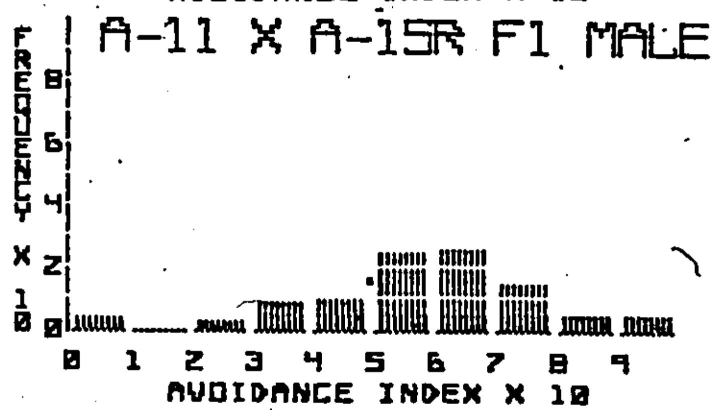
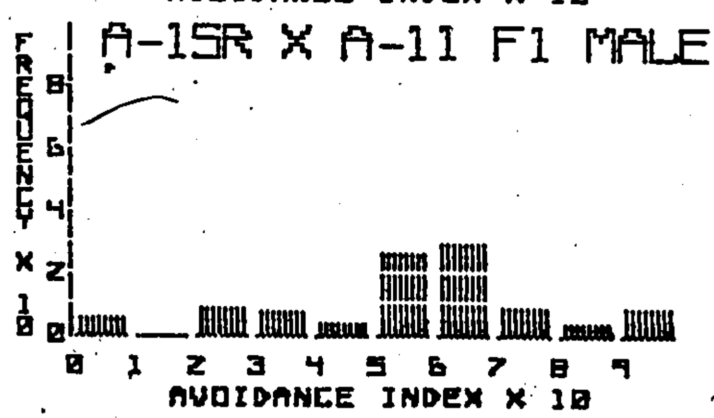
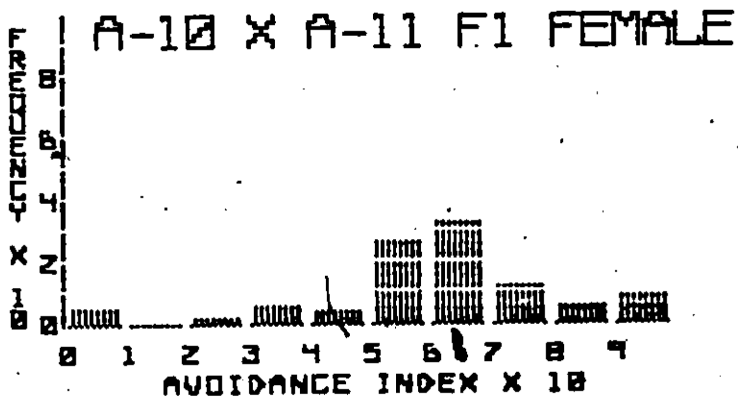
AVOIDANCE INDEX X 10

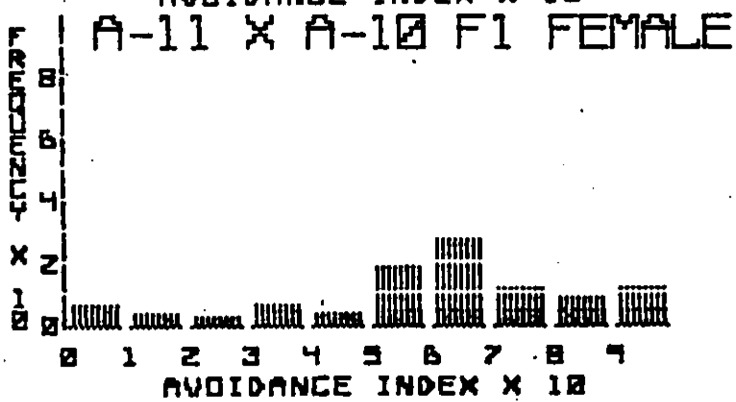

A-11 X J-11 F1 MALE
FREQUENCY X 10
AVOIDANCE INDEX X 10

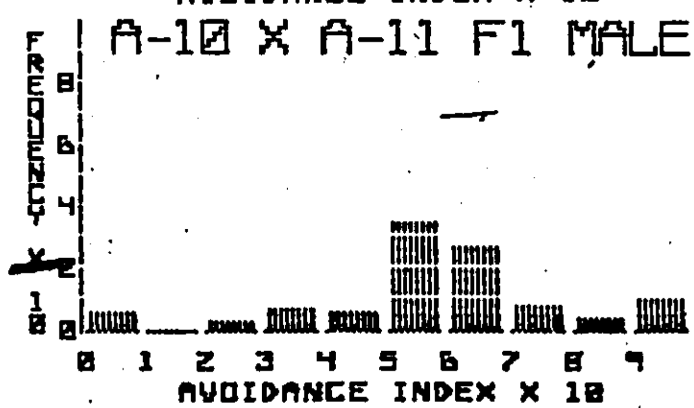

J-11 X A-11 F1 MALE
FREQUENCY X 10
AVOIDANCE INDEX X 10

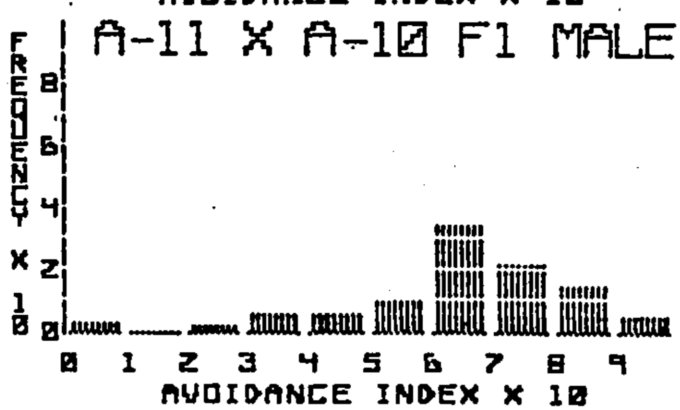

A-11 X A-15R F1 MALE
FREQUENCY X 10
AVOIDANCE INDEX X 10

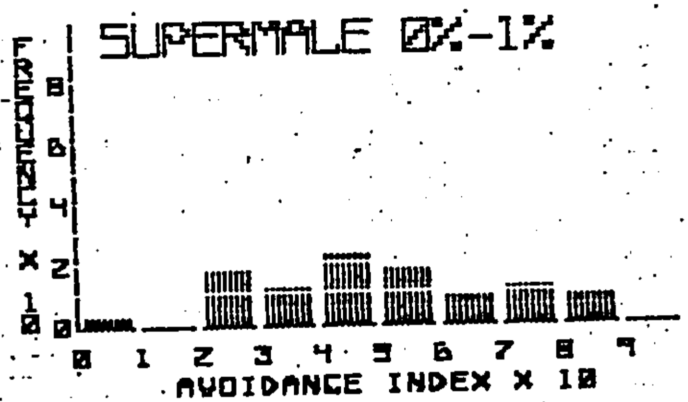

A-15R X A-11 F1 MALE
FREQUENCY X 10
AVOIDANCE INDEX X 10


A-10 X A-11 F1 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10

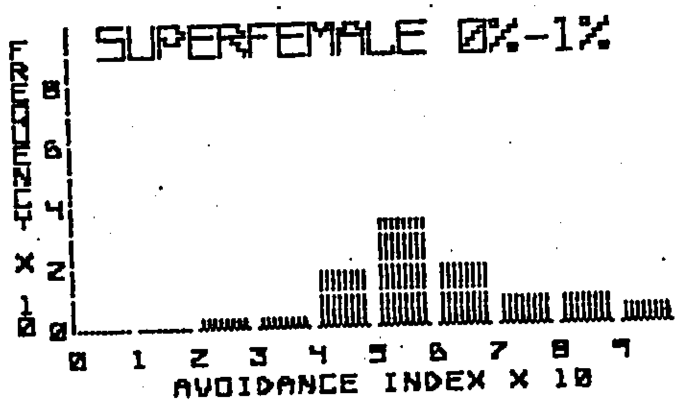

A-11 X A-10 F1 FEMALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-10 X A-11 F1 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10


A-11 X A-10 F1 MALE
FREQUENCY X 10
0 2 4 6 8
0 1 2 3 4 5 6 7 8 9
AVOIDANCE INDEX X 10



$\bar{A}$ = 0.48 s.d. = 0.20



$\bar{A}$ = 0.58 s.d. = 0.17

Results for F_3 flies from crosses 5 and 6.

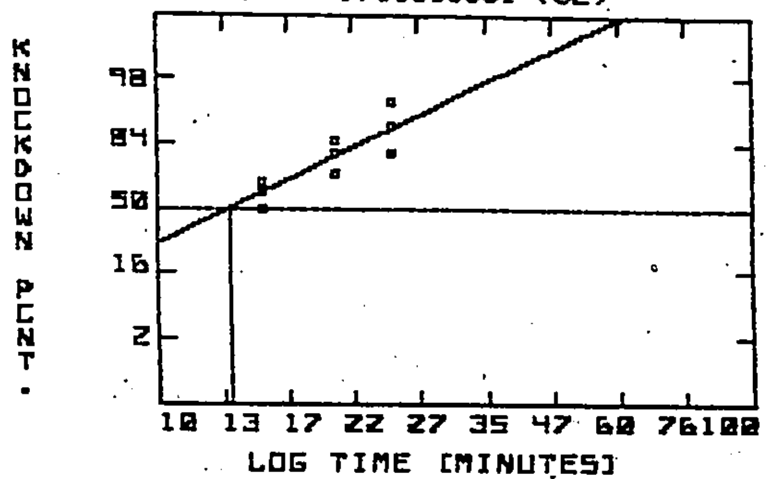
ii) Resistance testing: the raw data from the vacuum-injection resistance assay testing of F_1 and F_2 flies are listed on pages following, along with the computer generated knockdown curves. In each case the dose applied was 1% malathion, and the numbers used to designate the groups are the same as those given in Table F.2.

Time:		5	10	15	20	25	30
F_1's:							
1	m	2		12	16	18	
		1		10	14	16	
		2		13	16	18	
		1		13	17	20	
	f	1		4	16	17	
		2		10	18	19	
2	m	0	3	10	15	16	20
		1	2	8	12	16	18
		2	3	12	16	18	
	f	2	4	13	18	19	
		2	3	11	14	16	
		3	3	11	16	18	
3	m	3	11	17			
		4	12	18			
		4	13	20			
		3	14	19			
		3	14	19			
	f	5	12	18			
		3	9	17			
		4	9	19			
		4	8	18			
		5	11	20			

# down		5	10	15	20	25	30	40	50	60
4	m	5	11	15	19					
		5	10	16	17					
		2	5	14	16					
	f	3	14	18	19					
		3	8	14	17					
		2	12	16	18					
5	m	2	5	11	16	19				
	f	3	5	12	17	19				
6	m	2	6	12	15		18			
		2	8	15	18		20			
	f	0	1	5		12	14	16	18	
		0	1	5		12	14	16	17	
		1	1	2		8	11	14	16	

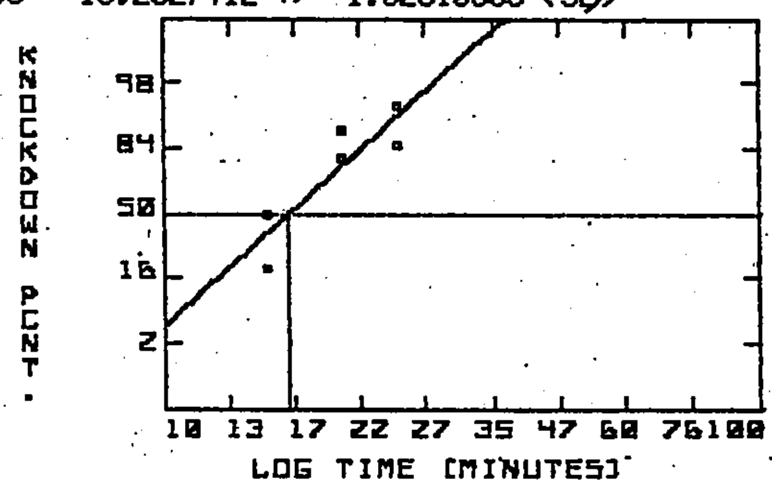
CROSS 1 F1 MALE 1%VAC

KT-50 = 13.1965325 +/- .788905891 (SE)



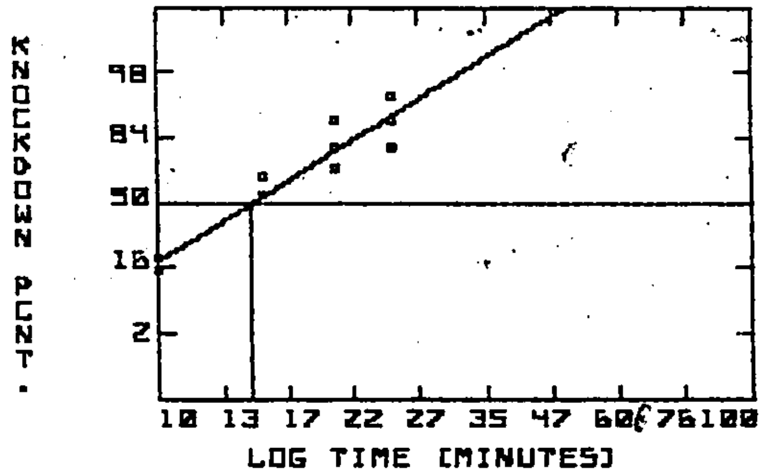
CROSS 1 FEMALE 1% VAC

KT-50 = 16.2327412 +/- 1.02810669 (SE)



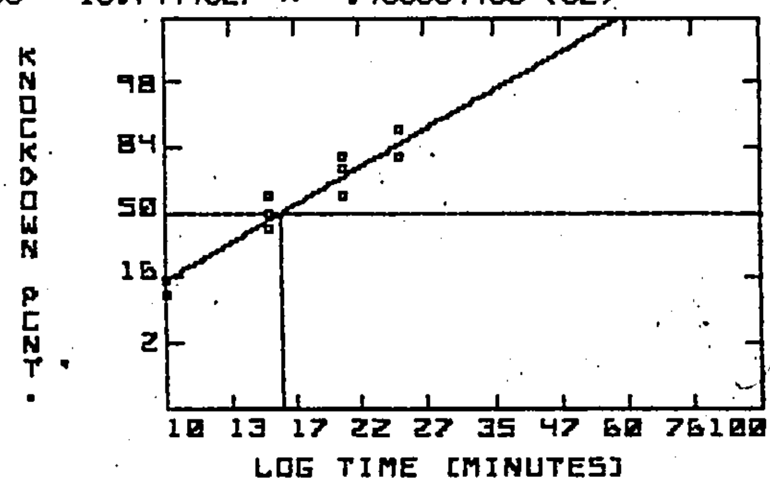
CROSS 2 FEMALE 1% VAC

KT-50 = 14.3829923 +/- .486404145 (SE)



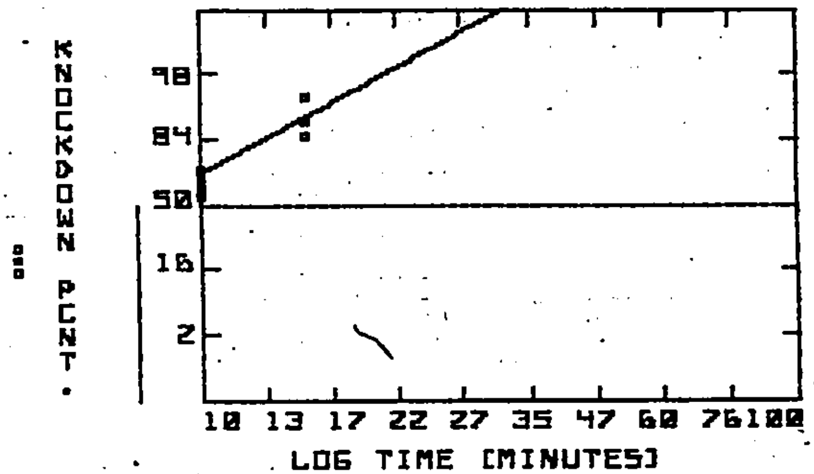
CROSS 2 MALE 1% VAC

KT-50 = 15.7444927 +/- .453064453 (SE)



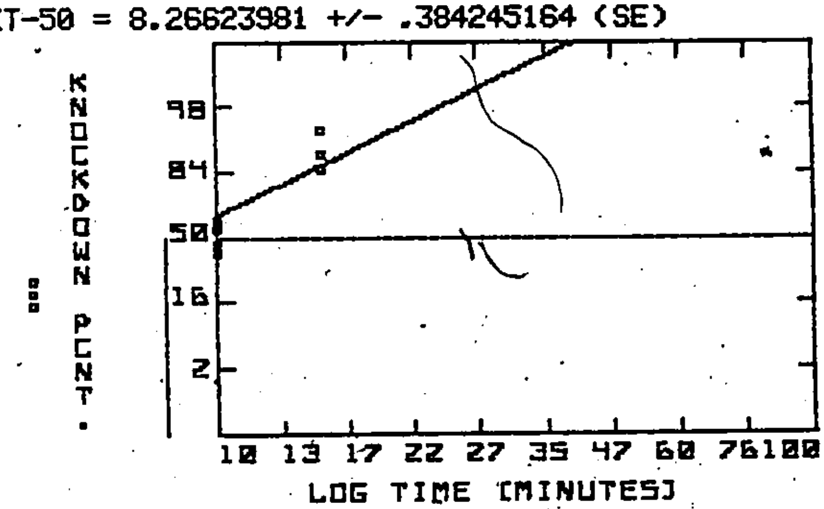
CROSS 3 MALE 1% VAC

KT-50 = 7.9065926 +/- .224722557 (SE)



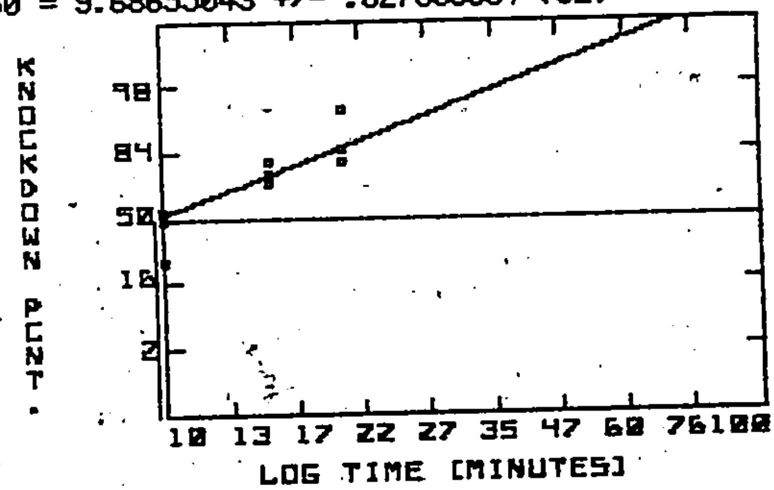
CROSS 3 FEMALE 1% VAC

KT-50 = 8.26623981 +/- .384245164 (SE)



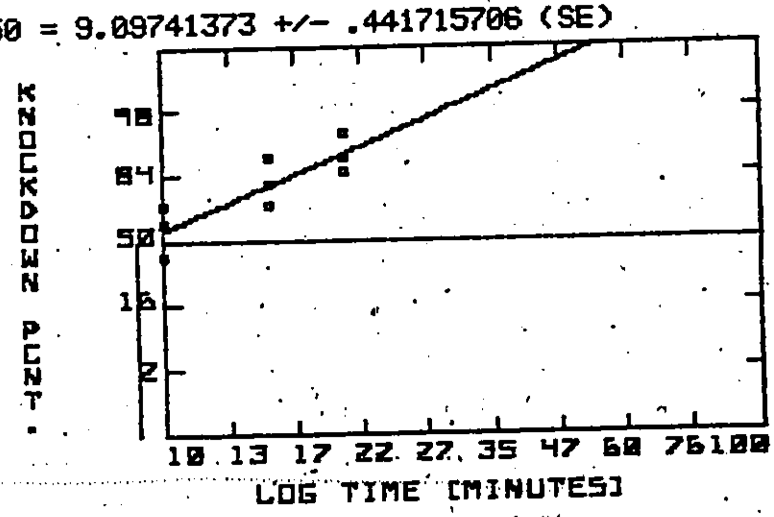
CROSS 4 MALE 1% VAC

KT-50 = 9.68655043 +/- .627655564 (SE)



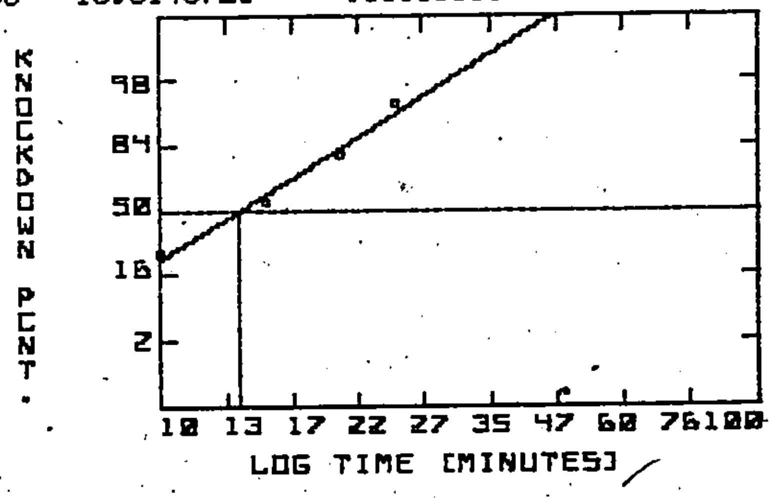
CROSS 4 FEMALE 1% VAC

KT-50 = 9.09741373 +/- .441715706 (SE)



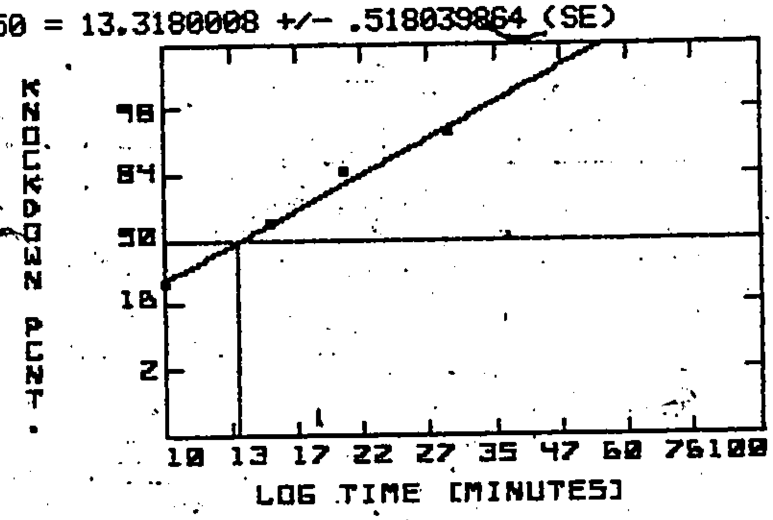
CROSS 5 MALE 1% VAC

KT-50 = 13.6146728 +/- .508899057 (SE)



