STUDIES ON THE SUSCEPTIBILITY OF *ANOPHELES* TO INFECTION AND INSECTICIDES

by

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ABSTRACT

The feasibility of reversing or delaying the evolution of insecticide resistance in mosquitoes by releasing susceptible males into a resistant population was demonstrated in Anopheles gambiae sensu stricto under laboratory cage conditions. Such releases produced a great reduction in the DDT resistance level of an initially resistant population. A control without releases showed a much slower decline in resistance. When DDT selection was applied to the females of the resistant population, continued male releases were able to hold the susceptibility level of the population constant.

The males for these release experiments carried a genetic sexing system based on translocation of an insecticide resistance gene on to the Y chromosome. This allows production of batches of over 99% males for release. This system was found to be reliable and stable. The use of this translocation system was also shown to introduce semisterility into the population

In three DDT selected strains, the resistance was made homozygous and this allowed the degree of dominance to be tested after crossing to a susceptible strain. There is no DDT dose at which one can fully discriminate homozygotes and heterozygotes . The dieldrin resistance genes also carried by these three strains varied from semi-dominant to almost completely dominant. An experiment to test for linkage of DDT and dieldrin resistance gave conflicting results.

Studies on Plasmodium infection showed that there was a tendency for loss of P. yoeli nigeriensis infection in older mosquitoes

and the parasite appeared to damage young mosquitoes. A series of experiments gave no evidence of any effect of sublethal doses of DDT, malathion or Dimilin on susceptibility for P. y. nigeriensis or on the rate of development in refractory and susceptible strains of An. gambiae s.s and a susceptible strain of An. stephensi.

A selection experiment showed no correlation of DDT resistance to P. y. nigeriensis infection. However, DDT selection pressure produced slight cross-resistance to permethrin and higher permethrin resistance was obtained by selection with the latter compound.

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INTRODUCTION

The campaign to control or eradicate malaria by the spraying of residual insecticides has achieved great successes, with a reduction in the global incidence of malaria from an estimated of 300 million cases annually before 1946 to about 120 million cases annually in 1973 (Brown et.al., 1976). Malaria still persists in almost all developing countries with some serious setbacks having been reported in recent years. One quarter of mankind is still at risk from this major disease. Malaria eradication in several tropical developing countries is unlikely to succeed in the near future because of both administrative and technical obstacles. One of the main technical problems is due to the fact that many species of anopheline mosquitoes have developed resistance to one or more insecticides (Brown and Pal, 1971; Pal, 1974).

Over the past two decades much data have been compiled on the formal genetics and biochemical mechanisms involved in resistance, and on the number of mosquitoes and other disease vectors, and geographical areas affected. However, unfortunately, this information has helped little in finding solutions to the practical problem posed by insecticide resistance. Several alternative methods of vector control have been investigated, but none of these has so far been able to replace the immensely beneficial effects of DDT. New alternative insecticides have also been developed, but the cost of the newer compounds is very high and they have therefore rarely been used in large scale projects, particularly in those developing countries which are relatively poor and which also have the highest levels of endemicity of malaria. In addition, cross-resistance has also been encountered with respect to some new

insecticides. For example, the gene for DDT resistance may also confer resistance to newer insecticides when these first come to be used (WHO, 1976; Chadwick et.al., 1977; Prasittisuk and Busvine, 1977). For these reasons, prolonging the effective usefulness of DDT is highly desirable.

There is some evidence that following withdrawal of DDT spraying, reversion to lower resistance levels occurs due to the reduced fitness of resistant genotypes relative to susceptibles. However it appears that populations do not revert to a level of susceptibility similar to that of untreated populations(Raghavan et.al., 1967 b). In fact, in the field situation, exposure to DDT or other insecticides is generally not completely withdrawn because of uncontrollable agricultural usage of insecticides, and there is evidence that selection pressure for insecticide resistance of mosquito populations can arise as a side effect of agricultural usage of insecticide (Georghiou, 1975).