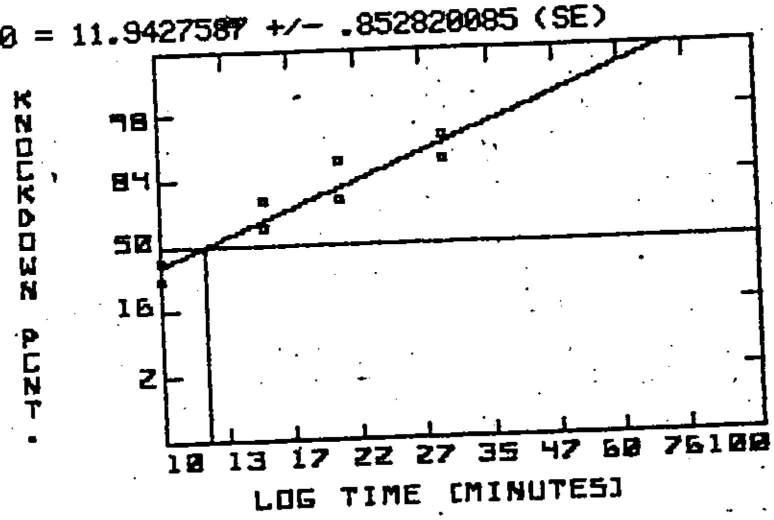
CROSS 5 FEMALE 1% VAC

KT-50 = 13.3180008 +/- .518039864 (SE)



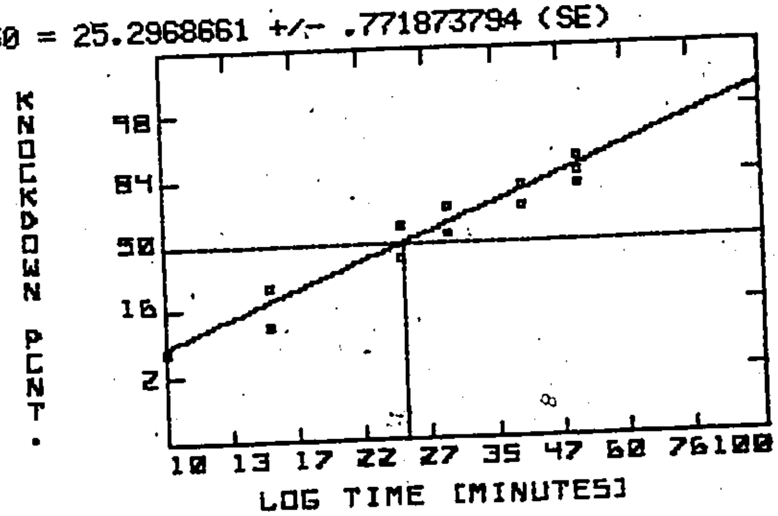
CROSS 6 MALE 1% VAC

KT-50 = 11.9427587 +/- .852820085 (SE)



CROSS 6 FEMALE 1% VAC

KT-50 = 25.2968661 +/- .771873794 (SE)



Results of resistance testing - F_2 flies.

down

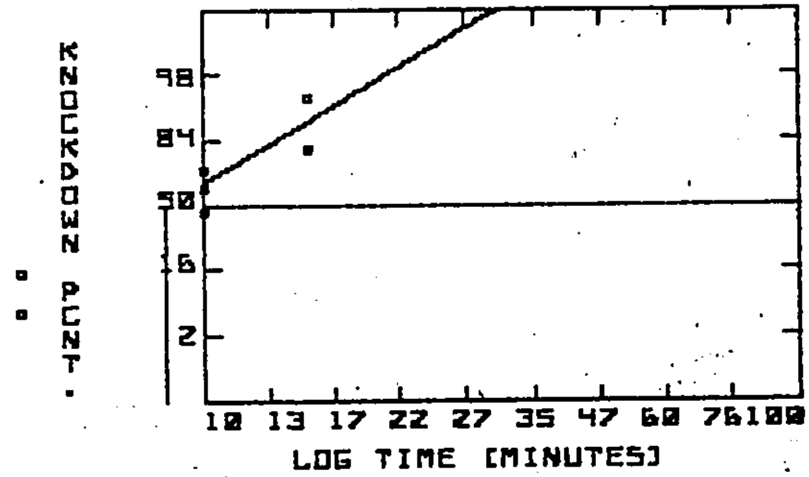
Time:	5	10	15	20	25	30
1 m	1	9	16	18		
	3	12	20			
	3	14	20			
f	1	3	10	16		17
	2	9	16	18		20
	2	4	11	14		16
2 m	4	15	19	20		
	4	14	20			
	4	12	18	19		
f	3	10	18		19	
	2	8	16		20	
	3	8	16		18	
	3	13	18		20	
3 m	6	7	12	13		16
	3	12	15	17		20
	6	8	17	19		20
	2	3	12	16		17
f	2	8	17			20
	3	6	12			18
	2	6	16			20
	4	8	13			18

Time:	5	10	15	20	30	40	60	75	105	120
4 m	4	7	14	17						
	2	11	20							
	2	6	17	20						
	1	7	18	20						
f	3	8	15	17						
	2	8	17	19						
	3	7	18	20						
5 m		4		16		17			17	17
		4		13		17	18		18	18
f		3		6	8			15	16	16
		3		11	14			19	19	19
		4		9	12			14	15	16
		3		7	10			13	14	14
6 m		3		9	12		16			17
		0		4	5		9			13
		3		6	9		10			13
				6	9		12			16
f		2		8	10		12			14
		4		7	9		10			10
		5		13	14		17			19

total survivors from 5 and 6 F_2 = 58 out of 260 flies tested.

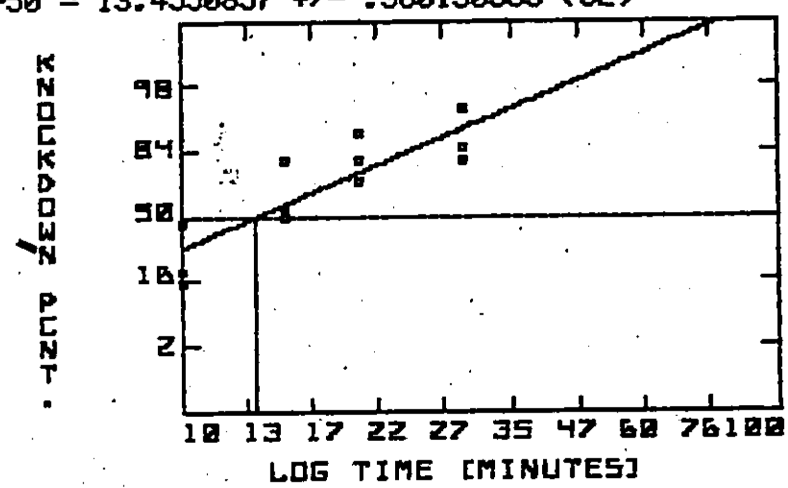
CROSS 1 F2 MALE 1% VAC

KT-50 = 8.63957457 +/- .437182527 (SE)



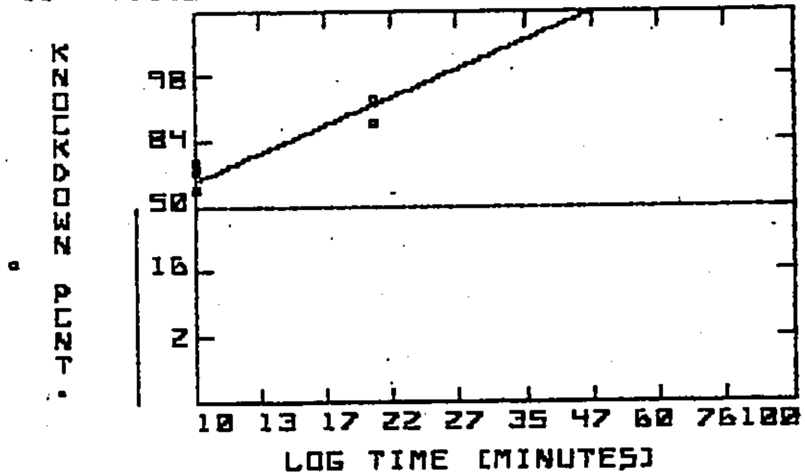
CROSS 1 F2 FEMALE 1% VAC

KT-50 = 13.4550857 +/- .980190365 (SE)



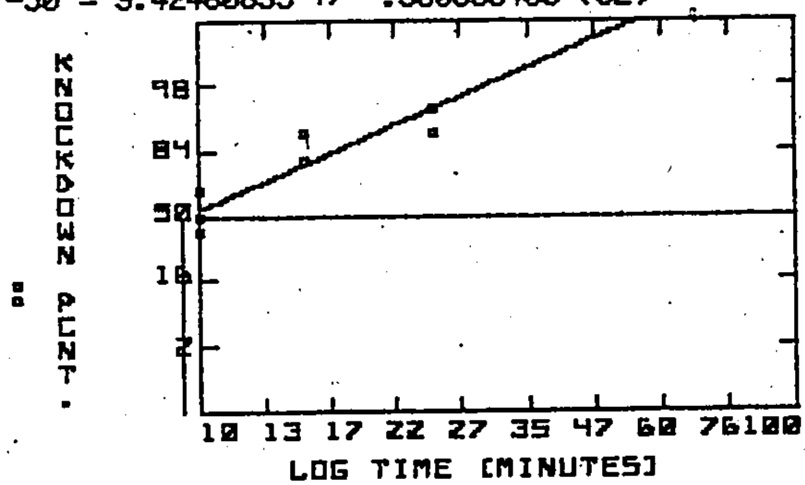
CROSS 2 F2 MALE 1% VAC

KT-50 = 7.96255437 +/- .292858179 (SE)



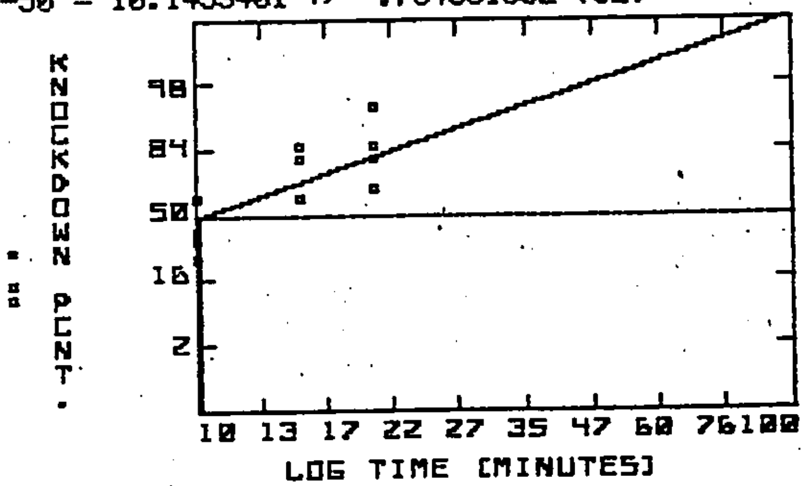
CROSS 2 F2 FEMALE 1% VAC

KT-50 = 9.42460835 +/- .380695403 (SE)



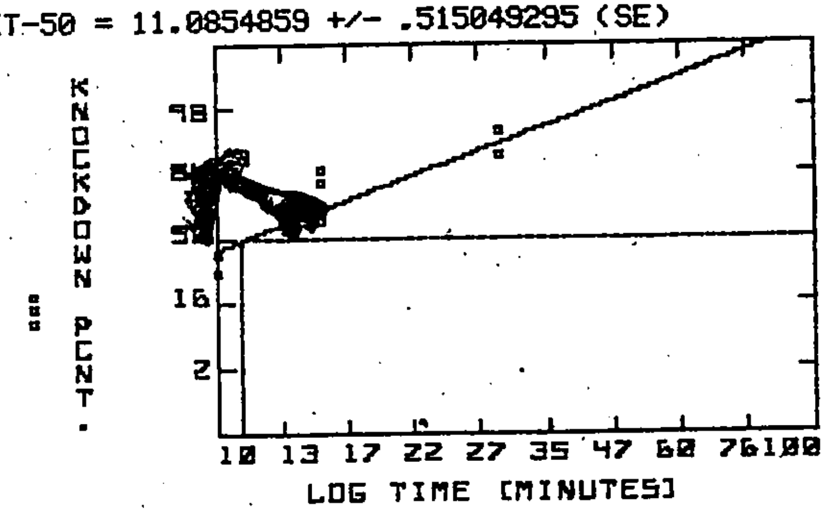
CROSS 3 F2 MALE 1% VAC

KT-50 = 10.1453401 +/- .704901092 (SE)



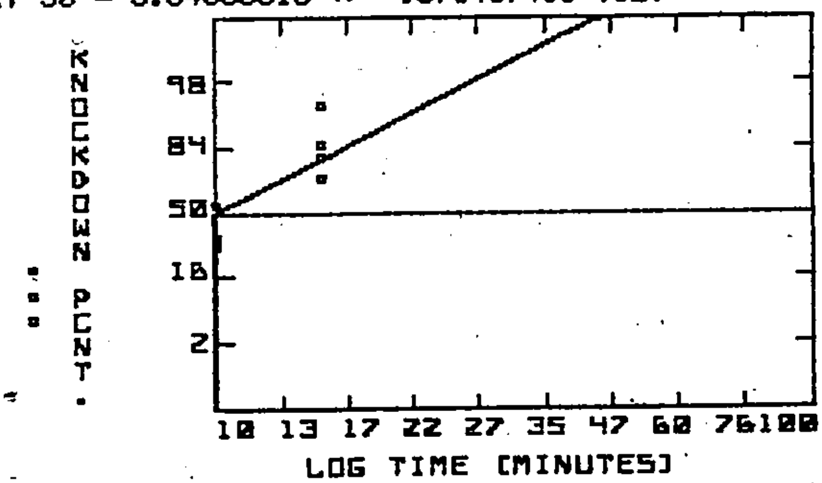
CROSS 3 F2 FEMALE 1% VAC

KT-50 = 11.0854859 +/- .515049295 (SE)



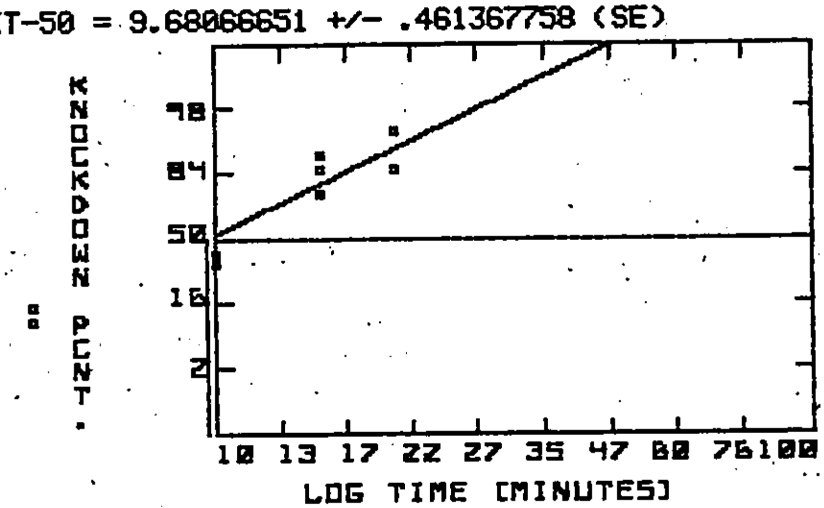
CROSS 4 F2 MALE 1% VAC

KT-50 = 9.94685518 +/- .570497466 (SE)



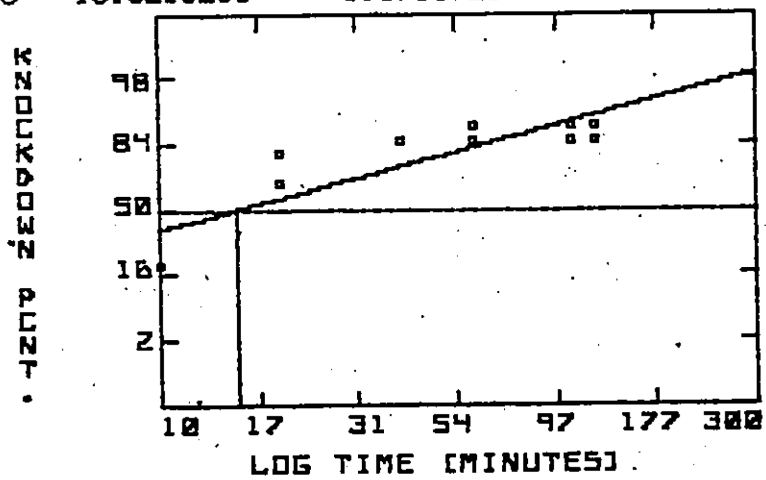
CROSS 4 F2 FEMALE 1% VAC

KT-50 = 9.68066651 +/- .461367758 (SE)



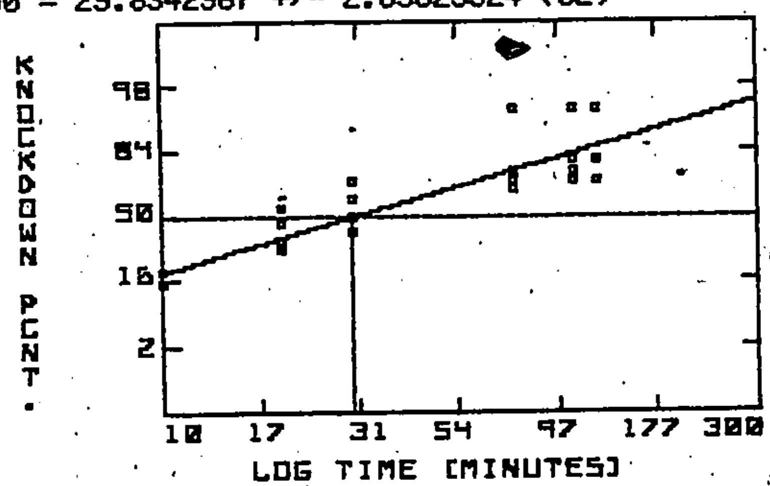
CROSS 5 F2 MALE 1%

KT-50 = 15.6213209 +/- 3.17107214 (SE)



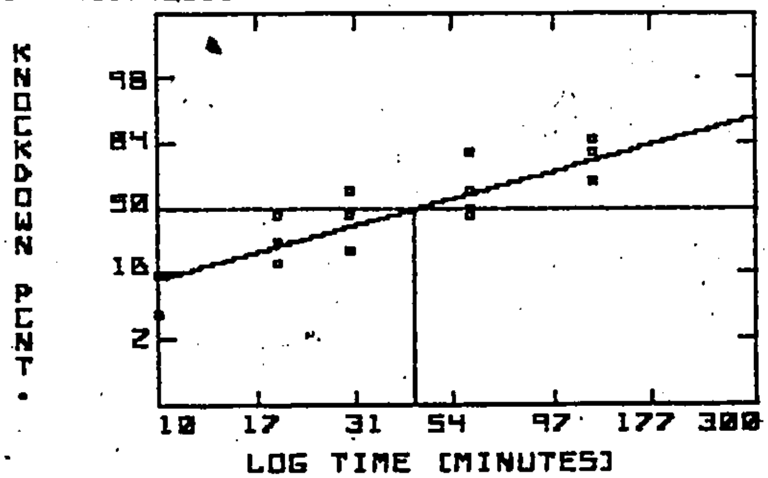
CROSS 5 F2 FEMALE 1% VAC

KT-50 = 29.8342967 +/- 2.85829924 (SE)



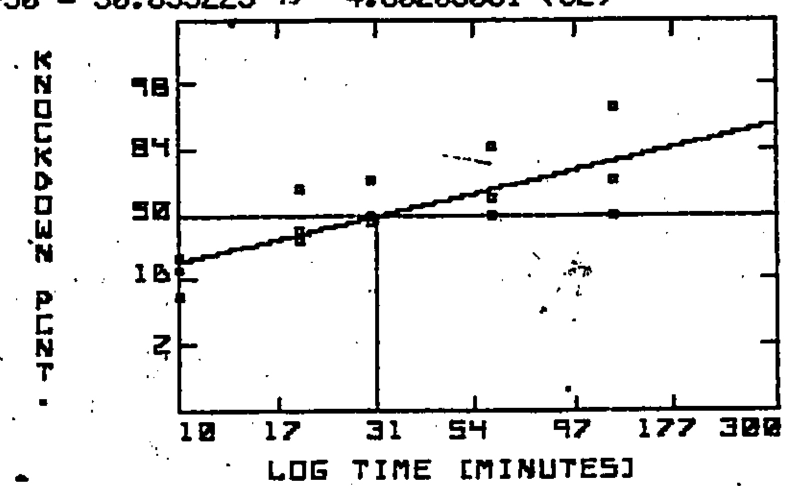
CROSS 6 F2 MALE 1% VAC

KT-50 = 43.7421667 +/- 4.13260363 (SE)



CROSS 6 F2 FEMALE 1%VAC

KT-50 = 30.895223 +/- 4.80205031 (SE)



APPENDIX G

Mutant Screening: i) Rough screen - the results of the rough screening of attached-X lines are given in the pages following. Those lines whose knockdown profiles differ obviously from the others are indicated by an asterisk, and these lines were kept for further testing.

ii) Vacuum assay screen - lines retained for testing after the rough screen were assayed for resistance to a dose of 1% malathion, and the raw data are given following the rough screen data, while the knockdown curves are plotted in Figures G.1 and G.2. Of the lines tested, six were found to be similar to Canton S in resistance (72, 123, 277, 145, 141, 127) and 2 others showed step curves indicative of split-populations for resistance, hence none of these lines were kept except for 127, which showed obviously defective behaviour.

Of the lines retained after the second screen, (127, 63, 99, 151, 59, 12) lines 63 and 99 subsequently gave results indicating that they were split populations with respect to resistance, and thus the final group of mutant lines carried forward consisted of 151 (less resistant than Canton S), 12 and 59 (more resistant than Canton S), and 127.