For these reasons some attention has been given to the theoretical advantages of deliberate re-introduction of susceptibility genes by making releases into wild populations. Most of these studies have concerned the idea of using negatively heterotic systems to force population replacement. However, such systems are not yet available in anophelines. More recently introduction into populations of genes favourable to man by simply "dilution", i.e. release of males carrying desired genes but otherwise the same as the wild population,has been proposed (Curtis, 1978a). The release of genetically normal and insecticide susceptible males would be much simpler than a negative heterosis system though it would be less efficient, i.e. more prolonged releases

would be necessary. The release of males could be used as a supplement to a "grid" or "mosaic" system of spraying unrelated insecticides in adjacent areas, which has been proposed by Muir (1975, 1977) and Comins (1977). The release of males would not add to the biting population but, provided that the released males competed for mates, it would result in the reduction of the frequencies of resistance genes among the wild females of the later generations. If such reversals in the evolution of insecticide resistance are possible they would contribute greatly to overcoming the recent setbacks in the world-wide malaria control programme.

The mass production of males for such a release programme would require a genetic sex separation system and such systems have been established in three species of anophelines, including Anopheles gambiae sensu stricto (= An. gambiae species A). The systems are based on the translocation of insecticide resistance genes on to the Y chromosome so that it can be arranged that males are resistant but females are susceptible and can be killed as young larvae, leaving the males to be reared and prepared for release.

Although it is well recognized that laboratory experiments are only preliminary "models" of practical operations, studies were made in this laboratory with An. gambiae s.s. to investigate the possible effects in a cage environment of release into a resistant population of susceptible males, which were obtained by a genetic sexing system. A partially resistant strain was made highly resistant by selection and the strain was maintained as a cycling population. When this "target" population had reached equilibrium, a regular

schedule of introductions of susceptible males were commenced. Later, the joint effect of releases and renewed selection for resistance was tested. Under laboratory conditions it has often been found that the resistant colony after being maintained for some time becomes more susceptible. Therefore a control population with artificial selection relaxed but with no releases was also investigated.

The effectiveness of such release procedures in reducing the resistance level of a population is greatly dependent on the degree of recessitivity of the resistance genes. In other words, if the resistance gene is effectively recessive under field conditions, the resistance could be overcome more easily and the releases need not be frequent to maintain a given level of susceptibility. Studies on the inheritance of DDT resistance in the An. gambiae s.s. used for the cage experiment were therefore carried out to investigate the dominance relations and other genetic properties. In addition, genetic studies of DDT and dieldrin resistance of two other strains of An. gambiae s.s.. which had been selected for DDT resistance, were also carried out in order to determine whether different types of resistance gene were selected when selection pressure for DDT resistance was applied to strains of different geographical origin.

For the study of the dominance of resistance, pure homozygous susceptible and homozygous resistant colonies are required. If resistance is dominant it is difficult to determine accurately what the dominance relations really are. In such a case, a homozygous resistant stock can only be produced by repeated inbreeding and testing of the progeny of individual females.

As already mentioned, the experimental releases were of males separated by a genetic sex separation system. The experiment therefore constituted a test of the stability and reliability of such a separation system in routine use. Moreover, use of such a sexing system would also involve the introduction of semisterility in the form of male-linked translocations into the population, which may contribute to vector control. This system was also tested under "laboratory model" conditions.

According to Macdonald's classic mathematical statement (1957), susceptibility to infection of man-biting anophelines is one of the components determining vectorial capacity of the population. The mean length of time for which a mosquito remains infective is also a relevant factor. Macdonald (1957) considered that the important variables with respect to the mosquito are the incubation period of infectivity within the mosquito, which depends on temperature and species of parasite, the effect of infection on the mosquito's viability and the duration of infectivity. He stated that the mosquito's viability is not found to be influenced by the parasite and the infectivity of mosquito is permanent once established. It is still not known whether the last two presumptions are true or not. Studies were therefore carried out to detect any possible changes in the infectivity of An. gambiae s.s. and An. stephensi after different incubation periods.

Genetic and environmental factors can affect mosquito susceptibility to infections (Garnham, 1964; Macdonald, 1967). Sublethal doses of insecticides have various physiological effects and there are precedents for them effecting vector susceptibility. If DDT

or any other insecticide has effects on vector susceptibility under field conditions, the consequences of spraying campaigns might include not only the shortening of the average life span and a density reduction resulting from their lethal effects, but also changes in the susceptibility to infection of the surviving mosquitoes. Such changes might be in the direction of increased or reduced susceptibility. Recently, results obtained by Gaaboub and Busvine (1975, 1976) showed that sublethal doses of DDT or Dimilin (PH60:40) can increase the susceptibility of a poor vector strain, Ae. aegypti, for Brugia pahangi. If this is a general phenomenon, DDT or Dimilin (which have totally different biochemical effects), or any other insecticides, might have unexpected effects in the mosquito population. It should be emphasized that if these insecticides can truly enhance vector susceptibility of strains or species which are normally poor vectors, it might have important adverse effects on campaigns against insect-transmitted diseases. Many anopheline mosquitoes were found not to be malaria vectors in the past, although they are anthropophilic species, e.g. An. campestris in Thailand (Gould et.al., 1967), and some species of anopheline which used to be malaria vectors in Europe; e.g. An. labranchiae, An. atroparvus and An. sacharovi were found not to develop infections when fed on African Plasmodium falciparum (Ramsdale and Coluzzi, 1977; Dashkova, 1977). Unfortunately, if sublethal doses of insecticides can affect susceptibility to infection of those mosquitoes mentioned above, this may have a serious effect on the future malaria eradication and control programme. Furthermore, if sublethal treatments interfered with refractoriness, the possibility of controlling vector populations by the introduction of refractoriness may prove not feasible. On the other hand, sublethal effects might operate in a

favourable direction, i.e. towards reduction in susceptibility of fully susceptible strains as also found by Gaaboub and Busvine (1975, 1976).

Mosquitoes of the An. gambiae species complex are the main vectors of malaria in Africa and An. stephensi is an important malaria vector in the Indian sub-continent and the Middle East. Strains of An. gambiae s.s. which had been selected for refractoriness and susceptibility to P. berghei berghei, were available in this laboratory. In addition, a strain of An. stephensi with highly susceptibility to rodent malarias, was also available. Therefore, a study was made to investigate the possible effects of sublethal insecticide treatment on the extrinsic cycle of rodent malaria, P. yoeli nigeriensis, and the possible effects on the vectorial capacity of both susceptible and refractory strains of An. gambiae s.s. and with the highly susceptible strain of An. stephensi. DDT and malathion, which are the most widely used insecticides in mosquito control and Dimilin, which is one of the promising compounds for replacing DDT, were initially chosen for the study of possible effects on vector susceptibility.

The selection for one character often affects another, either because of a physiological connection between the characters, i.e. pleiotropic effects of the gene concerned, or alternatively because of the linkage of the alleles concerned. Therefore a study was carried out to show whether selection of An. gambiae s.s. for DDT resistance would change its susceptibility to infection with P. y. nigeriensis.

The use of synthetic pyrethroids as insecticides is presently undergoing great expansion, because of the discovery of new highly active compounds. Pyrethroids possess many desirable properties, including high toxicity to insects, rapid action and high biodegradibility (Barlow and Hadaway, 1975; WHO, 1976; Elliott et.al., 1978). In view of the data on cross-resistance in Ae. aegypti reported recently (Chadwick et.al., 1977), it is important to know whether DDT selection pressure has any effect on the pyrethroid tolerance level in anophelines. If strains of anophelines already possess pyrethroid resistance, as a by-product of the DDT selection pressure to which they have been exposed, this would mean the loss of an alternative insecticide, which might have been valuable in several fields. Studies were therefore carried out to investigate possible correlated effects of DDT selection pressure on pyrethroid tolerance levels of An. gambiae s.s.

LITERATURE REVIEW

1. Historical background

The introduction of potent and stable synthetic insecticides into public health programmes, particularly DDT, has provided a rapid and efficient means of combating the ravages of insect vectors of disease. Specific data can be quoted from diverse areas of the beneficial effects of insecticides in controlling typhus, filariasis, trypanosomiasis and leishmaniasis. Malaria is one of the most common of all the vector-borne diseases. About 1945 million people live in originally malarious areas of which approximately 73 per cent are in areas where malaria has been eradicated or where attempts are being made to eradicate; the remaining 27 per cent live in areas where there is no eradication programme (WHO, 1975). Bruce-Chwatt (1971) pointed out that malaria eradication programmes, which were based entirely on residual spraying of insecticides, particularly DDT, almost certainly prevented the occurrence of more than 2,000 million cases of malaria between the years 1961-1971. He also estimated that 15 million people were saved from dying between 1955 and 1965. The results of malaria eradication and control throughout the world have been reviewed by Brown et.al. (1976). The substantial reduction of malaria, with the saving of many millions of lives can be largely credited to DDT.