Rough screening results

% down (males)

Time:	5	10	15	20	25	30	35	Sample Size
32	27	64	73		100			45
*59	4	10	26		64		100	50
60	18	34	46		95		100	56
58	12	19	31		65			78
14	11	34	59		95			77
16	15	36	51		96			76
3	36	59	71		91			75
5	30	52	69		92			77
*12	40	82	100					68
48	21	39	53		83			78
61	53	77	93		100			73
*57	60	92	99					73
167	51	91	97					78
162	43	87	96					67
159	29	86	93					59
156	22	88	94					74
135	18	84	92					49
132	19	85	93					75
105	18	83	93					40
*63	4	54	86	96				56
*151	7	53	66	86	97			29
169	30	72	97	99				43
136	40	86	94	100				43
166	12	92	97	100				38
116	42	86	94	100				38
150	53	93	97	100				31
*127	5	65	86	92		95		37
171	29	87	95	100				38
125	66	92	97	100				36
*250	44	81	84	92		95		38
*145	13	69	79	87	90	92		39
*59	16	60	88	96	98			50
61	50	86	92	100				36
160	58	90	95	100				40

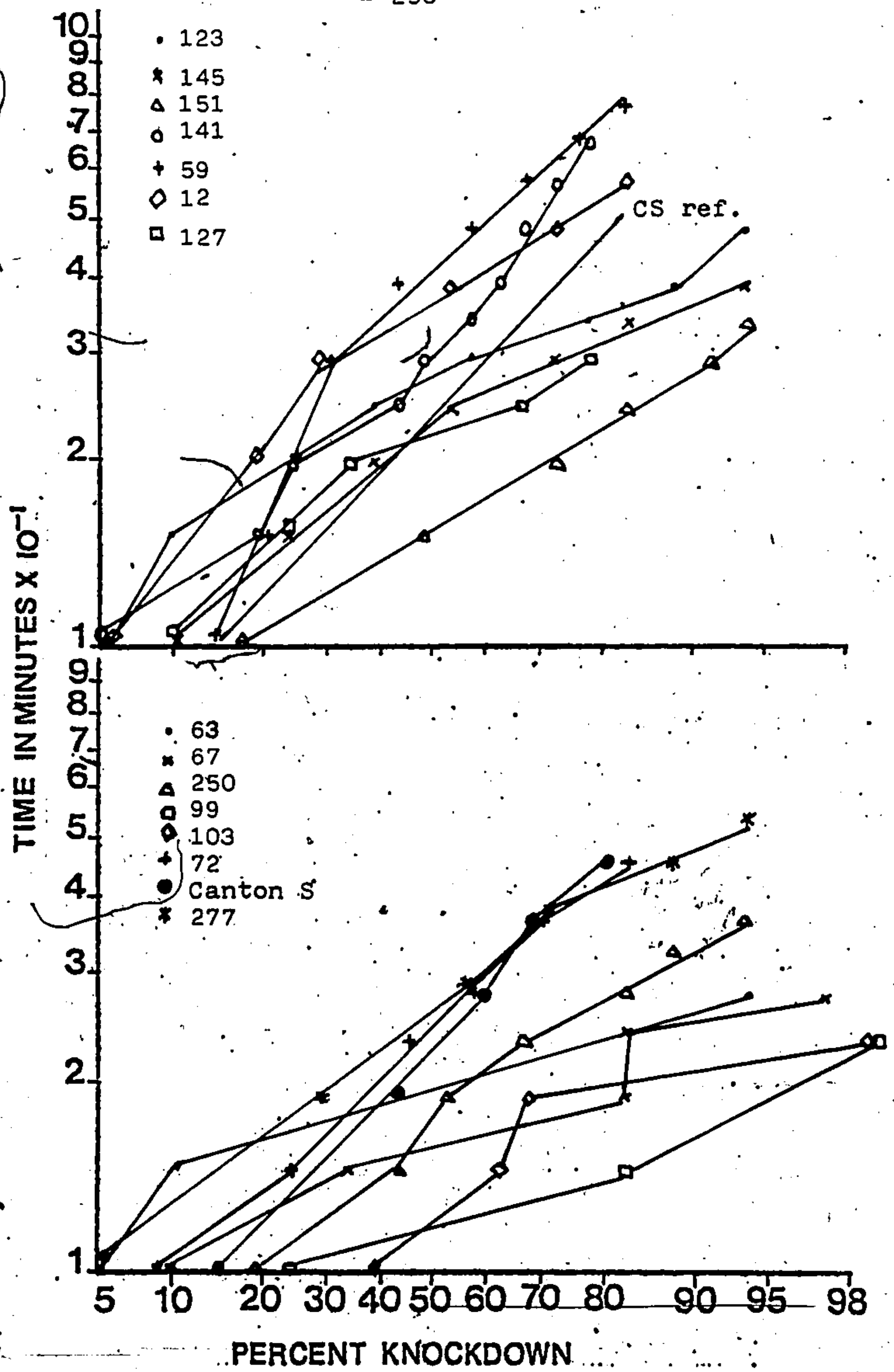
Time:	5	10	15	20	25	30	Sample Size
45	20	83	90	95			40
*111	5	48	65	83	88	93	40
115	28	82	87	93			47
*277	2	29	56	69	73	81	41
153	15	85	93	97			48
129	52	92	94	96			49
248	14	64	82	90			48
175	29	75	88	92			46
258	6	47	71	88			17
173	56	83	98	100			41
113	52	86	92	98			46
110	32	88	92	98			48
267	4	68	82	96			51
101	16	80	92	96			49
154	45	88	95	97			43
257	19	77	85	87	94		46
122	45	84	88	92			47
145	44	88	91	97			48
48	6	46	80	96	100		50
3	3	52	76	86	96		50
60	12	62	80	94	96		50
*72	0	3.	42	58	78	89	36
89	2	41	78	89	100		46
67	2	32	68	88	96		50
*103	35	100					26
58	2	74	94	100			50
157	45	85	90	95			40
68	7	70	92	97			37
104	29	85	91	97			34
90	36	85	97	100			33
*141	78	100					37
275	10	37	73	93			30
*99	52	92	100				50
*123	6	29	50	88			49
163	14	78	89	95			37
274	15	58	96	100			47

Rough screen results (cont.)

	% down (males)								
Time: (min.)	10	15	20	25	30	35	40	50	Sample size
48		5	16	45		56		100	44
47	3		17	31		66		100	35
46	0		16	47		75		100	32
45	3		13	32		67		100	32
18			12	36		81		100	33
32	0	7	23	39		71		100	31
1		6	13	38	56	94	100		16
2			9	22	56	75	85	100	32
7				10	23	57	83	100	30
9			3	15	33	64	85	100	33
10			9	22	47	69	94	100	26
15		7		38	61	77	100		26
17		7	17	36	63	70	93	100	30
22			12	19	42	73	85	100	26
23			3	25	35	52	84	100	31
Canton S			8	16	36	76	96	100	50
" "			10	16	46	84	92	100	50

Results of vacuum assay testing - 1% malathion dose, 4-6 day old males tested at 22°C.

Time: (min.)	# down 10	15	20	25	30	35	40	50	60	70	80	
Canton S	3	8	9		14		15	17				
	3	7	9		11		14	15				
	4	8	10		12		13	14				
	3	8	9		11		13	15				
72	2	6		10	12		14	17				
	2	3		9	12		15	17				
123	1	3	5	8	12	14	17	19				same as CS
277	1		6		12		14	16				
145	2	5	8	11	15	17	18					
141	1	4	6	9	10	12	14	15				
127	2	5	7		14		16					
63	1	2		17	19							
99	5	17		20								
151	3	9	14	15	16	17						sensitive
	4	11	16	18	19	20						
67	2	7	17	17	20							split-population
103	8	13	15	20								
59	3	4	5		6		9	12	16	17		resistant
12	1		4		6		11	15	17			



Figures F.1 and F.2 - knockdown curves.

APPENDIX H

Mutant resistance testing: the results of individual vacuum-injection assay runs for the mutant lines are given on the pages following, along with the knockdown curves for each line at each testing dose. Testing was done at a temperature of 23-24°C in each case.

Significance testing - the results of intra- and inter-strain comparisons with the Kolmogorov-Smirnov test are given in Table H.1 below.

Table H.1 results of significance testing

Compared	D_{max}	D_{crit}	D_{max}	D_{crit}
	Dose: 1%		2%	
Male vs. Female:				
59cs	5	18	35*	18
12cs	10	14	33*	21
Canton S	18*	15	34*	23
151cs	6	16	60*	23
Female vs. Female:				
59cs,12cs	8	15		
59cs,Cs	30*	16		
59cs,151cs	56*	16		
12cs,CS	22*	15		
12cs,151cs	50*	15		
CS,151cs	35*	16		
Male vs. male:				
59cs,12cs	8	17		
59cs,CS	25*	17		
59cs,151cs	42*	17		
12cs,CS	26*	14		
12cs, 151cs	40*	14		
CS,151cs	20*	15		

*$p<0.05$

Results of resistance assay runs - 2% malathion dose.

 # down — Female

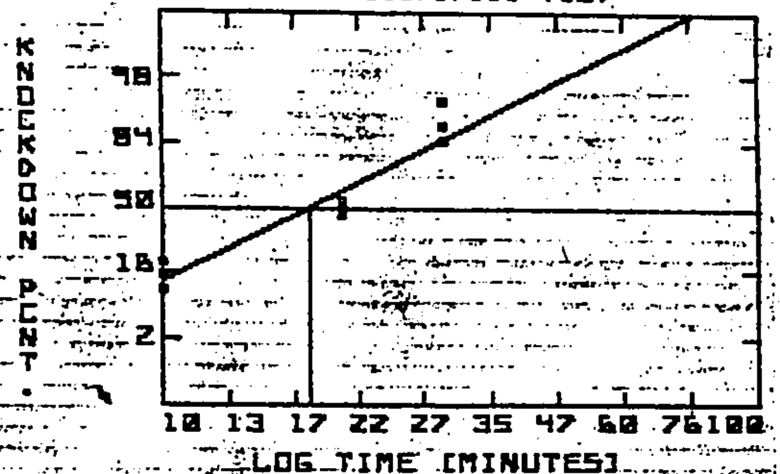
Time (min):	10	15	20	25	30	40
59cs	3		10		17	20
	3		9		17	20
	4		10		18	20
	2		11		19	20
%	15		50		89	100
12cs	4		8		17	19
	3		11		18	19
	2		8		12	16
%	15		45		78	90
CS	4		11		18	
	3		14		20	
	5		14		19	
%	17		65		95	
151cs	5	14	19	20		
	8	16	20			
	2	12	17	20		
%	25	70	93	100		

 # down — Male

Time (min):	10	15	20	25	30	40
59cs	6	13	17		19	
	8	15	19		20	
	3	12	17		19	
	6	12	15		19	
	6	13	16		18	
	4	10	14		17	
%	28	63	85		93	
12cs		12	15	18		
		13	16	19		
	9	15	18	20		
		11	14	17		
		10	15	18		
		12	16	18		
		13	15	18		
%		61	78	91		
CS	10	14	18			
	12	16	18			
	10	17	19			
	9	18	19			
%	51	81	93			
151cs	16		20			
	17		20			
	18		20			
	19		20			
%	88		100			

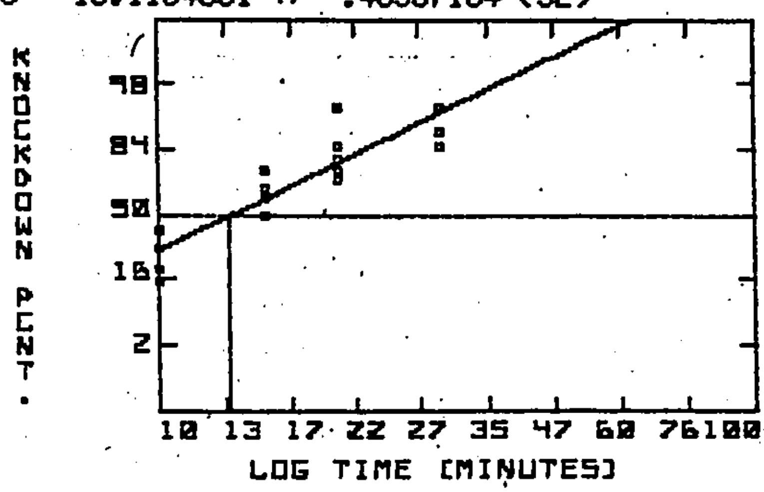
59CS F 2% VAC

KT-50 = 17.5815006 +/- .681717355 (SE)



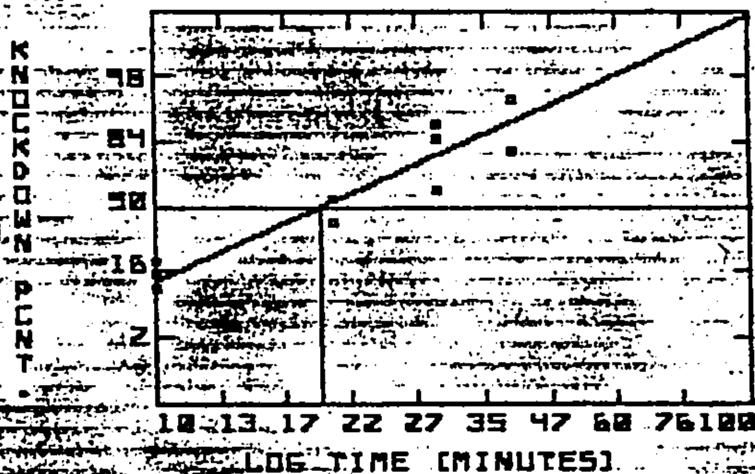
59CS M 2% VAC

KT-50 = 13.1104531 +/- .46967184 (SE)



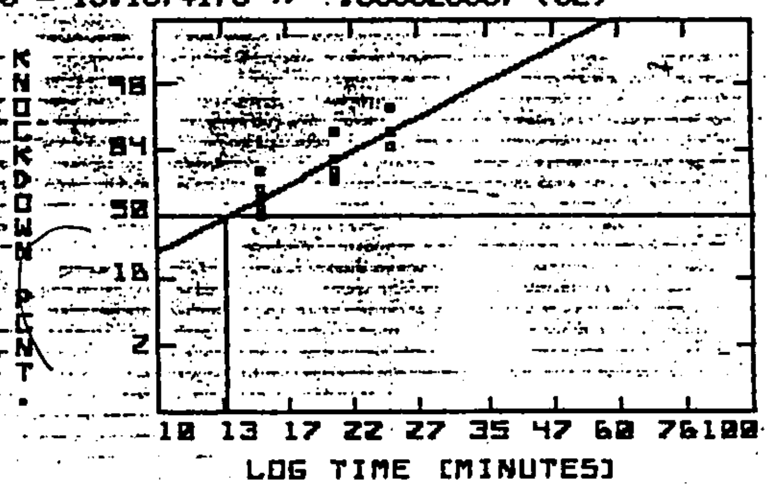
12CS F 2% VAC

KT-50 = 19.0806108 +/- 1.12075587 (SE)



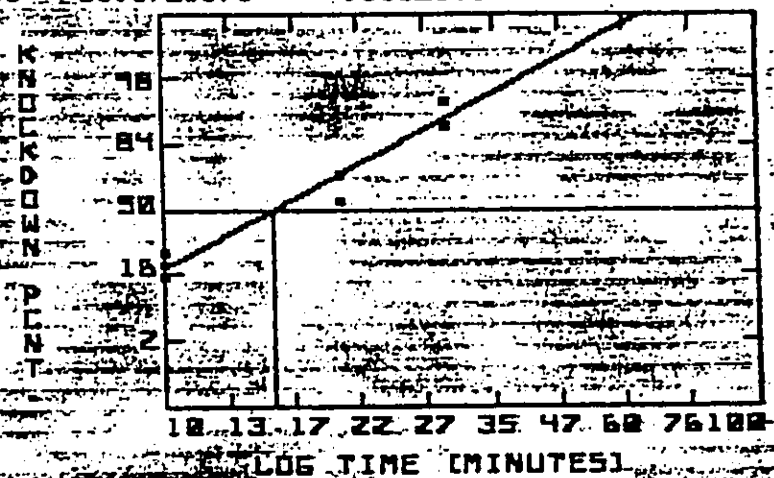
12CS M 2% VAC

KT-50 = 13.1674178 +/- .559326697 (SE)



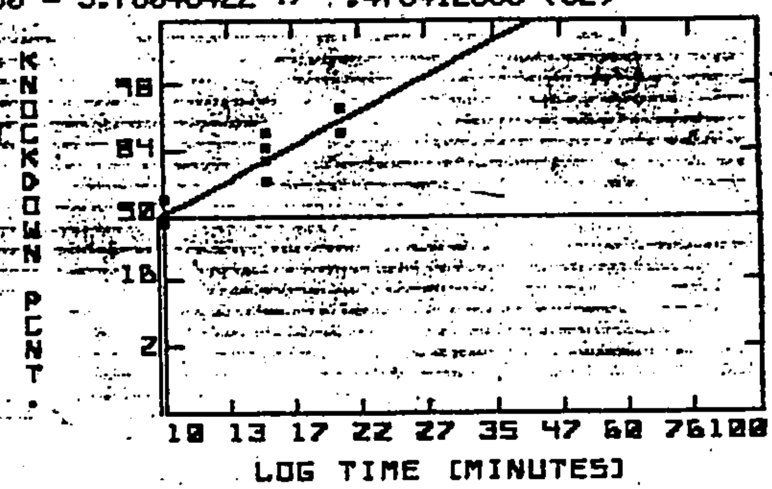
CS F 2% VAC

KT-50 = 15.3728576 +/- .591219911 (SE)



CS M 2% VAC

KT-50 = 9.78048422 +/- .478412963 (SE)



Results of resistance assay runs - 1% malathion dose

\# down

Female

Time: (min.)	10	20	30	40	50	60	75	90
59cs	3	5	9	13	15	17	19	20
	0	2	9	11	14	17	18	19
	2	4	8	11	14	16	19	20
	3	4	8	10	12	13	16	18
	0	4	8	9	12	13	15	17
	0	2	6	8	11	12	16	18
	1	4	8	9	10	13	16	18
%	6	18	40	51	63	72	85	93
CS	2	5	12	15				
	2	6	10	17				
	3	8	13	18				
	2	6	12	16				
	2	7	13	16				
	2	5	12	14	18			
	3	6	13	16	18			
%	11	31	61	80				

12cs	3	5	9	15	17	18
	2	8	12	15	18	19
	5	8	10	13	17	19
	4	9	11	13	15	17
	3	10	12	15		
	3	10	13	15		
	4	11	16	18		
	5	12	15	17		
	6	13	16	18		
%	19	48	63	77	85	91
151cs	1	12	18			
	8	17	19			
	3	13	20			
	5	15	20			
	6	20				
	5	13	17			
	4	17	20			
%	23	76	96			

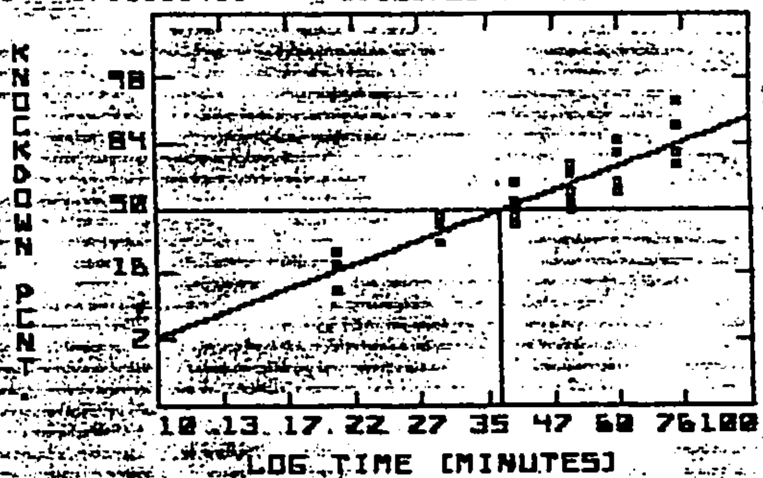
Male

Time: (min.)	10	15	30	45	60	75
59cs		6	11	13	15	16
		4	10	14	16	17
		6	11	14	16	18
		5	11	15	16	18
		5	11	16	17	18
%		26	54	71	80	87
CS	3	10	17	20		
	6	9	15	19		
	4	7	16	19		
	4	7	17	20		
	5	9	17	20		
	3	6	15	20		
	6	9	14	18		
	4	7	16	19		
	5	9	15	18		
%	22	41	79	96		
12cs		4	13	15	17	
		6	13	14	17	
		5	13	16	18	
		5	10	13	16	
		6	11	14	17	
		5	12	14	17	
		6	11	15	18	
		5	10	13	16	
		6	11	13	16	
		3	10	13	15	
%		26	57	70	84	

151cs	4	82	96
	8	18	20
	4	15	19
	6	17	19
	7	16	19
	8	18	20
	5	16	20
	5	18	20
%	29	82	96

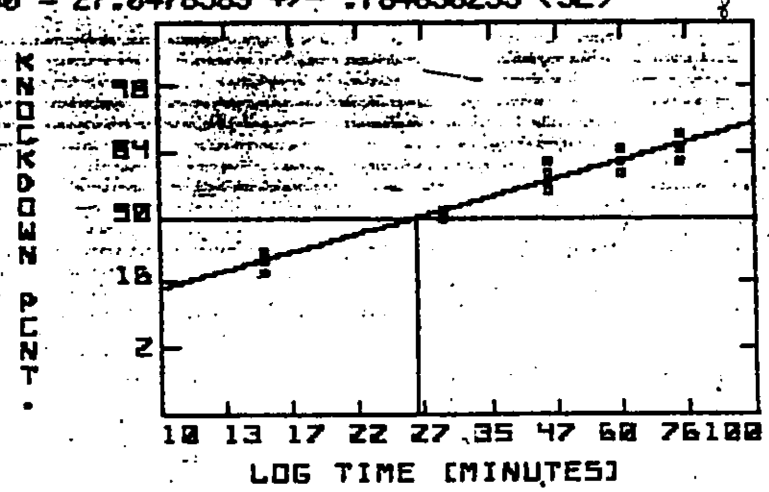
59CS F 1% VAC

KT-50 = 37.9018406 +/- 1.02672547 (SE)



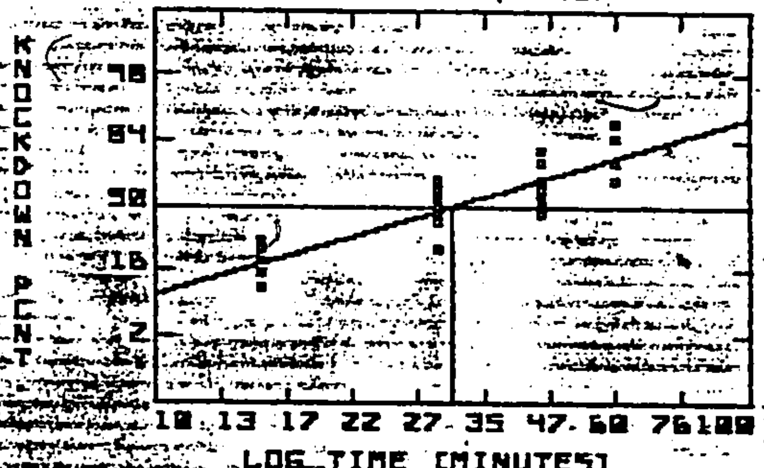
59CS M 1% VAC

KT-50 = 27.0478589 +/- .784698293 (SE)