Numerous attempts have been made to eradicate malaria since 1950 but eradication is now considered a practical impossibility in the remaining malarious countries (WHO, 1969, 1978). Several factors responsible for the persistance of transmission, lack of progress and possible future reverse of the global malaria eradication campaigns

have been reported by WHO and many workers. (WHO, 1969, 1976, 1978; Nalananda, 1973; Cheong, 1973, Lepes, 1974; Bruce-Chwatt, 1974). Administrative, socio-economic and political obstacles to eradication are very common in developing countries with inadequate basic health services and shortage of trained personnel. Human behaviour and habits, such as migration and refusal of DDT house spraying, have made the task of the malarial campaign a very difficult one. However, the most pressing problem is a lack of financial resources (Bruce-Chwatt, 1974; Brown et.al., 1976). Technical obstacles have also presented serious problems. These include the exophagic habits of some anopheline species, changes in the behaviour of some malaria vectors, resistance of the malaria parasite to antimalarial drugs and toxic hazards of insecticides to humans and animals due to environmental pollution. However, the main technical problem is the development of insecticide resistant strains of mosquito vectors.

According to the WHO Expert Committee on Insecticides (WHO, 1976), in 1975, a total of 42 anopheline species has been reported to be resistant, as compared with 38 in 1968: 41 species are resistant to dieldrin and 24 to DDT, 21 of the latter having developed double resistance. Resistance has also spread geographically. According to Brown and Pal (1971), Brown (1971), FAO (1970) and WHO (1976), more than 230 species of arthropods (including mosquitoes) of medical, veterinary and agricultural importance have shown resistance. Most of these are insects. The extent of resistance has increased each year as has been shown by several investigators (Busvine, 1970, 1972, 1976; Brown, 1971; Brown and Pal, 1971; Pal, 1974; WHO, 1976). Furthermore, cross and multiple resistance in certain strains have been found to

extend to certain chemicals even before they have been used in practice (Brown and Pal, 1971; Georghiou, 1972; Rongsriyam and Busvine, 1975). Environmental pollution, due to the widespread use of insecticides, has been increasingly obvious. These situations have persuaded scientists, biologists and entomologists to examine a wide variety of alternative control measures. These include the use of alternative insecticides such as malathion, propoxur (WHO, 1976); pyrethroids (Elliott et.al., 1978), Dimilin and Altosid (WHO, 1976, Staal, 1975). Mechanical, environmental, ecological, biological and genetic control measures were also investigated but none of those measures has yet approached the stage of effectively replacing the immensely beneficial effect of insecticides. Busvine (1976) stated that "insecticides should continue to be used but extra care and vigilance are needed". The final solution to the vector-borne diseases or pest control must come from a combination of methods, strategically applied. (Davidson, 1974; Busvine, 1976).

2. Genetic Studies of DDT and Dieldrin Resistance in Anopheles Mosquitoes

Progress in understanding the mechanisms causing resistance has been mainly due to improvement in the techniques available; these include standardization of testing techniques (Georghiou, 1969), improved methods of genetic analysis in some insect species with the increased availability of marker genes and biochemical techniques for studying the enzyme systems which metabolize insecticides. A number of literature reviews have been produced on the results of these studies (Oppenoorth, 1965; Brown, 1958, 1967; Georghiou, 1967, 1969; Brown and Pal, 1971; Plapp, 1976; Kitzmiller, 1976).

The genetics of DDT resistance in anopheline mosquitoes has been studied in the following eight species, An. sundaicus (Davidson, 1958), An. quadrimaculatus (Davidson, 1963a), An. stephensi (Davidson and Jackson, 1961a, 1961b; Davidson, 1966a), An. albimanus (Davidson, 1963b), An. pharoensis (Davidson, 1964a; Kamel et.al., 1970; Hamed et.al., 1973). An. pseudopunctipennis (Kitzmiller, 1976) and An. gambiae species A and B (Haridi, 1970, 1971, 1972). It was concluded that resistance in most of the species investigated was due to single incompletely dominant genes. DDT resistance in An. stephensi was reported to be monofactorial but of intermediate dominance (Davidson and Jackson, 1961a, 1961b). Subsequently a case of nearly complete dominance of resistance in An. stephensi from Iran was reported by Davidson (1966a). DDT resistance in An. pharoensis has been reported to be due to a single autosomal recessive gene (Kamel et.al., 1970; Hamed et.al., 1973). This result is different from Davidson (1964a) who stated that resistance in this species is controlled by a single incompletely dominant gene. Haridi (1970, 1972) reported that there are possibly two different resistance genes in the An. gambiae complex, a dominant in the Tog strain of An. gambiae species A and the Sudan strain of An. gambiae species B and an incompletely dominant gene in the Upper Volta strain of An. gambiae species A.