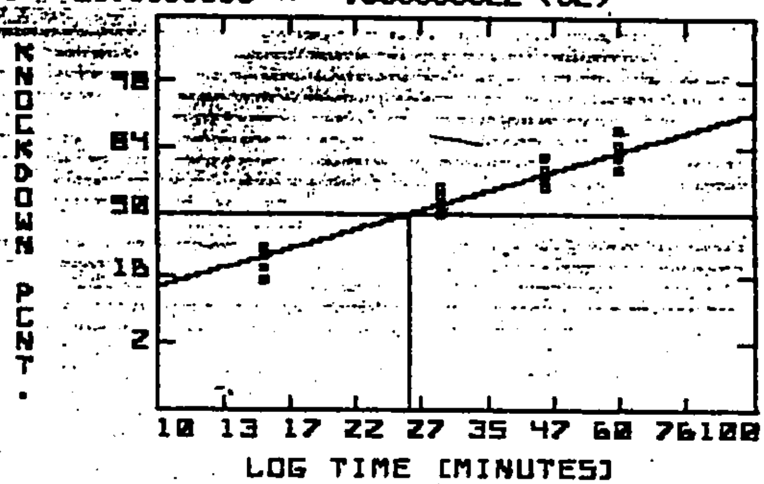
12CS F 1% VAC

KT-50 = 31.8066043 +/- 1.24290797 (SE)



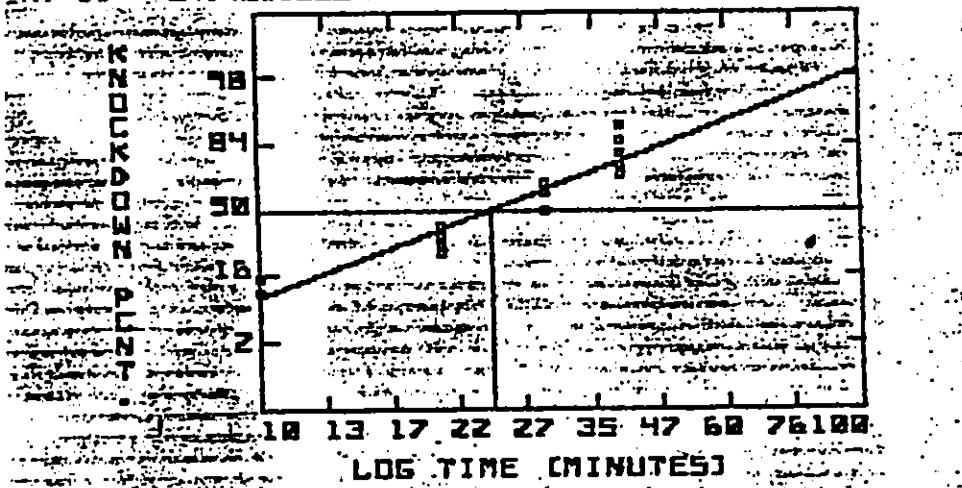
12CS H 1% VAC

KT-50 = 26.5868159 +/- .656685922 (SE)



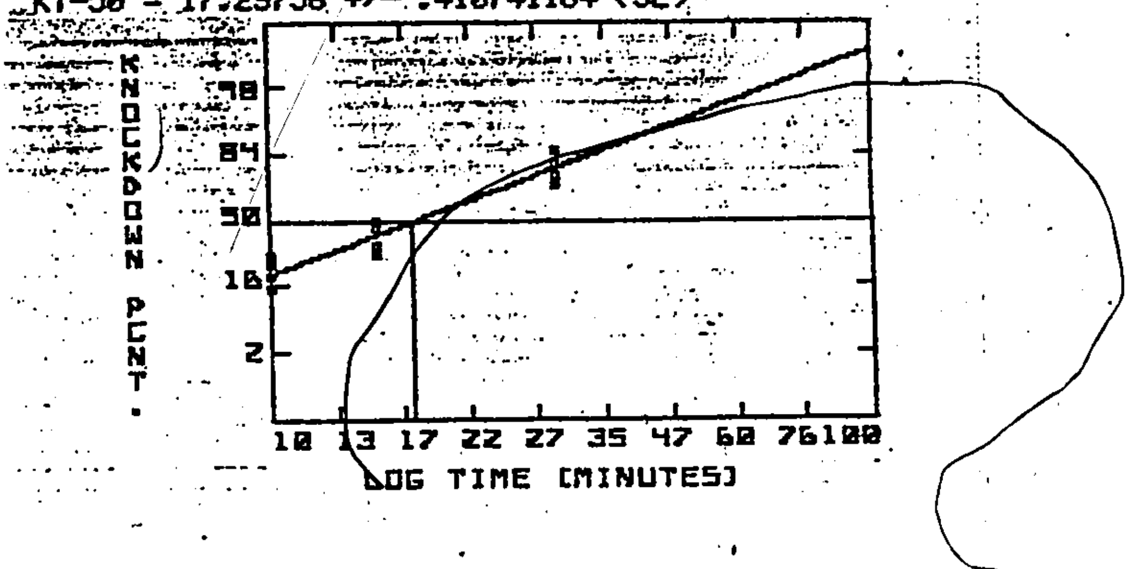
CS F 1% VAC

KT-50 = 24.4219222 +/- .662553251 (SE)



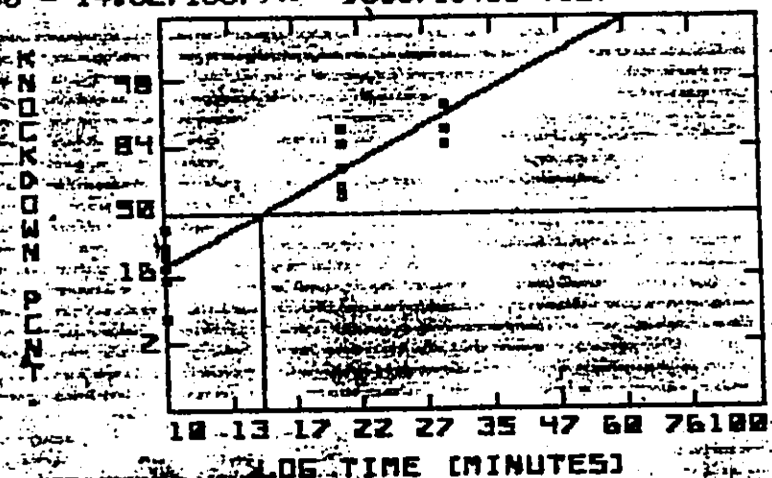
CS H 1% VAC

KT-50 = 17.29756 +/- .410741184 (SE)



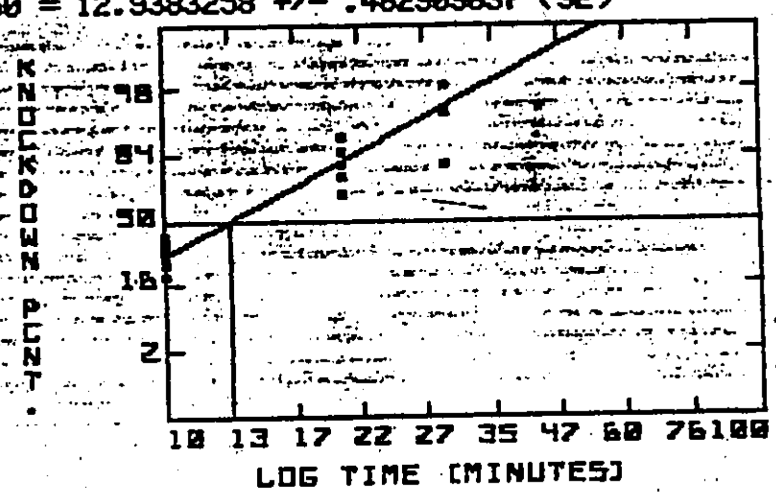
151CS F 1% VAC

KT-50 = 14.5271087 +/- .566713411 (SE)



151CS H 1% VAC

KT-50 = 12.9383258 +/- .482909637 (SE)



Results of resistance assay runs - 0.5% malathion dose, females.

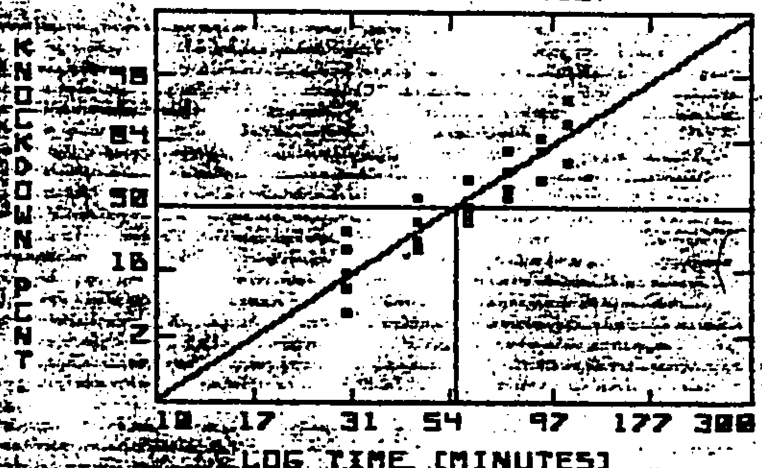
		# down								
Time (min):		15	30	45	60	75	90	105	120	135
59cs		2	7	11	13	16	17	18		
		0	2	6	8	11	13	15		
		2	5	6	9	12	16	18		
		2	3	8	10	12	16	18		
		0	1	5	10	14	17	19		
	%	6	18	36	50	65	79	88		
CS		3	8	13	16	17	19			
		1	6	13	16	18	20			
		1	6	12	14	17	18			
		2	7	13	15	17	18			
		1	8	13	16	18	19			
		4	9	14	17	18	19			
		5	9	11	14	15	17			
		1	6	12	14	15	17			
		2	5	7	11	15	17			
	%	10	35	60	74	83	91			
12cs		1	4	5	6	7		11	13	16
		1	2	5	6	10		14	16	18
		0	5	6	8	12		13	14	16
		1	5	7	9	11		16	17	19
		3	7	10	12	13		16	17	18
		2	10	12	14	16		18	19	19
		3	9	10	11	13		15	16	17
	%	8	30	39	47	59		74	80	88
151cs		1	10	18	19					
		1	12	20						
		2	9	11	14					
		3	10	14	17					
		3	12	16	19					
		5	11	17	19					
	%	13	53	80	90					

Results of resistance assay runs - 0.5% malathion dose, males.

	# down								
Time (min):	15	30	45	60	75	90	105	120	135
59cs	2	4	8	11	13	14	17	19	
	2	5	6	9	11	13	16	19	
	2	3	8	12	14	15	18		
	3	6	9	13	15	17	20		
	1	8	11	13	15	17	19		
%	10	26	42	58	68	76	90		
CS	2	9	11	13	14	16			
	6	11	15	17	18	19			
	3	11	14	16	18	19			
	3	7	10	14	16	18			
	3	8	11	13	15	17			
	5	11	16	17	19	20			
	5	12	16	18	19	20			
%	19	49	66	77	8[illegible]	[illegible]			
12cs	2	6	8	10	13	15		16	
	3	7	10	12	14	16		18	
	5	9	11	13	14	15		17	
	6	9	11	14	15	16		17	
	5	11	12	14	15	15		18	
%	21	42	52	63	71	77		86	
151cs	5	16	19						
	4	15	18						
	3	13	17						
	1	14	17						
	4	15	19						
%	17	73	90						

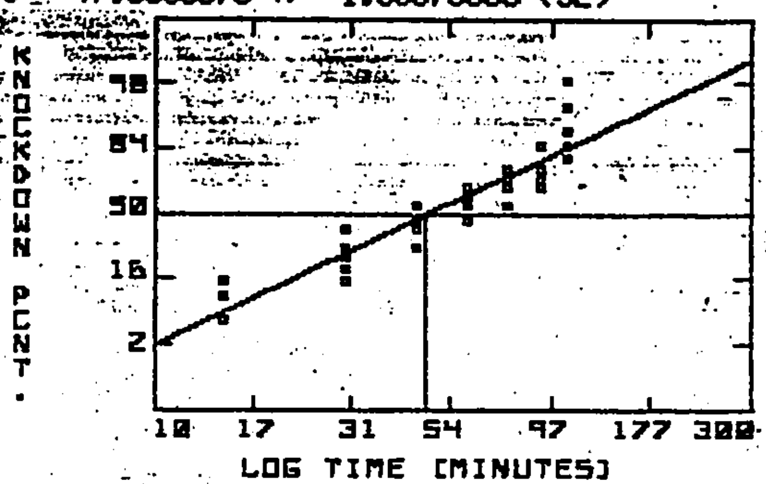
59CS F 0.5% VAC

KT-50 = 55.9511619 +/- 1.80840716 (SE)



59CS M 0.5% VAC

KT-50 = 47.3809379 +/- 1.90873883 (SE)



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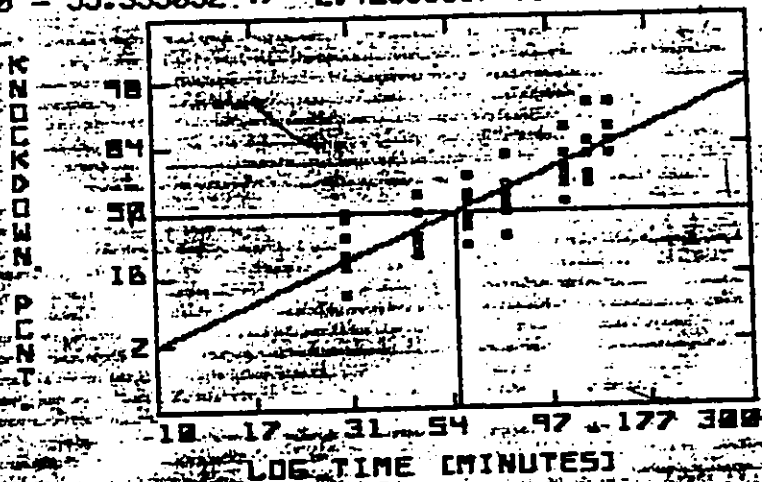
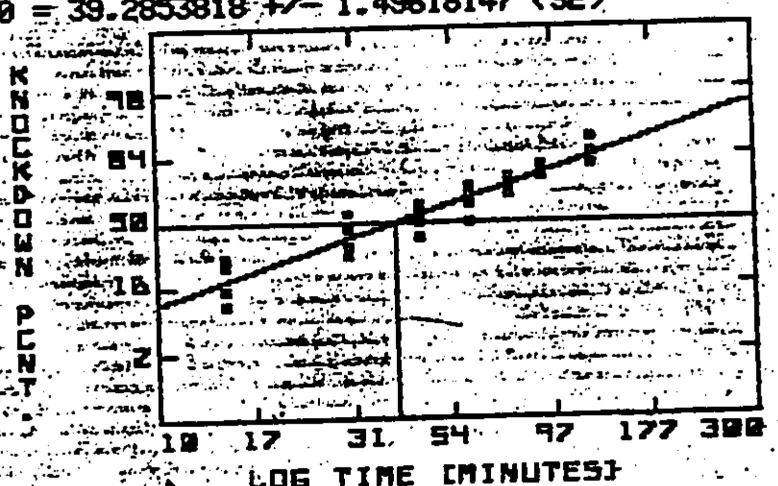
12CS F 0.5% VAC

KT-50 = 55.993032 +/- 2.42953697 (SE)



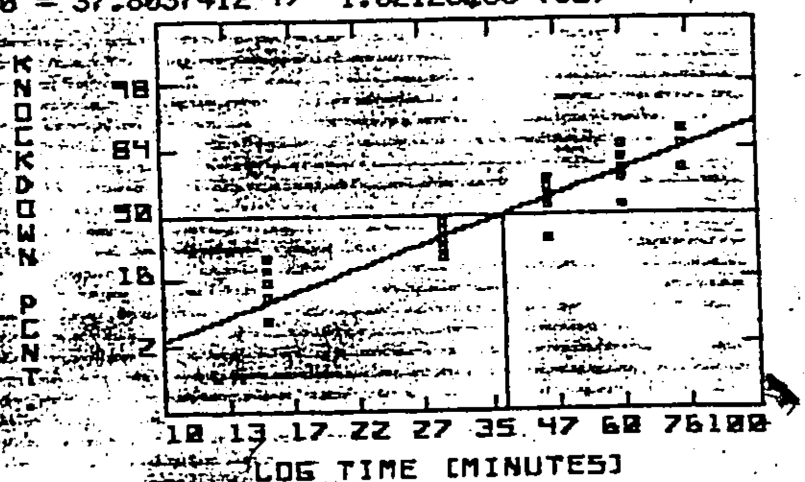
12CS M 0.5% VAC

KT-50 = 39.2853818 +/- 1.49618147 (SE)



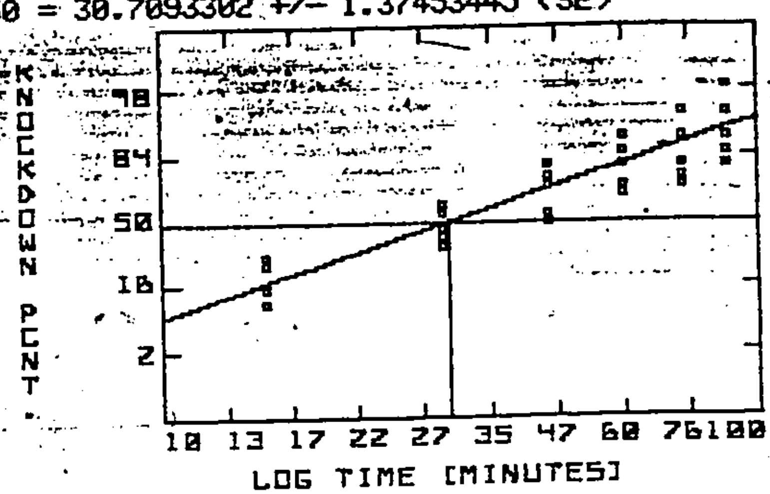
CS F 0.5% VAC

KT-50 = 37.8037412 +/- 1.02125866 (SE)



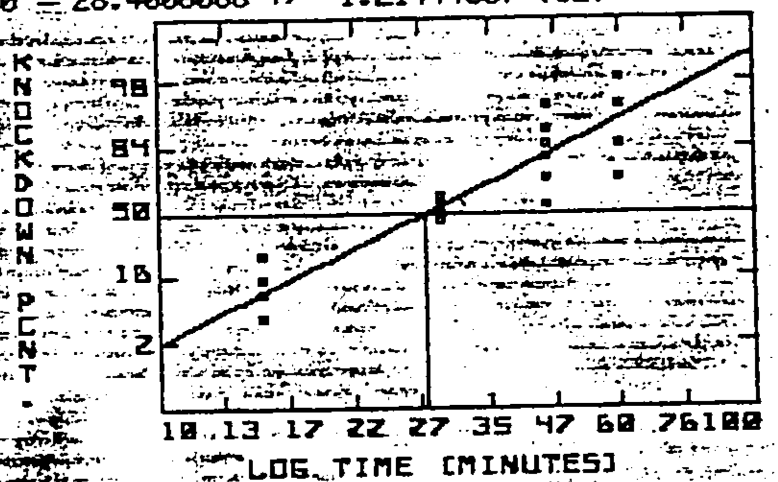
CS H 0.5% VAC

KT-50 = 30.7093302 +/- 1.37453445 (SE)



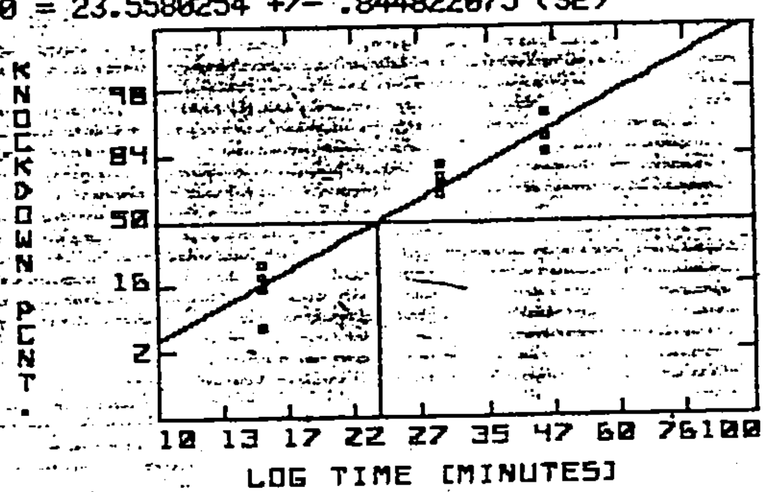
151CS F 0.5% VAC

KT-50 = 28.4006086 +/- 1.21444507 (SE)



151CS M 0.5% VAC

KT-50 = 23.5580254 +/- .844822075 (SE)



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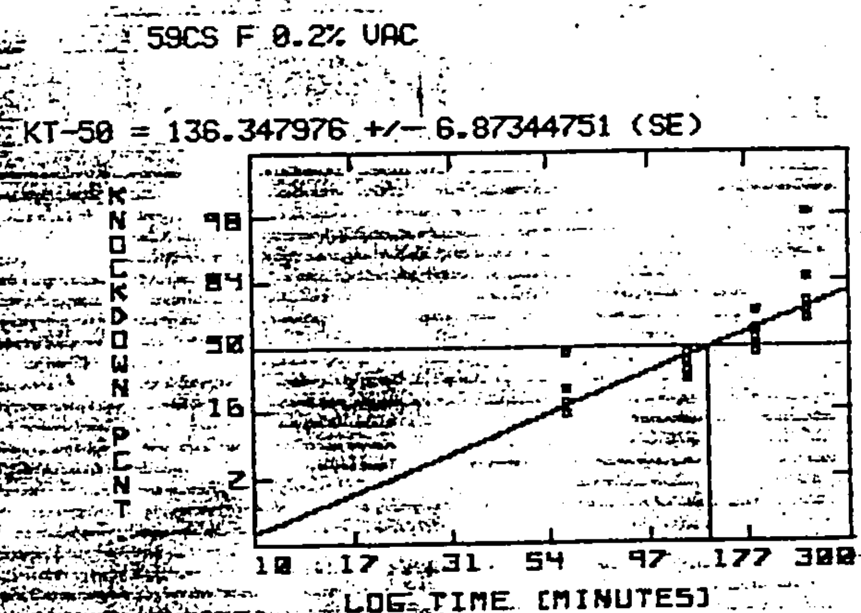
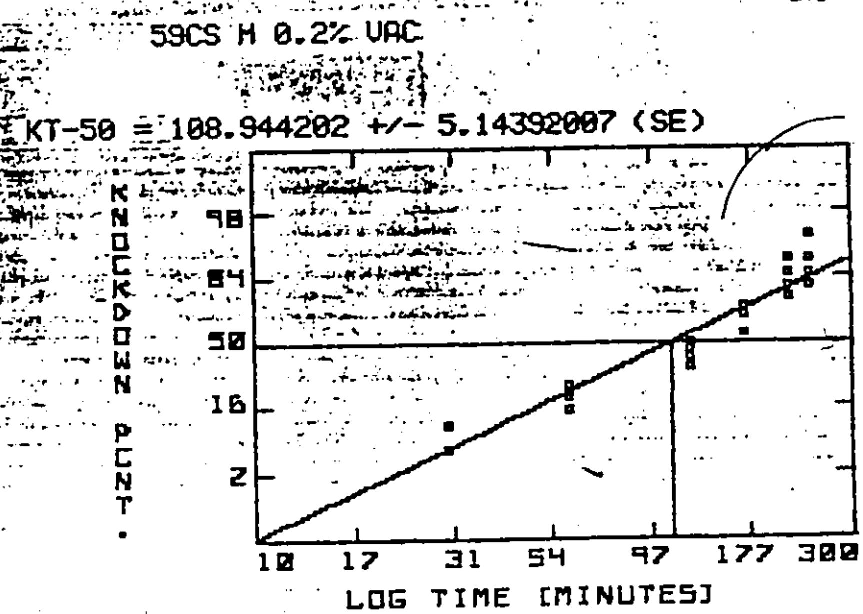
Results of resistance assay runs - 0.2% malathion dose, females.

				# down				
Time (min):	15	30	45	60	90	120	180	240
59cs		2		4		8	9	13
		0		4		8	11	14
		4		9		9	10	15
		3		5		8	11	15
		1		5		7	10	15
		0		4		6	14	20
		0		3		7	11	15
		1		3		7	12	17
% (mean)		6		23		38	55	78
CS		7		10		11	13	16
		3		4		11	13	16
		6		11		12	15	18
		7		10		11	13	16
		3		4		11	13	16
		2		8		9	13	17
		3		11		14	16	17
% (mean)		22		41		56	69	83
12cs		2		3		6	9	16
		2		4		5	6	13
		3		4		6	9	13
		3		5		10	14	16
		4		9		12	16	18
		4		8		11	18	20
		3		7		8	11	13
		3		7		8	13	17
% (mean)		15		29		41	60	79
151cs	1		4	8	15			
	5		9	13	16			
	3		6	8	13			
	1		8	11	16	19		
% (mean)	12		34	50	75	95		

Results of resistance assay runs - 0.2% malathion dose, males.

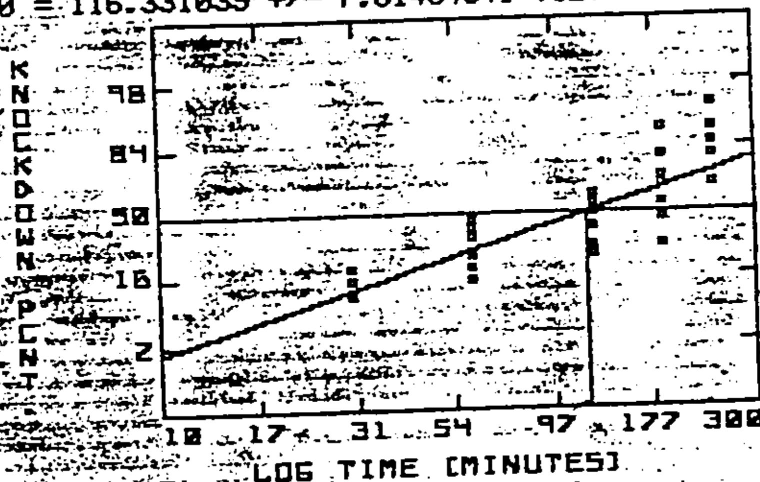
		# down										
Time (min):		15	30	60	75	90	120	150	165	180	210	240
59cs			1	3			7		14		17	18
			2	4			9		14		18	19
			2	3			10		13		16	17
			2	5			8		11		15	16
	$\overline{\%}$		9	19			43		65		82	88
CS			2	6				16				20
			5	6				13				18
			4	7				11				15
			3	10				15				18
			2	8				13				16
			7	10				12				15
			5	8				12				15
	$\overline{\%}$		20	40				66				84
12cs			2	7			11			13		14
			5	12			14			15		16
			5	8			11			12		15
			2	5			7			13		15
			3	6			7			13		14
			3	8			8			13		16
			5	11			13			18		20
	$\overline{\%}$		17	40			51			69		79
151cs		3	7	14	17	18						
		4	8	13	16	17						
		3	9	12	16	18						
		2	6	12	15	16						
		6	8	15	16	17						
	$\overline{\%}$	18	38	66	80	86						


59CS F 0.2% VAC
KT-50 = 136.347976 +/- 6.87344751 (SE)
KNOCKDOWN PCNT
98
84
50
16
2
10 17 31 54 97 177 300
LOG TIME [MINUTES]


59CS M 0.2% VAC
KT-50 = 108.944202 +/- 5.14392007 (SE)
KNOCKDOWN PCNT.
98
84
50
16
2
10 17 31 54 97 177 300
LOG TIME [MINUTES]

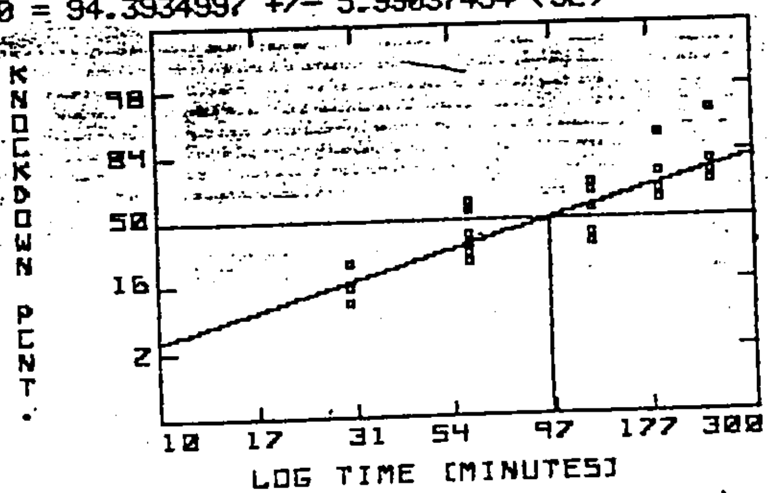
12CS F 0.2% VAC

KT-50 = 116.331039 +/- 7.81484941 (SE)



12CS M 0.2% VAC

KT-50 = 94.3934997 +/- 5.99037434 (SE)



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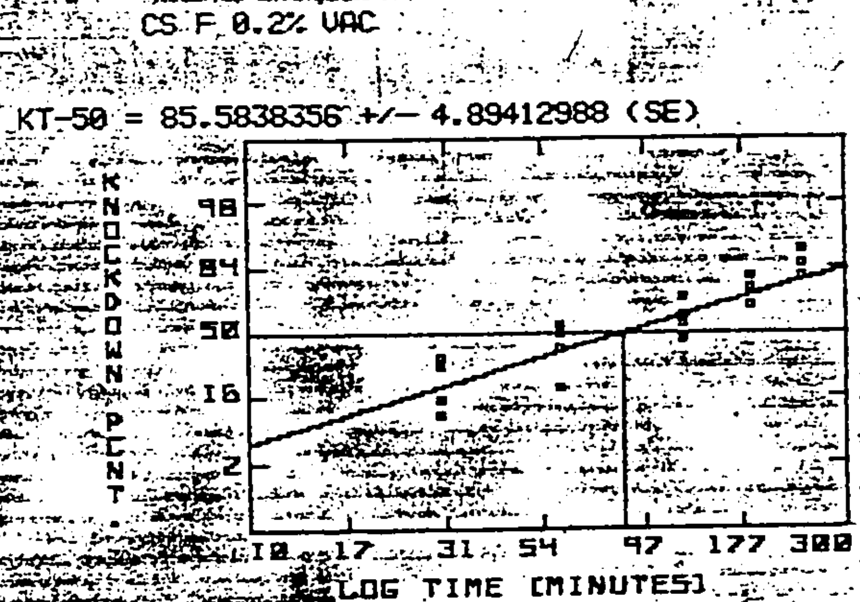

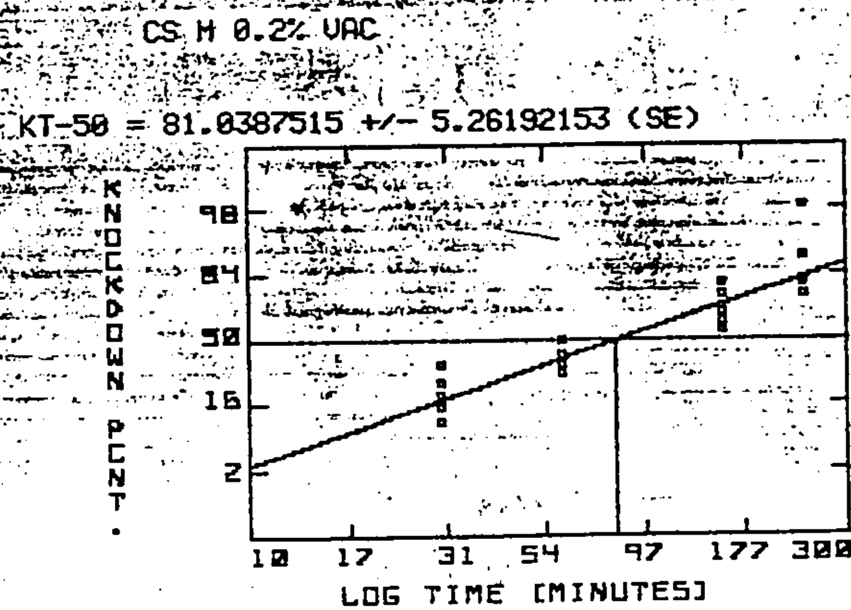

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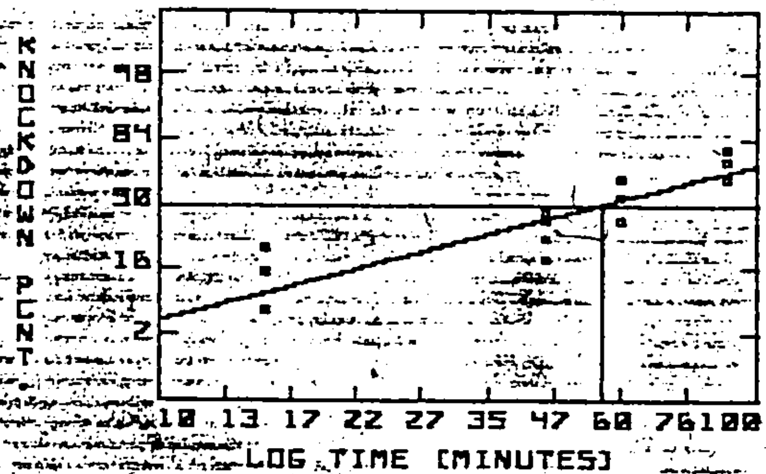
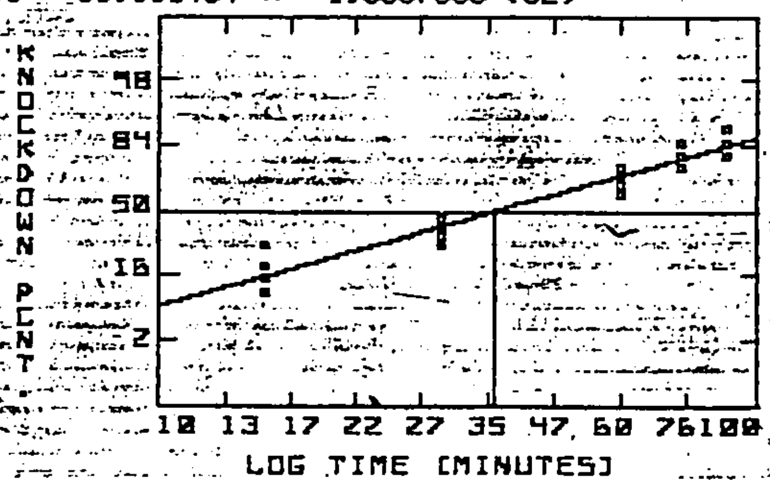
151CS F 0.2% VAC

KT-50 = 55.6930816 +/- 4.60559131 (SE)



151CS H 0.2% VAC

KT-50 = 36.881454 +/- 1.3307065 (SE)



APPENDIX I

Mutant line avoidance data: the raw data and parameter tables for the mutant line avoidance and control runs are listed on the pages following. Where the runs are not listed separately, they are separated by brackets (I).

Analysis - the results of homogeneity testing within groups is shown in the table below (since the variances of run data within each group was homogeneous this testing was done via anova). None of the F values are significant, and the results of runs within each group were pooled for further comparisons. The results of intersex comparisons are also shown on the table (done via the means test since the variances of Canton S and 151cs were inhomogeneous between sexes - from Bartlett's test the chi-square values were 24 and 20 for df=1 respectively).

Table I.1 - results of comparisons

Line	#runs	Homogeneity df	F	Male vs. Female df	F
CS female	3	2,70	1.8	1,5.1	2.4
male	5	4,109	1.4		
12cs female	2	1,60	0.8	1,5.0	11*
male	5	4,63	0.6		
59cs female	3	2,80	0.02	1,5.1	8.7*
male	3	3,50	2.1		
151cs female	3	2,48	2.3	1,5.0	11.3*
male	3	2,45	0.2		

*$p<0.05$

151C-F-A
0% 11, 11, 12, 8, 7, 10, 10, 8, 8, 9, 8, 7, 11, 8, 7, 4,
7, 5,
1% 2, 1, 3, 2, 3, 1, 0, 2, 1, 0, 0, 1, 0, 1, 0, 2,
3, 1,

151C-F-B
0% 7, 6, 10, 8, 6, 7, 4, 3, 2,
1% 2, 2, 1, 0, 1, 1, 2, 0, 0,

151C-F-C
0% 5, 3, 5, 6, 4, 3, 3, 3, 2, 2, 0, 3, 2, 2, 1, 1,
1, 1, 1, 2, 1, 1, 1, 1,
1% 4, 2, 1, 2, 0, 1, 0, 1, 2, 0, 2, 0, 1, 1, 2, 0,
0, 0, 1, 0, 1, 0, 0, 1,

151C-M-A
0% 7, 5, 7, 6, 6, 8, 7, 3, 7, 7, 6, 5, 6, 7, 7, 5,
7, 8, 7, 7, 6, 8, 9, 9, 6, 5, 4, 6, 6, 6, 5,
1% 1, 1, 1, 1, 3, 1, 0, 1, 1, 1, 0, 0, 0, 1, 2, 1,
0, 0, 0, 0, 1, 1, 1, 1, 1, 0, 0, 0, 0, 0, 0,

151C-M-B
0% 5, 6, 7, 4, 4, 4, 6, 4, 3,
1% 0, 0, 0, 0, 1, 1, 0, 0, 1,

151C-M
0% 8, 5, 5, 3, 4, 3, 3, 3,
1% 1, 0, 0, 2, 0, 0, 0, 0,

CS-F-A
0% 7, 8, 8, 7, 8, 7, 8, 7, 6, 7, 9, 11, 9, 9, 7, 7,
9, 8, 6, 7,
1% 3, 2, 1, 5, 3, 1, 4, 1, 2, 3, 4, 3, 3, 3, 3, 2,
3, 3, 2, 3,

CS-F-B
0% 4, 6, 4, 6, 6, 7, 6, 8, 7, 5, 4, 6, 7, 3, 6, 4,
5, 4, 3, 5,
1% 2, 2, 3, 2, 0, 2, 3, 1, 7, 2, 1, 0, 2, 1, 0, 1,
2, 1, 1, 1,

CS-F-C
0% 7, 4, 8, 9, 4, 4, 5, 5, 8, 8, 9, 8, 9, 8, 7, 7,
7, 5, 9, 7, 7, 7, 9, 9, 7, 6, 6, 8, 7, 6, 5,
1% 1, 3, 3, 3, 3, 2, 1, 1, 0, 0, 2, 1, 2, 3, 1, 2,
2, 1, 2, 2, 2, 1, 0, 1, 3, 2, 2, 1, 2, 2, 1,

CS-M
0% 8, 8, 7, 9, 6, 5, 4, 5, 4, 4, 7, 0, 3, 4, 5, 6,
6, 4, 6, 5, 7, 8, 6, 5, 6, 8, 8, 5, 3, 4, 6, 6,
8, 8, 7, 5, 3, 3, 5, 3, 8, 5, 10, 8, 7, 5, 7, 4,
4, 4, 4, 6, 9, 7, 8, 6, 5, 6, 6, 5, 4, 5, 7, 7,
8, 10, 9, 6, 11, 9, 7, 7, 9, 8, 7, 9, 5, 7, 6, 7,
7, 8, 5, 6, 6, 7, 7, 6, 5, 4, 2, 3, 2, 2, 2, 3,
6, 6, 7, 5, 4, 6, 4, 3, 5, 3, 2, 4, 4, 0, 0, 1,
3, 3, 3,
1% 3, 1, 4, 0, 3, 3, 1, 0, 4, 1, 2, 1, 0, 3, 1, 4,
4, 1, 3, 1, 1, 1, 0, 1, 1, 2, 3, 2, 2, 2, 3, 6,
0, 3, 1, 3, 1, 1, 2, 1, 2, 3, 2, 3, 3, 2, 2, 3,
2, 2, 2, 2, 1, 2, 3, 3, 1, 1, 2, 1, 4, 3, 2, 1,
1, 2, 3, 3, 3, 3, 3, 2, 1, 3, 0, 0, 3, 3, 3, 3,
3, 5, 3, 2, 1, 0, 0, 1, 2, 3, 2, 1, 1, 2, 2, 2,
4, 1, 3, 2, 1, 1, 0, 0, 1, 1, 2, 1, 1, 1, 1, 1,
1, 1, 1,

12FA

0% 5, 4, 4, 6, 5, 5, 4, 5, 6, 3, 5, 6, 0, 5, 3, 6,
7, 6, 4, 2, 5, 4, 4, 2, 5, 6, 5, 2, 4, 5, 6,

1% 3, 1, 2, 0, 3, 2, 2, 2, 2, 4, 2, 1, 2, 0, 1, 2,
3, 5, 2, 2, 1, 0, 1, 1, 3, 2, 1, 1, 1, 2, 1,

12FB

0% 0, 2, 2, 1, 4, 2, 4, 4, 3, 2, 1, 3, 2, 4, 4, 2,
3, 4, 1, 2, 3, 3, 3, 4, 4, 3, 6, 4, 3, 3, 4,

1% 2, 1, 2, 1, 2, 1, 1, 0, 1, 2, 2, 1, 2, 1, 1, 4,
0, 2, 1, 3, 2, 2, 0, 1, 2, 1, 2, 0, 1, 1, 1,

12CS-M

0% 1, 3, 0, 7, 5, 1, 1, 1, 2, 2,[5, 4, 5, 3, 6, 2,
1, 3, 2, 3,]3, 7, 8, 5, 5, 5, 4, 5, 4,[7, 6, 4,
5, 7, 4, 4, 2, 0, 2, 1, 1, 2,[6, 4, 2, 1, 3, 2,
1, 4, 3, 2, 2, 2, 3, 1,[4, 4, 7, 7, 4, 5, 4, 3,
4, 3, 2, 3,

1% 7, 6, 3, 5, 4, 4, 2, 1, 1, 0,]4, 4, 3, 4, 5, 7,
4, 3, 1, 1,]2, 4, 3, 3, 3, 3, 3, 2, 3,[4, 4, 2,
3, 1, 3, 1, 0, 1, 1, 1, 2, 1,[3, 4, 2, 3, 1, 3,
1, 3, 0, 1, 0, 1, 3, 1,[5, 5, 3, 5, 5, 2, 3, 1,
1, 0, 1, 1,

59CS-F-A

0% 7, 5, 5, 4, 2, 5, 5, 5, 3, 8, 8, 3, 5, 4, 4, 4,
8, 7, 4, 5, 5, 4, 6, 4, 3, 5, 4, 4, 1, 4, 5,

1% 5, 3, 3, 4, 2, 4, 2, 3, 2, 3, 2, 1, 1, 3, 2, 3,
2, 3, 3, 3, 2, 4, 3, 2, 2, 1, 0, 3, 4, 2, 3,

59CS-F-B

0% 5, 3, 3, 5, 5, 3, 2, 3, 4, 4, 3, 3, 6, 6, 5, 5,
5, 6, 3,

1% 2, 3, 2, 1, 4, 2, 3, 4, 4, 3, 3, 1, 2, 4, 0, 3,
1, 1, 1,

59CS-F-C

0% 6, 3, 2, 6, 7, 5, 6, 5, 4, 5, 7, 6, 4, 3, 5, 6,
3, 5, 5, 1, 4, 4, 4, 1, 2, 4, 1, 2, 3, 3, 4,

1% 3, 0, 2, 2, 4, 1, 1, 2, 3, 3, 2, 4, 3, 4, 6, 5,
1, 2, 0, 1, 2, 2, 1, 1, 2, 3, 2, 2, 1, 2, 3,

59CS-M

0% 2, 2, 5, 3, 5, 4, 3, 2, 4, 5, 2, 3, 4, 2,]4, 2,
3, 3, 2, 5, 2, 4, 3, 2, 2, 3, 2,[6, 3, 5, 2, 2,
2, 1, 3, 2, 3, 3, 2, 2,]2, 3, 4, 6, 3, 4, 2, 2,
1, 1, 2, 2, 1, 1, 1,

1% 4, 5, 6, 4, 3, 4, 4, 2, 3, 3, 3, 2, 1, 3,]2, 3,
3, 3, 0, 0, 2, 2, 3, 4, 2, 2, 1,]7, 4, 1, 2, 1,
1, 1, 1, 2, 1, 2, 3, 0,[8, 4, 4, 3, 1, 2, 2, 3,
3, 2, 1, 1, 2, 1, 1,

R-151C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
151C-F-A	8.388	2.145	1.277	1.074	.8680	.1097	6.565
151C-F-B	5.888	2.522	1.000	.8660	.8706	.1207	5.888
151C-F-C	2.250	1.539	.9166	1.017	.7460	.2712	2.454
151C-M-A	6.387	1.333	.6451	.7093	.9156	.0898	9.899
151C-M-B	4.777	1.301	.3333	.5000	.9277	.1092	14.33
151C-M	4.250	1.752	.3750	.7440	.9361	.1412	11.33

R-CS-F

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
CS-F-A	7.750	1.208	2.700	1.031	.7474	.0739	2.870
CS-F-B	5.300	1.417	1.700	1.525	.7755	.1298	3.117
CS-F-C	6.935	1.590	1.677	.9087	.8046	.1037	4.134
CS-M	5.530	2.213	1.895	1.187	.7296	.1767	2.917

R-12CSFEMALE

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
12FA	4.483	1.524	1.774	1.116	.7103	.1864	2.527
12FB	2.903	1.247	1.387	.8823	.6645	.2206	2.093
12CS-M	3.441	1.949	2.602	1.694	.5774	.2149	1.322

R-59CS-F

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
59CS-F-A	4.709	1.636	2.580	1.057	.6447	.1387	1.825
59CS-F-B	4.157	1.258	2.315	1.249	.6509	.1614	1.795
59CS-F-C	4.064	1.711	2.258	1.365	.6437	.1570	1.8
59CS-M	2.800	1.282	2.509	1.620	.5491	.1777	1.115

151
0% 2, 3, 4, 4, 4, 5, 6, 1, 0, 2, 3, 4, 1, 2, 4, 3,
2, 3, 2, 5, 2, 7, 3, 4, 8, 6, 2, 6, 6, 6, 5, 1,
5, 3, 3, 5, 2, 1, 3, 3, 2, 4, 2, 5, 2, 4, 3, 2,
1, 1, 2, 2, 2, 3, 2, 2, 4, 4, 5, 3, 4, 5,
0% 2, 4, 3, 2, 2, 2, 1, 4, 5, 6, 6, 7, 5, 8, 7, 7,
8, 5, 6, 2, 5, 2, 9, 8, 2, 3, 3, 3, 4, 3, 5, 2,
2, 2, 1, 1, 1, 2, 1, 0, 0, 3, 6, 0, 6, 5, 2, 3,
4, 5, 4, 3, 4, 5, 4, 4, 4, 4, 4, 4, 4, 4,