The inheritance of dieldrin resistance has been investigated in at least nine species of Anopheles. An. gambiae (Davidson, 1958; Davidson and Jackson, 1961a, 1961b; Davidson and Hamon 1962), An. albimanus, (Davidson and Jackson, 1961b; Davidson, 1963b, 1967; Georghiou et.al., 1967), An. quadrimaculatus (Davidson and Jackson 1961a, 1961b; Davidson 1963b, French and Kitzmiller, 1963), An. stephensi (Davidson and Mason 1963; Davidson, 1964a, 1965), An.

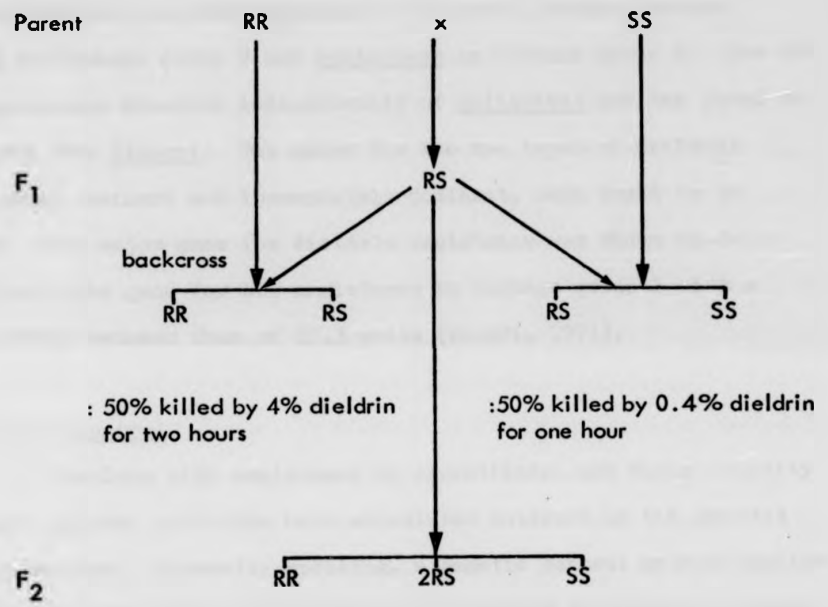
sundaicus (Davidson, 1964a), An. pharoensis (Davidson, 1964a), An. funestus (Davidson, 1966b), An. sacharovi (Davidson, 1968) and An. pseudopunctipennis (Davidson, 1966c; Martinez-Palacios and Davidson, 1967). In each case the resistance appears to be a case of monofactorial inheritance. The resistance in all of the species investigated, except some strains of An. gambiae was found to be incompletely dominant. The exceptional strains of An. gambiae species A show dominance of resistance (Davidson, 1964a; Davidson and Hamon, 1962). Except in the latter case, Davidson (1958, 1963a, 1963b, 1964a) showed that all three genotypes for dieldrin resistance/susceptibility can be clearly distinguished by discriminating doses of dieldrin (see Figure 1) as follows:

1) One hour's exposure to 0.4% dieldrin is used to kill the susceptible homozygote (SS); heterozygote (RS) and homozygote resistant (RR) individuals survive at this dose.
2) Two hours' exposure to 4% dieldrin is used to kill the susceptible homozygote (SS) and heterozygote (RS), only homozygote resistant individuals survive this dose.

A clear discrimination of genotypes has also been reported in larvae of An. albimanus (Gilotra, 1965) and An. quadrimaculatus (French and Kitzmiller, 1963).