CS
0% 4, 5, 6, 3, 3, 1, 2, 1, 0, 6, 3, 7, 3, 5, 4, 1,
5, 2, 5, 7, 6, 4, 6, 6, 3, 2, 4, 6, 5, 5, 4, 5,
5, 6, 5, 5, 5, 6, 8, 6, 5, 10, 7, 6, 7, 8, 3, 3,
2, 2, 3, 2, 3, 1, 2, 3, 4, 3, 5, 4, 6, 6,
0% 4, 5, 5, 4, 4, 3, 3, 3, 2, 4, 3, 3, 5, 5, 6, 4,
5, 3, 5, 3, 4, 3, 4, 3, 3, 5, 2, 3, 2, 2, 1, 10,
9, 9, 11, 9, 10, 3, 3, 2, 4, 4, 3, 3, 3, 4, 6, 5,
5, 6, 5, 6, 5, 4, 5, 5, 5, 4, 4, 5, 3, 4,

12CS
0% 2, 6, 5, 3, 5, 3, 3, 8, 4, 3, 3, 4, 3, 5, 4, 7,
6, 5, 8, 4, 4, 6, 8, 2, 6, 6, 6, 6, 6, 7, 7, 3,
4, 6, 4, 3, 4, 2, 6, 6, 2, 3, 1, 3, 4, 6, 5, 4,
4, 0, 1, 0, 1, 2, 4, 2, 8, 2, 2, 3, 6, 7,
0% 5, 4, 6, 4, 3, 4, 7, 2, 5, 3, 6, 5, 6, 1, 6, 6,
6, 2, 3, 3, 1, 2, 2, 6, 5, 2, 3, 3, 1, 4, 3, 3,
5, 5, 1, 5, 5, 7, 3, 4, 0, 3, 4, 3, 5, 6, 3, 4,
3, 3, 6, 6, 7, 5, 2, 4, 1, 5, 5, 5, 1, 3,

59CS
0% 5, 7, 7, 7, 3, 8, 3, 6, 5, 8, 4, 4, 6, 4, 7, 11,
12, 8, 8, 6, 7, 7, 8, 7, 11, 12, 9, 10, 7, 7, 11, 4,
7, 5, 6, 4, 5, 4, 6, 6, 7, 5, 5, 8, 4, 4, 9, 9,
5, 6, 8, 11, 7, 7, 11, 8, 7, 9, 9, 7, 8, 12,
0% 5, 5, 4, 4, 7, 9, 8, 11, 9, 6, 7, 7, 8, 4, 8, 7,
6, 7, 9, 9, 6, 2, 9, 8, 9, 8, 10, 8, 9, 10, 10, 7,
7, 8, 7, 7, 5, 8, 11, 10, 10, 11, 10, 10, 8, 8, 8, 7,
8, 7, 7, 6, 7, 8, 6, 9, 10, 6, 8, 9, 10, 6,

151

1% 3, 3, 1, 1, 2, 1, 1,[3, 2, 2, 1, 0, 1, 0,[3, 1,
2, 1, 1, 5, 2, 1, 2, 1, 1, 2, 1, 0, 2,

1% 0, 1, 1, 4, 0, 1, 1,[4, 3, 2, 2, 1, 0, 2,]2, 1,
2, 3, 0, 0, 1, 1, 3, 3, 1, 1, 0, 1, 0,

CS

1% 1, 2, 3, 0, 0, 1, 0,[2, 0, 2, 1, 1, 1, 2, 1, 1,
1, 1, 0, 1,]2, 1, 2, 3, 2, 1, 2, 1, 1, 1, 2, 1,
1, 1,

1% 2, 3, 2, 1, 1, 1, 1,[1, 1, 0, 0, 0, 1, 0, 1, 3,
1, 2, 1, 0,]0, 1, 2, 2, 1, 1, 0, 1, 0, 1, 0, 1,
1, 0,

12CS

1% 3, 2, 0, 0, 2,[5, 3, 1, 1, 1,]4, 3, 2, 5, 1, 2,
0,

1% 2, 0, 2, 3, 0,[2, 1, 3, 1, 1,]3, 3, 2, 3, 1, 1,
1,

59CS

1% 1, 5, 3, 2, 1,[4, 4, 3, 2, 2, 3, 4, 2, 4, 3, 2,
1, 2, 3, 3, 2, 2, 3, 3, 3, 1,]2, 2, 3, 2, 2, 4,
1, 1, 0, 4, 1, 2, 2, 3, 1, 2, 2, 4,

1% 5, 0, 4, 3, 2,[4, 3, 1, 4, 4, 2, 7, 4, 2, 5, 1,
2, 0, 1, 1, 2, 0, 2, 0, 1, 0,]3, 3, 3, 5, 5, 5,
4, 3, 4, 1, 3, 1, 1, 1, 2, 4, 0, 0,

R-59CS-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
59CS	2.409	1.106	2.454	1.784	.5470	.2696	.9814

R-151-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
151	1.586	1.086	1.413	1.210	.5498	.3175	1.121

R-CS-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
CS	1.235	.7807	.9705	.8343	.5691	.3389	1.272

R-12CS-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
12CS	2.058	1.599	1.705	1.046	.5104	.3038	1.206

R-59CS-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
59CS	7.064	2.332	7.709	1.893	.4739	.1080	.9163

R-151-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
151	3.306	1.684	3.758	2.116	.4851	.2284	.8798

R-CS-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
CS	4.354	2.017	4.435	2.069	.4830	.1740	.9818

R-12CS-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
12CS	4.241	2.046	3.887	1.756	.5172	.2211	1.091

APPENDIX J

Mutant line F_1 data: i) avoidance testing - the raw data from the tests of F_1 flies from the mutant line crosses is given in the pages following. The first page gives the results for males from the crosses of mutant line males to Canton S females, while the second page contains the results for both sexes from crosses of Canton S males to mutant line females. (The results of mean test comparisons of males from these crosses to the maternal line males were: 59cs - $F_{(1,3.3)} = 0.7$; 12cs - $F_{(1,3.3)}=6.5$; 151cs$_{(1,3.3)}=4.0$; in each case p 0.05).

The results of anova homogeneity testing of the avoidance results are listed in the table below.

Table J.1 results of anova comparisons of runs within groups.

Line	#runs	df	F	$\bar{A}$
59csMxCSF F_1 male	3	2,81	0.01	0.74
12csMxCSF F_1 male	3	2,94	0.13	0.74
151csMxCSF F_1 male	3	2,90	0.68	0.73
all F_1 males + CS		3,331	0.15	0.74
59csFxCSM F_1 female	3	2,80	2.0	0.63
12csFxCSM F_1 female	3	2,46	0.24	0.68
151csFxCSM F_1 female	2	1,47	0.07	0.74

ii) resistance testing - raw data and knockdown curves can be found following the avoidance data.

HRH1F1
0% 7, 8, 6, 5, 5, 7, 6, 7, 7, 2, 2, 1, 2, 1, 2, 4,
7, 3, 2, 3, 2, 1, 1, 4, 5, 6, 3, 5, 4, 5, 2, 6,
4, 4, 5, 5, 3, 4, 5, 4, 5, 5, 8, 6, 7, 8, 4, 6,
5,
1% 2, 2, 2, 3, 0, 1, 1, 2, 1, 3, 2, 2, 0, 3, 1, 2,
3, 2, 0, 1, 2, 1, 0, 1, 1, 5, 4, 2, 3, 3, 1, 1,
4, 4, 1, 1, 1, 1, 2, 2, 1, 3, 3, 1, 1, 2, 0, 2,
3,

151F1
0% 4, 4, 5, 4, 2, 2, 4, 3, 4, 3, 3, 1, 1, 1, 5, 4,
2, 3, 2, 3, 2, 2, 2, 2, 2, 1, 1, 4, 3, 2, 2, 4,
2, 4, 5, 4, 3, 2, 1, 2, 3, 3, 3, 4, 4, 5, 4, 3,
5, 3, 6, 7, 4, 5, 6, 3, 1, 3, 2, 3, 3, 1, 2, 1,
3, 2, 3, 3, 3, 3, 2, 5, 3, 4, 3, 4, 4, 3, 3, 5,
1, 4, 2, 2, 3, 5, 2, 4, 3, 2, 3, 3, 2,
1% 2, 2, 1, 1, 0, 1, 2, 5, 2, 2, 1, 1, 0, 0, 2, 0,
3, 2, 0, 1, 1, 0, 0, 2, 1, 0, 0, 1, 3, 1, 0, 1,
3, 1, 1, 2, 1, 1, 3, 1, 0, 3, 0, 0, 1, 1, 3, 1,
2, 4, 4, 0, 0, 1, 1, 1, 2, 1, 1, 1, 1, 1, 3, 1,
1, 1, 1, 0, 0, 1, 0, 5, 3, 4, 0, 0, 1, 1, 0, 3,
3, 1, 0, 0, 4, 3, 3, 2, 2, 3, 1, 1, 1,

12CSF1
0% 5, 6, 5, 5, 5, 6, 4, 6, 6, 6, 4, 5, 4, 7, 3, 7,
4, 4, 5, 3, 4, 3, 5, 4, 4, 5, 5, 4, 5, 5, 3, 4,
2, 5, 4, 7, 4, 6, 3, 4, 4, 4, 4, 4, 3, 5, 4, 5,
6, 5, 3, 2, 6, 5, 3, 6, 5, 4, 4, 2, 3, 4, 4, 2,
3, 0, 1, 1, 3, 2, 2, 4, 2, 2, 6, 5, 2, 2, 0, 6,
3, 5, 3, 5, 4, 4, 4, 5, 5, 2, 3, 2, 3, 2, 4, 2,
1,
1% 2, 1, 1, 2, 2, 1, 3, 2, 2, 1, 3, 3, 2, 4, 3, 0,
1, 0, 1, 2, 3, 3, 2, 0, 1, 0, 0, 0, 3, 1, 2, 2,
1, 1, 0, 4, 3, 3, 1, 2, 0, 1, 0, 0, 1, 3, 4, 3,
3, 1, 2, 4, 1, 2, 1, 0, 1, 2, 0, 0, 1, 1, 1, 1,
1, 1, 1, 0, 1, 1, 0, 1, 0, 12, 3, 3, 1, 5, 4, 2,
2, 1, 1, 2, 0, 1, 0, 2, 1, 0, 0, 0, 1, 1, 2, 0,

59CSF1
0% 5, 5, 4, 3, 3, 4, 3, 4, 1, 4, 3, 3, 1, 1, 1, 3,
2, 1, 6, 3, 2, 1, 1, 2, 3, 2, 1, 1, 1, 4, 8, 2,
5, 5, 5, 3, 5, 4, 6, 4, 2, 4, 2, 2, 3, 3, 2, 3,
5, 2, 3, 1, 1, 2, 2, 2, 2, 1, 6, 3, 7, 3, 3, 5,
4, 4, 3, 2, 4, 4, 3, 3, 4, 6, 7, 5, 5, 4, 3, 3,
6, 6, 6, 6,
1% 3, 2, 1, 2, 1, 1, 1, 1, 1, 0, 2, 0, 1, 0, 2, 1,
2, 0, 2, 1, 2, 0, 1, 1, 1, 0, 1, 0, 0, 3, 0, 4,
2, 1, 0, 1, 2, 2, 2, 1, 1, 3, 1, 2, 1, 0, 0, 3,
1, 1, 1, 1, 0, 0, 1, 1, 0, 1, 7, 5, 2, 3, 0, 1,
1, 0, 2, 2, 3, 1, 1, 1, 1, 2, 1, 0, 1, 0, 4, 2,
2, 1, 1, 1,

59CSF1-H
0% 4, 4, 4, 5, 4, 5, 4, 6, 5, 3, 3, 4, 4, 3,
1% 5, 4, 3, 4, 5, 2, 2, 3, 2, 2, 2, 3, 2, 1,
151F1-H
0% 6, 3, 7, 7, 3, 6, 7, 4, 4, 7, 5, 4, 6, 3, 4, 3,
2, 6, 4, 3, 3, 3, 3, 3, 3,
1% 2, 1, 2, 1, 1, 0, 2, 1, 1, 1, 0, 0, 0, 0, 1, 0,
0, 0, 1, 0, 2, 0, 1, 0, 0,
12CSF1-H
0% 6, 4, 3, 3, 3, 2, 4, 3, 4, 2, 3,
1% 1, 2, 3, 2, 5, 1, 3, 2, 2, 0, 2,
151F1-F-B
0% 7, 5, 4, 4, 8, 4, 4, 4, 8, 5, 5, 5, 5, 5, 4, 6,
5, 3, 2, 3, 4, 5, 3, 4, 4,
1% 2, 1, 5, 1, 2, 2, 2, 4, 5, 2, 1, 0, 1, 1, 1, 2,
3, 3, 1, 2, 1, 3, 0, 0, 1,
151F1-F-C
0% 5, 10, 8, 6, 7, 8, 10, 7, 13, 6, 8, 7, 5, 7, 7, 4,
4, 5, 3, 4, 5, 7, 4, 3,
1% 2, 4, 1, 1, 3, 3, 4, 4, 4, 6, 4, 1, 4, 1, 2, 2,
1, 4, 0, 2, 2, 1, 0, 1,

12CSF1-F-A
0% 5, 4, 2, 4, 2, 4, 3, 1, 3, 2, 3,
1% 3, 1, 2, 3, 4, 3, 1, 3, 1, 2, 0,
12CSF1-F-B
0% 7, 7, 6, 8, 6, 5, 6, 6, 4, 3, 6, 4, 7, 3, 5, 6,
5, 4, 3, 3, 5,
1% 4, 1, 6, 5, 2, 6, 3, 4, 3, 4, 3, 5, 2, 2, 3, 3,
3, 1, 3, 3, 3,
12CSF1-F-C
0% 4, 0, 4, 3, 4, 5, 5, 3, 1, 2, 3, 3, 5, 2, 4, 1,
0,
1% 4, 2, 4, 2, 5, 1, 3, 1, 1, 6, 3, 5, 4, 1, 5, 2,
0,

59F1-F-A
0% 6, 5, 5, 7, 5, 5, 7, 8, 7, 7, 5, 5, 4, 4, 3, 4,
3, 5, 5, 4, 4, 4, 3,
1% 3, 1, 2, 4, 3, 2, 3, 2, 5, 4, 4, 2, 1, 4, 2, 1,
1, 1, 3, 2, 2, 1, 2,
59F1-F-B
0% 5, 6, 3, 6, 4, 6, 5, 7, 8, 5, 4, 3, 5, 3, 2, 5,
3, 1, 4, 5, 6, 3, 5, 1, 4, 5, 2,
1% 5, 2, 4, 1, 2, 3, 3, 2, 2, 4, 3, 2, 0, 4, 3, 5,
2, 3, 0, 1, 3, 0, 1, 2, 1, 2, 1,
59F1-F-C
0% 5, 3, 4, 6, 6, 7, 4, 5, 6, 7, 9, 7, 6, 3, 6, 9,
4, 6, 5, 5, 5, 4, 7, 5, 6, 9, 5, 2, 4,
1% 3, 2, 3, 5, 2, 5, 3, 4, 3, 4, 2, 2, 1, 2, 4, 5,
8, 4, 3, 1, 6, 3, 3, 3, 4, 1, 4, 6, 4,
59F1-F-D
0% 2, 6, 2, 4, 3, 7, 2, 4, 5, 3, 1, 3, 5, 3, 3, 0,
1, 2, 1, 3, 1, 4, 4, 2, 3, 3, 4,
1% 2, 2, 2, 3, 4, 2, 2, 1, 2, 3, 2, 0, 1, 1, 2, 2,
3, 3, 1, 3, 1, 3, 1, 2, 3, 4, 2,

R-59F1-F

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
59F1-F-A	5.000	1.414	2.391	1.196	.6878	.0935	2.090
59F1-F-B	4.296	1.727	2.259	1.403	.6601	.1998	1.901
59F1-F-C	5.517	1.744	3.448	1.616	.6189	.1408	1.6
59F1-F-D	3.000	1.617	2.111	.9740	.5620	.2050	1.421

R-151F1-F

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
151F1-F-A	3.440	1.474	2.080	1.115	.6155	.2031	1.653
151F1-F-B	4.640	1.439	1.840	1.374	.7394	.1497	2.521
151F1-F-C	6.375	2.410	2.375	1.582	.7498	.1295	2.684

R-12CSF1-F

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
12CSF1-F-A	3.000	1.183	2.090	1.221	.6046	.2146	1.434
12CSF1-F-B	5.190	1.503	3.285	1.383	.6156	.1197	1.579
12CSF1-F-C	2.882	1.653	2.882	1.798	.4928	.1922	1

R-59CSF1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
59CSF1	3.357	1.697	1.321	1.213	.7397	.1886	2.540

R-151F1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
151F1	3.043	1.284	1.387	1.242	.7258	.2057	2.193

R-12CSF1

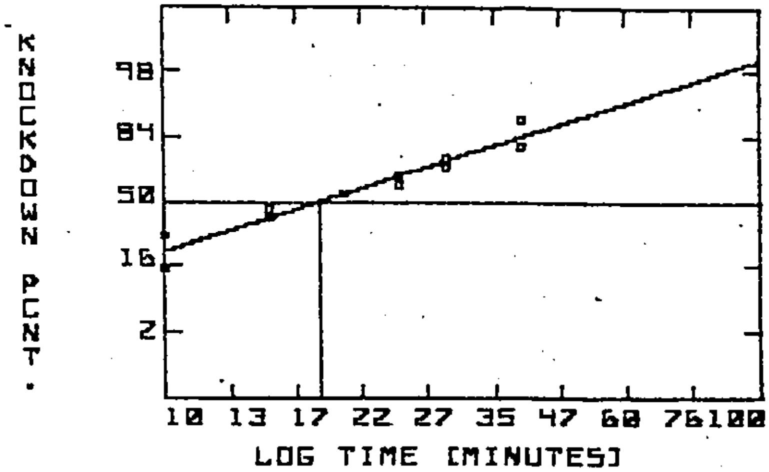
RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
12CSF1	3.968	1.475	1.572	1.614	.7443	.2063	2.523

Results of resistance assay runs - 1% malathion dose.

	# down										
Time (min):	5	10	15	20	25	30	40	50	60	70	90
Males from crosses of mutant males to CS females:											
59cs	2	6	9	11	12	14	16	18			
	.2	3	8	11	13	15	18	20			
12cs		3	9	12		16	18				
151cs		5	11	15		18	20				
F_1 flies from crosses of mutant females to CS males:											
59cs female		1		2		4	6		15	18	
12cs female		1		2		4	8		16		20
		1		3		4	7		17		20
		1		2		3	5		17		20
male		3		7		10	13		18		
		1		2		8	12		19		
151cs female		0		5		9	15	18			
		1		3		10	16	19			
male		2		8		17					

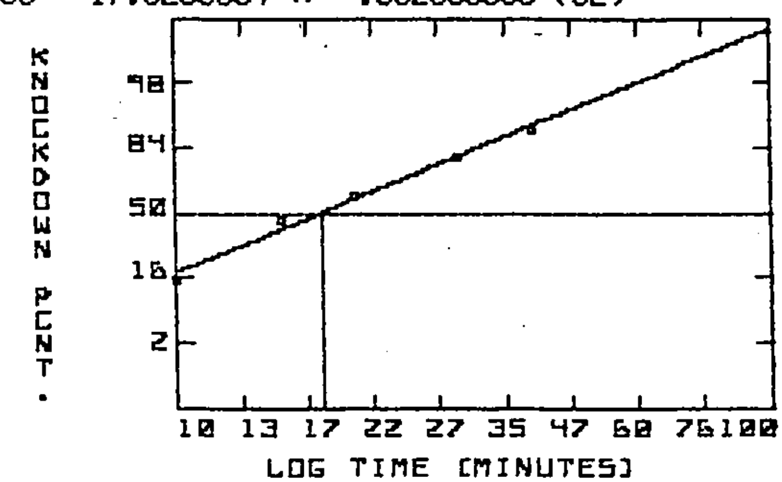
59CSMXCSF F1 MALE 1%VAC

KT-50 = 18.2516049 +/- .709514204 (SE)



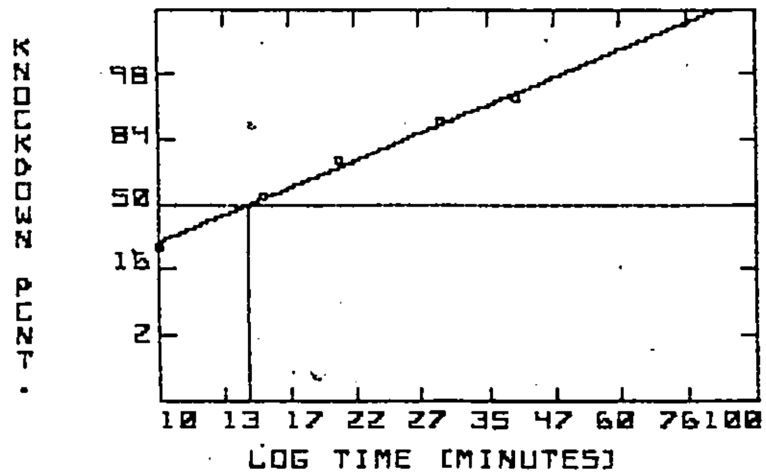
12CSMXCSF F1 MALE 1%VAC

KT-50 = 17.6299034 +/- .562963385 (SE)



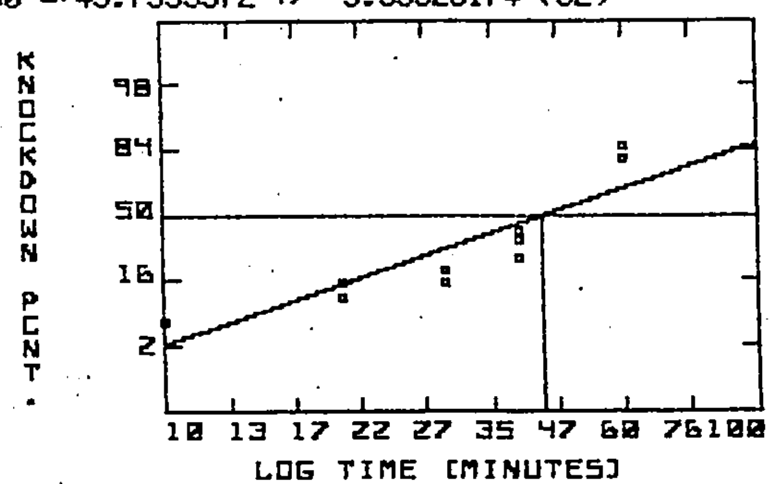
151CCMXCSF F1 MALE 1%VAC

KT-50 = 14.2239965 +/- .450905493 (SE)



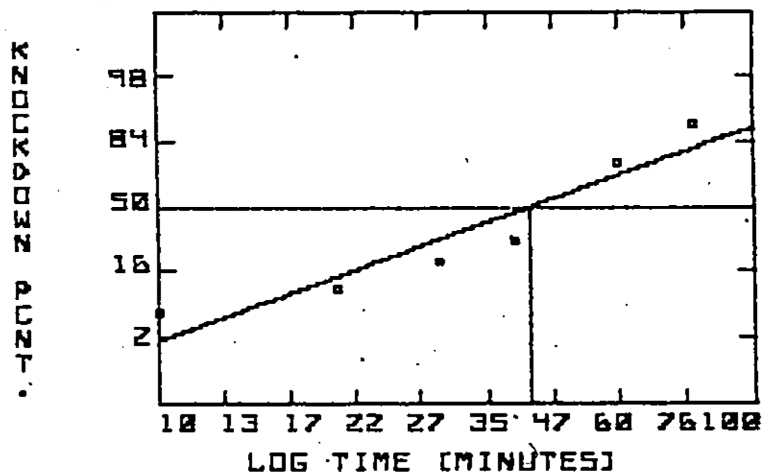
12CSFXCSM F1 FEMALE 1%VAC

KT-50 = 43.7933372 +/- 3.93323174 (SE)



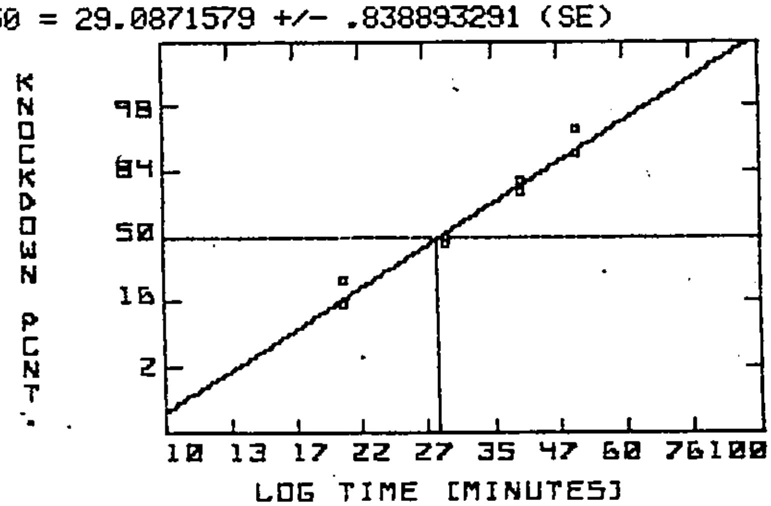
59CSFXCSM F1 FEMALE 1%VAC

KT-50 = 42.5490245 +/- 5.17165173 (SE)



151CSFXCSM F1 FEMALE 1%VAC

KT-50 = 29.0871579 +/- .838893291 (SE)



APPENDIX K

Mapping raw data: the results of resistance assay runs are listed on the following pages for the F_2 males (run numbers are the same as those used on the graphs in the results section - sample size is 20 flies except where otherwise indicated), and the attached-X lines raised from these males. The testing dose was 1% malathion except where otherwise indicated.

Results of resistance assay runs for F_2 males from the 12cs cross.

		# down										
Time (min):		10	20	30	40	50	60	75	90	120	150	180
++++	1.	2	3	11	14	18						
	2.	4	8	10	12	15						
	3.	2	6	11	13	15						
	4.	2	8	13	17	19						
	5.	3	7	9	13	17						
	6.	2	6	8	12	15						
	7.	1	4	8	12	15						
	%	12	31	48	64	78						
+++f	1.	0	4	8	10	11	13	14	18			
	2.	2	6	9	12	14	16	17	18			
	3.	3	4	7	9	11	13	13	13	15	16	18
	4.	3	6	7	9	9	9	11	12	14	15	16
	5.	1	2	3	3	5	5	7	11	16	19	
++vf	1.	4	7	9	11	13	14	15	16			
	2.	2	3	6	7	8	10	11	15			
	3.	1	4	6	11	13		14	15			
	4.	2	5	9	12	13	15	16	17			

Results from resistance assay runs for F_2 males from the 59 cs cross.

Time (min):	# down	10	20	30	40	50	60	70	80	90	110	120	150	180	#
++++	1.	3	6	10	15	16	18	19							
	2.	1	6	7	9	10	12	15							
	3.	2	3	9	12	14	15	16							
	4.	2	4	7	10	11	14	16							
	%	10	24	41	58	64	74	84							
+++f	1.	3	6	8	11	13	15	16							
	2.	2	3	4	6	8	12	13		15		18			
	3.	0	3	6	8			14		15		18			
	4.	2	5	7		10	13	15		16	17				
++vf	1.	2	5	6	9		9			9		12	15	16	18
	2.	1	3	3	6	9	9	11	14	15					
	3.	2	2	3	3	6	7		11	13		14	15	16	18
	4.	3	4	4	4	6	6		7	8		11	15	16	
	2 3 7	8	10	12	12	14	15								
	6.	2	2	4	6		8	11		12		15	19		

Results of resistance assay runs for F_2 males from the 151cs cross - the top set is for a dose of 0.5% malathion, the lower set for 1%.

Time: (min)		10	20	30	40	50	60	75	90	105	150	180	210	250	Sample Size
		# down													
++++	1.	3	6	11	16	18									
	2.	3	6	13	16	19									
	3.	2	9	14	16	19									
	4.	2	11	17	18	20									
	5.	2	6	13	17	19									
	%	12	38	68	83	95									
+++f	1.	1	3	4	4	4	4	5		8		17		18	20
	2.	1	1	1	2	4	5	6		7		13		15	19
	3.	1	3	4	4	5	5		8		11		15		20
	4.	2	4	5	6	7	8		11	13	13		14		20
++vf	1.	2	3	4	7		8		10		11		12	16	23
	2.	2	3	4	·	6		7	9	10	13		15	18	22
	3.	1		2	3	4	6		8		12	13	14	15	22

Time:		10	15	20	25	30	35	40	50	60	75	90	105	120	
++++	1.	4	7	17	18										
	2.	5	10	18	19										
	%	23	43	87	93										
+++f	1.	1		2	3	3	3	5	9	13	16	18			18
	2.	0	2	3	6	6	6	7	9	10	12	14	15		17
	3.	3	3	3	5	7	8	10	13	16		18			
++vf	1.	3		5	5	6	7	8		11	14	18	21		24
	2.			1	2	3	4	6	7	8	9	10	13	16	

Results from resistance assay runs for attached-X line males from the 151cs cross.

								# down							
Time: (min)	10	20	30	40	50	60	75		10	20	30	40	50	60	75
+++f lines:															
A	2	3	5	6	11	15	18	D	0	13	18				
	0	4	6	10	12	14	19	J	5	7	13	18			
B	2	8	9	10	11	13	15		5	6	13	19			
	4	7	7	9	10	12	15	M	2	13	16	19			
C	1	2	6	8	9	12		S	2	10	16	18			
E	1	6	11	12	13	14	16		4	12	18	20			
F	1	6	9	13	16	18	19		2	11	13	17			
I	5	8	11	14	16	17			5	15	19				
	3	6	11	14	19	19									
	0	3	8	14	17	18		F	2	5	9	13	15	18	
L	0	2	6	11	17	18			2	6	9	11	13	18	
N	1	4	6	8	14	17			1	4	10	13	16	17	
O	1	3	5	9	10	13	16		1	5	9	13	16	17	
P	1	6	8	11	17	19									
Q	1	2	7	9	12	15	17	S	4	12	17	20			
T	1	4	9	11	14	16	18		4	14	18	20			
H	3	5	10	11	16	19			3	10	15	18			
ycvv+ lines:															
A	3	6	14	18				F	3	11	19				
D	5	8	14	16					1	16	19				
	2	5	11	16				G	3	18	20				
J	5	10	13	18				H	3	12	19				
	3	6	12	18				N	2	13	20				
M	3	6	17	20				R	5	13	17				
O	2	9	14	17					4	15	19				
P	4	7	13	17				B	4	11	17				
	1	5	16	19											
Q	3	8	11	16											

Results from resistance assay runs for males of attached-X lines from the 12cs cross.

Time: (min)	15	30	45		# down 15	30	45	60
+++f males:								
1	2	16	18	7	3	7	13	14
5	3	14	18		3	7	10	16
	3	16	19		3	7	13	16
6	0	13	15	10	5	9	15	18
	2	13	18		2	7	11	15
11	3	12	18		2	11	13	15
	4	16	19	22	5	9	13	19
15	9	17	19		1	6	14	16
	8	18	20	29	5	7	14	15
16	9	17	19	30	0	6	13	17
17	5	18	20		0	5	13	15
	5	15	18	33	1	5	11	13
18	7	17	19	34	4	8	12	16
	6	18	20	41	2	5	11	15
19	11	17	20		2	4	9	14
	10	18	20	2	5	11	15	18
21	7	15	19		6	13	16	20
	8	19	20					
23	5	15	18	10	4	9	14	19
24	5	18	20		3	9	15	20
	3	17	20		5	8	12	18
25	7	17	20					
	10	19	20	5	8	15	19	
26	7	17	20		6	15	19	
	7	18	20		10	17	20	
32	5	17	20					
36	5	13	18					
	5	12	18	++vf males:				
37	5	16	18					
39	8	15	17		6	9	12	17
8	1	15	17		3	12	16	19
	0	13	17		1	11	14	18
4	4	15	19		2	12	16	19

Results from the resistance assay runs for males from the attached-X lines from the 59cs cross (++vf).

Time: (min)	15	30	45	60	# down	15	30	45	60	75
1	3	10	17	19	6	2	6	10	12	15
5	1	9	14	18	7	0	4	8	11	17
8	9	16	18	19		1	5	9	12	16
10	2	10	17	18	11	2	6	10	13	17
13	0	11	15	17	15	1	3	9	11	16
14	4	10	16	18		1	6	10	12	14
16	5	14	18	20	17	0	3	5	9	13
21	5	13	17	18	18	2	5	9	10	14
38	4	12	17	19	19	2	5	9	14	16
40	5	14	17	19		3	6	10	11	15
42	3	9	15	18	22	0	8	11	14	15
27	3	8	15	18		3	9	10	11	13
32	1	8	12	17	23	1	5	7	10	13
24	3	8	12	16	30	3	7	8	10	13
28	4	9	13	16	31	3	9	11	12	15
12	2	9	13	16	32	0	4	6	9	13
					36	2	4	6	11	13
40	5	12	17	20	39	3	6	9	12	14
	2	8	12	18	37	1	4	8	14	16
	6	13	16	19		1	3	4	12	16
						0	3	6	11	14

APPENDIX L

Other mutant lines: i) avoidance testing - the raw data from testing of lines MRM-1, cyloc and 127 are listed on the pages following, along with the parameter table and the index distribution histograms, and the control run results for line 127. The results of homogeneity testing and comparisons to Canton S (via anova) are given in the table below.

ii) resistance testing - the raw data and knock-down curves for the three lines can be found on the pages following the avoidance results.

Table L.1 results of comparisons.

Compared	# runs	df	F	p
Homogeneity:				
cyloc female	4	3,38	3.4	>.025
cyloc male	4	3,63	2.1	>.05
MRM-1 female	4	3,107	7.9	<.001
MRM-1 male	6	5,153	15.2	<.001
127 male	6	5,148	10.0	<.001
Pooled group result comparisons:				
Male vs. male:				
MRM-1, CS		1,179	20	<.001
cyloc, CS		1,180	12	<.001
127, CS		1,267	3.3	>.05
Female vs. female:				
MRM-1, CS		1,166	5.2	<.025
cyloc, CS		1,111	81	<.001

MRM1FA
0% 6, 5, 5, 8, 9, 8, 12, 6, 4, 7, 3, 4, 4, 2, 1, 2,
2, 0,
1% 2, 2, 2, 4, 1, 0, 0, 3, 5, 1, 1, 0, 3, 1, 0, 1,
0, 1,
MRM1FB
0% 7, 6, 7, 6, 9, 6, 3, 4, 4, 5, 4, 5, 6, 7, 3, 2,
6, 2, 1, 4, 5, 3, 4, 5, 6, 5, 3, 5, 4, 3, 4,
1% 1, 2, 2, 1, 0, 0, 1, 5, 4, 3, 1, 0, 0, 3, 2, 0,
4, 1, 1, 4, 3, 1, 1, 4, 3, 2, 1, 0, 1, 0, 1,
MRM1FC
0% 8, 6, 9, 8, 9, 7, 7, 7, 8, 8, 6, 6, 3, 4, 4, 8,
8, 5, 6, 5, 6, 7, 7, 9, 7, 8, 7, 8, 8, 7, 6,
1% 3, 1, 2, 1, 1, 1, 1, 1, 1, 0, 2, 1, 1, 1, 1, 1,
1, 1, 3, 1, 3, 0, 0, 1, 0, 1, 1, 1, 0, 0, 1,
MRM1FD
0% 7, 8, 8, 7, 6, 8, 9, 6, 5, 7, 8, 7, 4, 6, 4, 7,
7, 6, 8, 9, 7, 7, 5, 6, 8, 7, 6, 7, 4, 6, 6,
1% 1, 1, 1, 0, 1, 1, 1, 0, 0, 0, 1, 1, 0, 1, 1, 0,
0, 0, 4, 0, 2, 1, 0, 2, 0, 0, 1, 1, 1, 0, 1,
MRM1MA
0% 7, 8, 9, 8, 7, 7, 6, 9, 8, 7, 5, 5, 5, 4, 1, 3,
1, 4, 3,
1% 1, 0, 3, 3, 3, 2, 0, 1, 2, 3, 2, 1, 1, 1, 1, 1,
2, 0, 2,
MRM1MB
0% 2, 4, 6, 3, 1, 2, 5, 2, 3, 4, 4, 4, 3, 3, 2, 1,

1% 2, 5, 3, 1, 0, 0, 2, 2, 0, 2, 2, 2, 3, 1, 2, 1,

MRM1MC
0% 9, 8, 8, 9, 10, 8, 5, 5, 5, 5, 6, 7, 10, 2, 5, 4,
4, 4, 5, 5, 4, 3, 5, 6, 7, 8, 9, 9, 6, 5, 5,
1% 1, 1, 1, 1, 1, 0, 1, 2, 1, 0, 0, 1, 0, 1, 1, 1,
1, 1, 5, 1, 1, 1, 1, 1, 2, 2, 1, 0, 2, 2, 1,
MRM1MD
0% 6, 7, 8, 8, 4, 4, 5, 6, 8, 8, 8, 8, 8, 9, 9, 10,
11, 10, 11, 10, 11, 13, 14, 12, 12, 12, 11, 12, 11, 12, 12,
1% 1, 0, 0, 1, 2, 1, 0, 1, 0, 0, 0, 0, 1, 2, 0, 1,
1, 1, 1, 1, 0, 0, 1, 0, 0, 0, 0, 0, 1, 1, 1,
MRM1ME
0% 7, 5, 5, 2, 4, 3, 7, 6, 8, 9, 9, 7, 7, 7, 5, 5,
5, 8, 7, 9, 11, 11, 9, 11, 10, 9, 11, 12, 10, 9, 12,
1% 0, 2, 1, 0, 2, 0, 0, 1, 1, 0, 0, 0, 1, 0, 0, 0,
0, 2, 1, 0, 0, 1, 0, 0, 1, 0, 0, 1, 1, 1, 1,
MRM1MF
0% 6, 4, 6, 9, 8, 6, 5, 4, 7, 7, 7, 6, 4, 4, 3, 2,
3, 3, 7, 8, 5, 6, 6, 5, 6, 6, 5, 5, 7, 5, 7,
1% 1, 0, 1, 1, 0, 1, 1, 1, 1, 1, 1, 0, 2, 1, 0, 1,
1, 0, 2, 0, 2, 1, 1, 0, 1, 0, 0, 2, 2, 1, 1,

```
CYLOC-F
  0%  11, 8, 10, 9, 7, 7, 5, 8,
  1%  1, 1, 1, 2, 2, 0, 1, 0,
CYLOC-F-B
  0%  11, 14, 11, 10, 8, 9, 8, 7, 6, 5, 12, 8, 8, 6, 7, 5,
      6,
  1%  0, 1, 0, 1, 1, 0, 0, 0, 1, 2, 0, 0, 1, 0, 1, 0,
      1,
CYLOC-F-C
  0%  14, 15, 16, 16, 12, 14, 15, 14,
  1%  0, 0, 0, 0, 0, 1, 0, 1,
CYLOC-F-D
  0%  18, 15, 16, 17, 15, 13, 14, 16, 14,
  1%  0, 0, 0, 1, 1, 0, 0, 0, 1,
CYLOC-H-A
  0%  7, 7, 4, 6, 5, 5, 4, 5, 9, 6, 5, 3, 1, 2, 4, 3,
      2, 3, 4, 3, 3, 1, 5, 4, 3, 2, 4, 2, 2, 1,
  1%  2, 3, 1, 1, 0, 3, 2, 1, 2, 0, 1, 0, 0, 0, 1, 1,
      1, 0, 2, 2, 0, 0, 1, 1, 2, 0, 3, 2, 3, 1,
CYLOC-H-B
  0%  7, 8, 7, 6, 2, 3, 1, 5, 4, 5, 3, 5, 7, 5, 4, 2,
      3, 4,
  1%  1, 1, 2, 0, 0, 0, 2, 1, 1, 2, 2, 1, 2, 0, 0, 2,
      0, 1,
CYLOC-H-C
  0%  4, 5, 7, 4, 4, 2, 2, 4, 3, 3, 3, 2,
  1%  0, 2, 1, 2, 1, 0, 2, 0, 1, 1, 0, 0,
CYLOC-H-D
  0%  11, 7, 8, 9, 9, 10, 10,
  1%  2, 0, 0, 0, 0, 0, 1,
```

127M1
0% 5, 8, 7, 9, 7, 9, 11, 9, 10, 11, 5, 10, 11, 14, 10, 12,
10, 12, 11, 12, 9, 11, 10, 11, 8, 11,
1% 3, 2, 7, 6, 7, 5, 5, 4, 4, 7, 8, 7, 5, 7, 8, 5,
9, 8, 3, 3, 5, 2, 3, 4, 4, 6,
127M2
0% 12, 10, 11, 11, 11, 10, 14, 11, 10, 6, 9, 11, 9, 10, 14, 14,
11, 12, 7, 6, 5, 6, 8, 6, 5,
1% 0, 2, 3, 3, 5, 5, 4, 6, 5, 9, 9, 5, 4, 6, 2, 2,
5, 6, 6, 5, 4, 2, 5, 4, 3,
127M3
0% 12, 14, 15, 13, 12, 12, 15, 13, 13, 13, 14, 13, 12, 14, 14, 1
2,
10, 11, 10, 9, 11, 10, 12, 11, 10, 9,
1% 4, 5, 6, 8, 4, 2, 4, 6, 4, 1, 3, 2, 2, 4, 4, 3,
3, 3, 2, 4, 5, 6, 5, 5, 3, 4,
127M4
0% 6, 8, 5, 7, 8, 6, 7, 8, 7, 9, 10, 12, 12, 8, 6, 4,
3, 2, 4, 3, 3, 5, 7, 8, 6, 4, 4, 4, 5,
1% 7, 6, 4, 5, 4, 5, 2, 2, 2, 2, 1, 2, 3, 2, 5, 4,
6, 9, 8, 8, 7, 5, 5, 5, 5, 4, 4, 2, 1,
127M5
0% 6, 6, 7, 7, 6, 5, 5, 7, 5, 4, 5, 8, 6, 7, 4, 5,
3, 4, 4, 3, 3, 4, 5, 6, 7, 7, 7, 7, 8, 6, 6,
1% 2, 0, 0, 1, 1, 4, 3, 4, 2, 2, 1, 2, 2, 1, 1, 1,
1, 1, 0, 0, 0, 2, 3, 1, 1, 1, 1, 1, 2, 0, 1,
127M6
0% 4, 5, 5, 0, 1, 3, 2, 1, 3, 5, 3, 3, 3, 1, 4, 2,
1,
1% 1, 3, 3, 5, 5, 3, 2, 2, 2, 2, 2, 3, 0, 1, 1, 1,
0,

R-CYLOC

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
CYLOC-F	8.125	1.885	1.000	.7559	.8929	.0812	8.124
CYLOC-F-B	8.294	2.568	.5294	.6242	.9366	.0819	15.66
CYLOC-F-C	14.5	1.309	.2500	.4629	.9833	.0308	57.99
CYLOC-F-D	15.33	1.581	.3333	.5000	.9794	.0309	45.99
CYLOC-M-A	3.833	1.931	1.200	1.030	.7866	.1791	3.194
CYLOC-M-B	4.500	1.977	1.000	.8401	.8185	.1887	4.499
CYLOC-M-C	3.583	1.443	.8333	.8348	.8379	.1674	4.299
CYLOC-M-D	9.142	1.345	.4285	.7867	.9650	.0624	21.33
MRM1FA	4.888	3.103	1.500	1.465	.7436	.2506	3.259
MRM1FB	4.645	1.742	1.677	1.469	.7550	.1719	2.769
MRM1FC	6.838	1.507	1.064	.8138	.8661	.0917	6.424
MRM1FD	6.645	1.330	.7419	.8550	.9089	.0912	8.956
MRM1MA	5.631	2.476	1.526	1.020	.7681	.1674	3.689
MRM1MB	3.062	1.388	1.750	1.290	.6765	.1890	1.75
MRM1MC	6.161	2.130	1.129	.9216	.8384	.1088	5.457
MRM1MD	9.354	2.640	.5806	.6204	.9365	.0789	16.11
MRM1ME	7.741	2.657	.5483	.6752	.9352	.0893	14.11
MRM1MF	5.548	1.650	.8709	.6704	.8669	.1047	6.370
127M1	9.730	2.127	5.269	2.011	.6530	.1069	1.846
127M2	9.560	2.785	4.400	2.081	.6835	.1325	2.172
127M3	12.07	1.741	3.923	1.572	.7594	.0751	3.078
127M4	6.241	2.572	4.310	2.189	.5908	.1926	1.448
127M5	5.580	1.455	1.354	1.081	.8202	.1253	4.119
127M6	2.705	1.571	2.117	1.452	.5841	.2590	1.277

R-MRM1-1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
MRM1	3.590	2.554	3.568	2.214	.4603	.2323	1.006
127	6.000	2.749	6.547	1.876	.4788	.0837	.9163

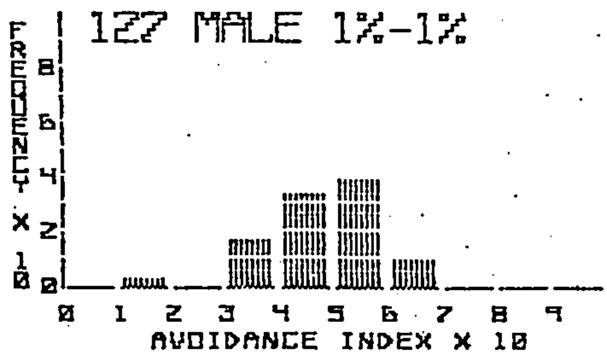
R-MRM1-C

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
MRM1	2.225	1.086	2.419	1.148	.4551	.1985	.92
127	8.016	3.138	14.41	2.761	.3497	.0895	.5559