Resistance to both DDT and dieldrin occurring in the same species has been examined in An. albimanus, An. quadrimaculatus, An. stephensi, An. pharoensis (Davidson, 1964b) and An. gambiae (Haridi, 1974).

Figure 1: Demonstration of the monofactorial inheritance of Dieldrin resistance of Anopheles mosquitoes.



Because of the shortage of suitable markers in Anopheles, little work on linkage has been attempted but in An. gambiae, the autosomal mutants diamond and collarless, were described (Kitzmiller and Mason, 1967; Mason, 1967). Linkage studies of An. gambiae species A showed that the two autosomal markers belonged to different linkage groups: diamond on linkage group 2 and collarless on linkage group 3. The DDT resistance gene assorted independently of collarless and was found to be linked with diamond. The genes for the two types of dieldrin resistance, dominant and incompletely dominant, were found to be allelic. The major gene for dieldrin resistance was shown to be linked with the gene for DDT resistance in linkage group 2 with a map distance between them of 35.3 units (Haridi, 1974).

3. Genetic Control

Problems with resistance to insecticides and their toxicity and environmental pollution have stimulated interest in the genetic control methods. Generally speaking, a genetic control method implies the release into a wild population of genetically altered males which will mate with the wild females and have the effect of reducing the density or even eliminating the population or changing its genetic composition so as to make it harmless or more easily controllable in some other way. Davidson (1974) has reviewed the many different genetic control mechanisms which have been proposed or tested in wild populations. In this thesis work is described relevant to the possible use of male-linked chromosome translocations in genetic control and this review will therefore concentrate only on this aspect of genetic control.

The genetic mechanism whereby chromosomal translocations cause inherited semisterility was originally explained in plants, and this explanation appears to be applicable to insects also. The potential usefulness of translocations for insect vector control was first proposed by Serebrovsky (1940). Much more recently interest has been renewed in the theoretical possibility of using translocations to insect control as a result of the work of several geneticists. Curtis (1968a) made a theoretical comparison between the effectiveness of Glossina control by translocations and by sterile males and concluded that on certain assumptions the translocation method might have an economic advantage. The partial sterility due to translocations will tend to be inherited for a few generations after introduction into wild populations but eventually it will be selectively eliminated. There would be similar selective elimination from captive colonies except in two circumstances: (a) colonies homozygous for translocations, (b) colonies when all the males carry a heterozygous translocation firmly linked to the Y chromosome or male determining gene. One or other of these conditions is necessary for the mass production of translocations for release. Curtis (1968b) suggested that the homozygous translocations might be better used as transport mechanisms for desirable genes such as those for non-susceptibility to human pathogens rather than a means of population suppression or eradication. The use of viable translocation homozygotes in this way is an example of the use of a negatively heterotic system which has the property that once the released translocation reaches a majority in the population natural selection will then favour it at the expense of the wild type chromosome. Later, Whitten (1970, 1971a,b) proposed the use of negative heterotic multiple translocation homozygotes to replace the field population. These would have the advantage over

the single translocation system of faster selection of the introduced genes. Replacement of a field population with insecticide susceptible homozygotes has also been suggested. It has been emphasized by Curtis (1975a) that the property of selection following the establishment of a translocation in a population only applies to viable translocation homozygotes: in the case of fully male-linked translocations where homozygotes cannot exist, selection will always tend to eliminate them from populations.

Translocations have already been isolated in several insect species of medical importance. This was done by irradiation and testing for the pseudo-linkage of markers, inherited partial sterility or by making cytological examinations. In the C. pipiens complex, induction of chromosomal translocations was reported by Laven and his co-workers (Laven, 1969; Laven et.al., 1971a, 1971b). Young males were X-ray irradiated and then crossed to untreated females. The inheritance of partial sterility was followed by measuring the egg hatchability of the outcross progeny. In some lines all males were found to be partially sterile, while females showed normal fertility when crossed with normal males. This indicated that a translocation had occurred between the male-determining chromosome and one or other of the two autosomes. Krishnamurthy, et.al.,(1977) obtained fully male-linked translocation complexes in C. p. fatigans with up to 80 per cent sterility. These levels of sterility were maintained if selection was applied for high sterility, but where selection was relaxed the sterility levels gradually declined to 50%. A more rapid decline in sterility level of two initially highly sterile translocations in C. p. fatigans was reported by Bhalla et.al., (1974).