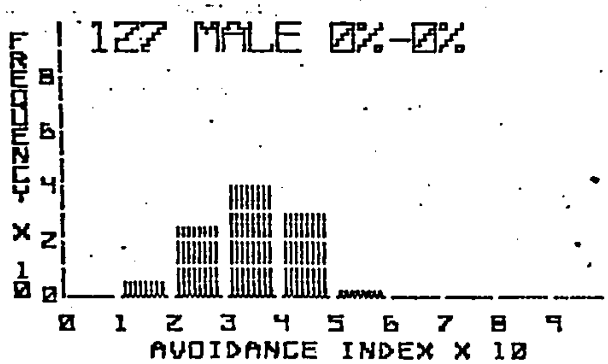
R-MRM1F1

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
MRM1F1	4.469	2.011	1.816	1.166	.7129	.1779	2.460
127F1	3.607	2.065	1.785	1.606	.6901	.2311	2.02

127
1% 4, 5, 5, 6, 7, 9, 7, 3, 6, 4, 2, 11, 12, 10, 13, 10,
9, 6, 4, 6, 5, 6, 7, 7, 8, 7, 10, 7, 6, 4, 5, 4,
4, 4, 6, 5, 4, 4, 3, 4, 3,
1% 5, 6, 8, 9, 6, 7, 7, 5, 4, 3, 2, 7, 8, 7, 7, 9,
10, 6, 8, 8, 7, 7, 8, 8, 8, 8, 9, 6, 8, 9, 8, 6,
6, 8, 6, 6, 4, 4, 5, 4, 3, 5,



127
0% 4, 4, 8, 2, 6, 7, 10, 7, 7, 10, 11, 12, 13, 10, 8, 9,
12, 15, 13, 18, 11, 6, 3, 8, 8, 9, 9, 10, 13, 11, 10, 5,
5, 6, 4, 5, 5, 9, 8, 9, 8, 7, 9, 10, 11, 8, 9, 12,
7, 9, 5, 5, 7, 7, 5, 5, 3, 5, 3, 7, 7, 8,
0% 13, 10, 15, 17, 13, 15, 16, 16, 14, 13, 14, 15, 15, 14, 14, 14,
18, 18, 15, 14, 12, 8, 11, 12, 13, 15, 16, 17, 18, 13, 12, 8,
8, 11, 12, 13, 15, 13, 16, 18, 19, 19, 15, 16, 16, 16, 15, 13,
15, 14, 15, 18, 9, 11, 12, 18, 18, 14, 14, 18, 20, 15,

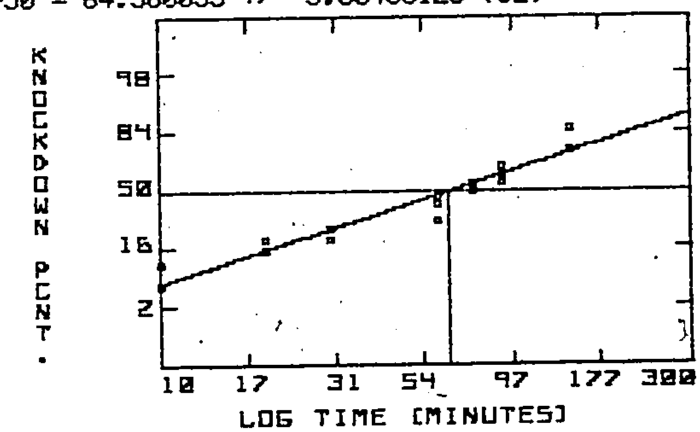


Results from resistance assay runs for lines and doses indicated.

Line	Time: 10 (min.)	15	20	25	30	35	40	50	60	70	80	90	100	110	170
	#down														
MRM-1 male															
2%	2	9	16	19											
	3	10	15	18											
1%	3		4		6		10		13			18			
	4		6		10		12		15			16			
	3		7		7		10		12			15			
	2		5		9		14		16			18			
0.7%	1		4		5				9		11	11		15	
	1		3		4				6		10	12		17	
	2		4		4				8		11	13		17	
cyloc male															
1%	6		18		20										
	3		13		18										
	3		13		18										
	5		11		18										
	5		11		17										
0.5%		4			10			11	13			15	17	19	
		2			8			11	13			14	16	17	
0.2%		1						4					7		16
		1						2					8		18
		1						4					7		16
		1						3					10		19

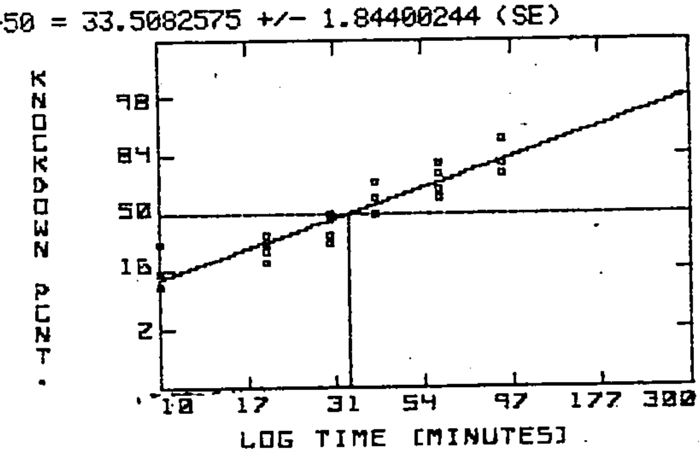
MRM-1 MALE 0.7% VAC

KT-50 = 64.560099 +/- 3.53490125 (SE)



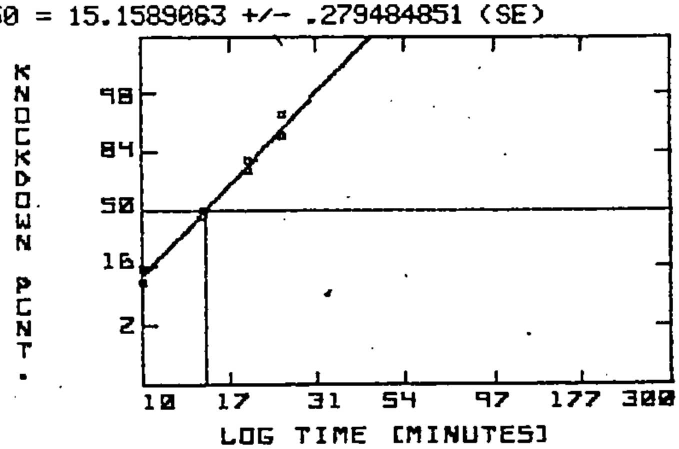
MRM-1 MALE 1% VAC

KT-50 = 33.5082575 +/- 1.84400244 (SE)



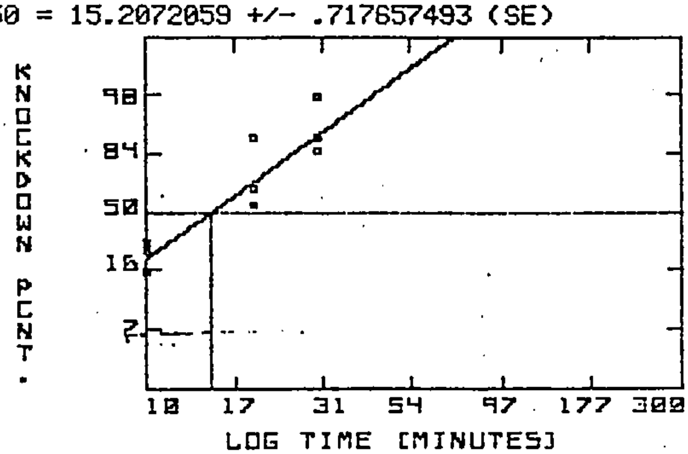
MRM-1 MALE 2% VAC

KT-50 = 15.1589063 +/- .279484851 (SE)



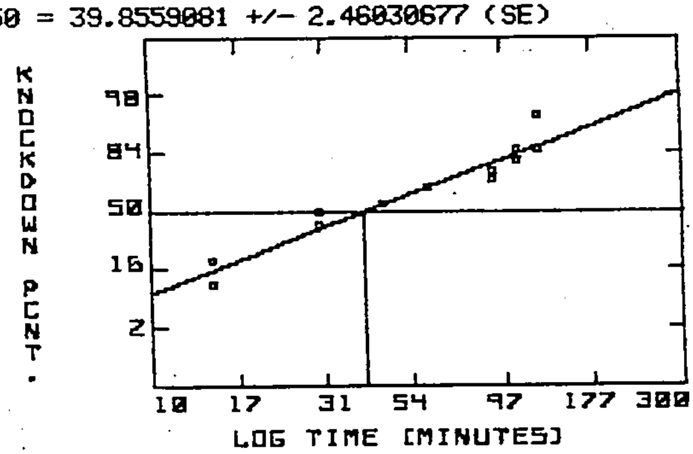
CYLOC MALE 1%VAC

KT-50 = 15.2072059 +/- .717657493 (SE)



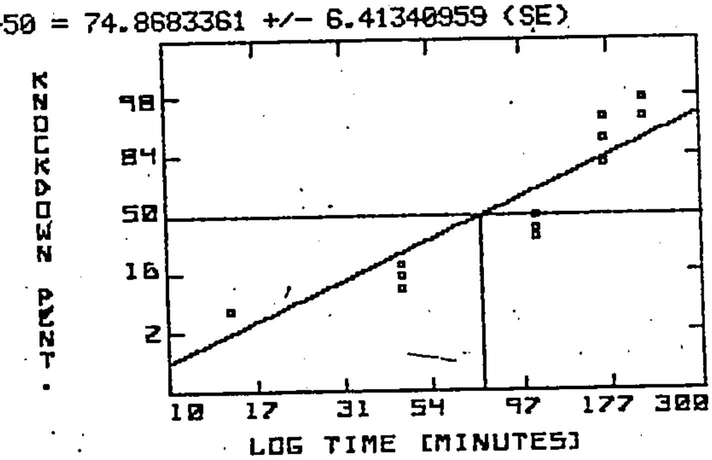
CYLOC MALE 0.5% VAC

KT-50 = 39.8559081 +/- 2.46030677 (SE)



CYLOC MALE 0.2%VAC

KT-50 = 74.8683361 +/- 6.41340959 (SE)

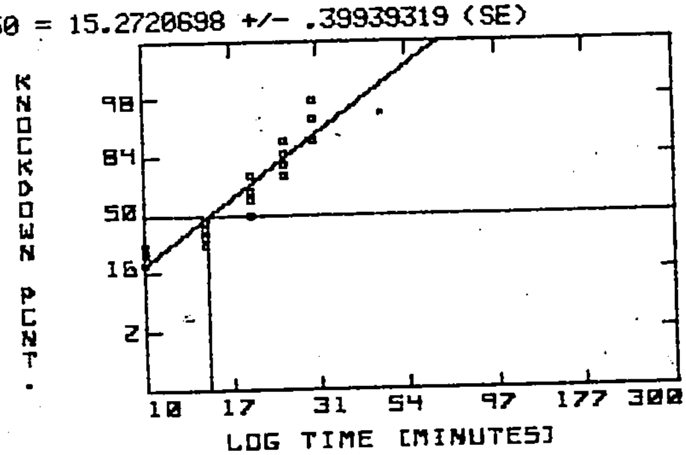


Results of resistance assay runs for 127 line males at doses indicated.

Line	#down Time: 10 (min.)	15	20	25	30	35	40	50	60	70	80	90	100	110	170
1%	4	9	15	16	18										
	6	9	15	17	19										
	4	9	13	18	20										
	6	7	13	17	19										
	5	6	10	15	18										
	6	8	12	18	19										
0.7%	3		8		12		13	16	17	18					
	3		9		10		11	13	15	17					
	2		6		8		11	14	16	18					
0.5%		1			6		10		12			17	18		
		2			3		6		10			13	15		
		2			5		9		13			16	19		
		2			6		10		13			15	17		
		2			6		9		10			13	15		
0.2%					2				11			14			17
					4				8			11			16
					3				7			10			13
					4				7			11			18

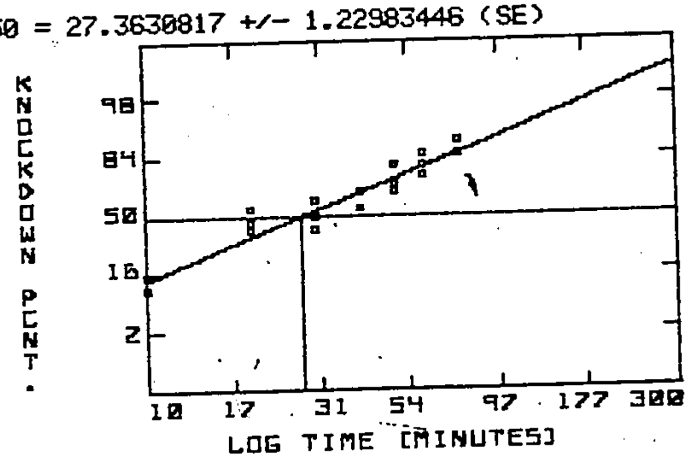
127 MALE 1%VAC

KT-50 = 15.2720698 +/- .39939319 (SE)



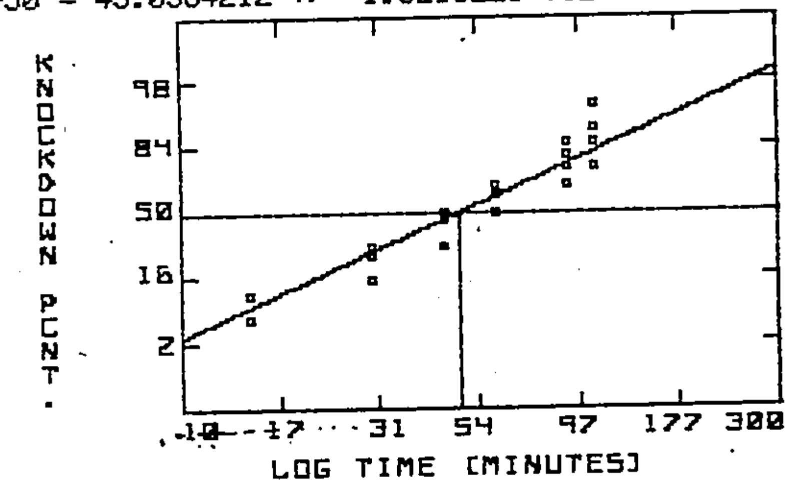
127 MALE 0.7% VAC

KT-50 = 27.3630817 +/- 1.22983446 (SE)



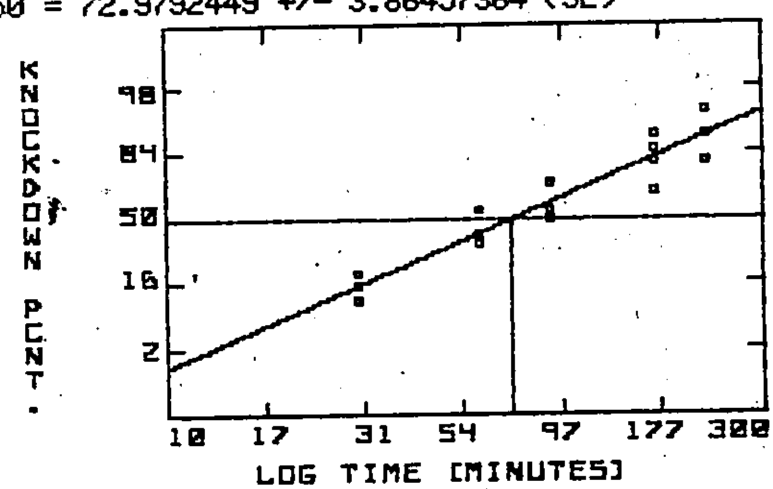
127 MALE 0.5% VAC

KT-50 = 49.0584212 +/- 1.8216228 (SE)



127 MALE 0.2% VAC

KT-50 = 72.9792449 +/- 3.86457364 (SE)



APPENDIX M

Activity test results: the data from counter-current apparatus runs are given below. Flies aged 4 to 6 days (all male) were run in groups of 60, starved 2.5-3hours (to light runs) and 3-3.5hours (away from light runs) at a temperature of 23°C in a darkened room at 30% relative humidity. The method used is described in the text, and the top of the tubes was kept 15cs from the light source, the intervals were 30 seconds long, and the tubes were tapped 5 times at each shift.

	No. flies in Tubes					
Line	1	2	3	4	5	6
TO LIGHT:						
Canton S	3	5	7	17	16	12
	9	4	12	14	13	8
127	12	3	7	11	12	15
	27	4	9	6	4	10
12cs	2	5	19	13	15	6
	5	5	6	12	13	19
	0	1	10	15	19	15
151cs	3	1	12	20	11	13
	5	2	5	11	22	15
59cs	1	1	3	5	16	34
	1	2	2	4	20	31
MRM-1	5	1	15	15	16	8
	8	0	11	9	19	13
AWAY FROM LIGHT:						
Canton S	24	13	9	10	4	0
127	59	0	1	0	0	0
12cs	29	15	8	7	1	0
151cs	48	6	5	1	0	0
59cs	29	11	8	8	4	0
MRM-1	32	17	8	3	0	0

APPENDIX N

Avoidance data for varied concentrations of malathion: the raw data and parameter table are on the page following for the runs whose avoidance index distributions are given in the text. The results of homogeneity testing are: for CS females run at 0.1% - $F_{1,60)}=0.71$;151cs females at 0.1% - $F_{(1,60)}=1.13$ (both compared via anova, both $p>0.05$); 12cs females at 0.1% - $F_{(1,1.3)}=0.3$ (means test, $p>0.05$).

A comparison of all three lines tested at 0.1% via the means test gave an $F_{(2,5.9)}$ value of 4.8, which is not significant.

RUN	M 0%	S.D.	M 1%	S.D.	M A	S.D.	M0/M1
CS-F-.1-A	5.466	1.756	3.833	2.069	.5981	.1688	1.426
CS-F-.1-B	5.258	2.393	3.290	1.883	.6135	.1967	1.598
CS-F-.5-B	9.258	2.323	3.903	1.813	.7059	.1265	2.371
CS-F-1	7.838	2.544	.4193	.5641	.9463	.0801	18.69
12-F-0.1A	5.096	2.992	4.032	1.940	.5435	.2063	1.264
12-F-0.1B	1.448	1.120	1.655	1.494	.5036	.3394	.875
151-F-0.1A	3.933	1.779	2.133	1.382	.6477	.1888	1.843
151-F-0.1B	3.566	1.832	2.633	1.711	.5701	.2336	1.354

CS-F-.1-A
0% 3, 9, 6, 3, 6, 6, 5, 4, 4, 5, 5, 4, 5, 5, 9, 6,
7, 6, 5, 8, 6, 6, 3, 6, 5, 3, 3, 5, 9, 7,
1% 4, 2, 7, 2, 6, 7, 7, 6, 5, 3, 4, 3, 6, 2, 0, 2,
3, 3, 3, 2, 5, 1, 2, 2, 7, 6, 6, 2, 2, 5,

CS-F-.1-B
0% 3, 5, 8, 1, 2, 5, 6, 5, 5, 3, 6, 2, 3, 4, 6, 4,
6, 7, 4, 11, 8, 10, 9, 8, 4, 5, 6, 5, 2, 4, 6,
1% 3, 3, 0, 3, 4, 4, 1, 5, 1, 3, 5, 3, 2, 1, 3, 2,
5, 5, 6, 1, 4, 2, 7, 3, 0, 4, 2, 3, 7, 4, 6,

CS-F-.5
0% 8, 4, 7, 7, 8, 7, 11, 9, 8, 11, 5, 9, 6, 11, 12, 7,
8, 10, 10, 9, 13, 11, 11, 13, 10, 9, 14, 10, 11, 9, 9,
1% 2, 5, 5, 5, 4, 4, 4, 6, 3, 1, 6, 7, 1, 3, 2, 3,
4, 5, 8, 5, 3, 5, 5, 3, 1, 4, 3, 2, 5, 6, 1,

CS-F-1
0% 7, 2, 4, 8, 8, 9, 8, 9, 10, 9, 7, 7, 7, 9, 5, 6,
3, 3, 10, 11, 9, 6, 6, 8, 9, 11, 12, 9, 9, 11, 11,
1% 0, 1, 1, 0, 0, 1, 1, 1, 1, 1, 1, 1, 1, 0, 0, 0,
0, 0, 0, 2, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0,

12-F-0.1A
0% 4, 2, 4, 1, 5, 3, 6, 3, 3, 4, 2, 2, 1, 1, 1, 5,
4, 8, 9, 7, 7, 9, 9, 7, 8, 9, 10, 10, 8, 3, 3,
1% 6, 2, 3, 0, 8, 2, 3, 2, 5, 4, 3, 5, 4, 6, 5, 3,
3, 4, 6, 4, 5, 2, 4, 5, 5, 2, 2, 2, 9, 6, 5,

12-F-0.1B
0% 0, 5, 2, 1, 2, 1, 2, 0, 2, 1, 1, 0, 0, 1, 1, 1,
1, 1, 2, 1, 1, 1, 2, 2, 1, 2, 1, 4, 3,
1% 2, 0, 0, 1, 3, 1, 0, 3, 1, 1, 1, 1, 1, 2, 2, 0,
5, 0, 0, 1, 1, 5, 0, 2, 2, 4, 4, 3, 2,

151-F-0.1A
0% 4, 6, 2, 2, 1, 2, 2, 3, 7, 7, 7, 3, 2, 3, 4, 5,
6, 5, 5, 2, 2, 5, 5, 5, 4, 5, 2, 2, 5, 5,
1% 3, 3, 4, 1, 2, 4, 1, 0, 1, 1, 1, 1, 1, 2, 3, 2,
1, 1, 0, 2, 1, 3, 2, 2, 4, 2, 3, 3, 6, 4,

151-F-0.1B
0% 4, 7, 5, 0, 7, 5, 1, 1, 2, 1, 3, 2, 4, 6, 5, 2,
3, 2, 4, 5, 5, 3, 2, 4, 6, 3, 2, 4, 5, 4,
1% 6, 7, 4, 3, 4, 4, 2, 5, 5, 3, 4, 4, 3, 1, 0, 2,
2, 1, 2, 3, 1, 1, 3, 1, 1, 2, 2, 1, 1, 1,

APPENDIX O

Media - the recipe for the medium used for maintaining cultures is listed below.

Carpenter's Medium

Solution A

900ml H_2O
15g agar
100g sucrose
50g brewer's yeast
1g potassium phosphate

this solution was autoclaved and added to solution B.

Solution B

200ml H_2O
8g potassium sodium tartrate
0.5g calcium chloride
0.5g sodium chloride
0.5g manganous chloride
0.5g ferrous chloride

once the mixture had cooled to 40°C, 5.5 ml of propionic acid (anti-fungal agent) was added and the medium was poured.

Medium for feeding resistance assay - the medium consisted of 1.5 g sucrose + 1g agar in 100 ml H_2O, to which malathion was added once the solution had cooled to 40°C. Resistance was assayed by putting groups of 50 flies into vials containing this medium and recording knockdown vs. time.

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