With regard to Ae. aegypti. Rai et.al., (1970), Rai and McDonald (1971, 1972) and McDonald and Rai (1970) described two radiation induced sex-linked translocations in this species. The translocations were isolated following x-irradiation of young adult males and these males were crossed with untreated females of the same strain and F_1 males were back crossed to parental females. Two types of translocation involving the male-determining chromosome were obtained, one was a translocation involving linkage group I and II and the other involved linkage group I and III. Lorimer et.al., (1972) produced two homozygote interchange lines from more than 40 reciprocal translocations in Ae. aegypti. Of the two homozygous viable translocations, one was partially sex-linked, involving chromosome 1 and 3, and the other autosomal involving chromosome 2 and 3; 19% hatchability rate was found in the first and 55% in the second. Recently, Rai et.al., (1974) reported that two out of 45 translocations induced in a stock from India yielded viable homozygotes in Ae aegypti. Bhalla (1973) has described seven sex-linked translocations on the basis of sterility, linkage alteration and cytological examinations of chromosomal configurations. Fertilities of seven translocation heterozygotes ranged from 18% to 71%.

Concerning Culex tritaeniorhynchus, extensive work on the isolation of translocations has been carried out by Baker et.al., (1970), Sakai et.al.,(1972) and Baker and Sakai (1974). Forty-six translocation stocks, which were derived from gamma radiation from a ^{60}Co source, were isolated. Autosome-autosome translocations were also produced (Baker et.al., (1970). Attempts were made to increase the sterility of the first isolated stocks by further irradiation (Sakai et.al., 1972). Higher

sterilities were found among males, this being due to an increase in the complexity of chromosomal aberrations. Furthermore, doubly heterozygous translocated males were also produced by crossing males and females which were heterozygous for different complex aberrations. Crosses between the resultant doubly heterozygous males and wild type females have shown sterilities of over 96%. Attempts to produce translocation homozygotes from the stocks mentioned above failed (Baker and Sakai, 1974).

Selinger (1972) was also able to produce nine lines carrying a male-linked translocation and six lines with autosomal interchanges in C. tritaeniorhynchus. These 15 lines were maintained for 16 generations in the heterozygous condition. Viability was found to be as good as in normal strains and the degree of sterility was also constant.

Regarding anopheline mosquitoes, two radiation induced translocations have been reported in An. maculipennis (Frizzi and Jolly, 1961). In An. stephensi, Aslamkhan and Aagil (1970) obtained chromosome translocations by gamma irradiation. They examined cytologically in one case and it was claimed to be a sex-linked translocation. Rabbani and Kitzmiller (1972) have presented the methods of induction, screening, maintenance and description of translocations in An. albimanus. Six reciprocal translocations, three being of the autosome-autosome type, two involving X-autosome interchanges and one involving Y-autosome interchanges, have been produced. Attempts to detect any translocation homozygotes were unsuccessful. Rabbani and Kitzmiller (1975) obtained translocation heterozygotes which showed 50-60% sterility, except for

one involving the Y chromosome and both autosomes, which showed about 78% sterility. Again, they failed to establish homozygote lines from 12 different autosomal translocations. Under the conditions used, they found that males carrying a Y autosome translocation were equal in mating competitiveness with wild type males.

In the An. gambiae species complex, Krafsur (1972a, 1972b) described isolation of translocations. Two lines of species A and three lines of species B carried translocations which involved the Y chromosome and one of the two autosomes and 13 lines were produced which involved autosome-autosome interchanges. These were characterized by fertility differences between females and males, female heterozygotes being more fertile than their translocated brothers (Krafsur and Davidson, 1972). Attempts to isolate homozygote translocation lines were unsuccessful. The presence of translocations was confirmed cytologically in the heterozygous stocks by Hunt and Krafsur (1972). Attempts to isolate and characterize reciprocal chromosome translocations in An. gambiae species A were carried out by Akiyama (1975). Translocations were induced in males with X-rays and determined by testing for occurrence of inherited partial sterility. Young males of a Y chromosome translocation line were re-irradiated with X-rays (at 4,000 rads). Several further interchanges were isolated. Among 120 F_1 males tested, four 3-chromosome double translocation lines were obtained. These lines were found to transmit their characteristic sterility to all their sons when outcrossed to the wild type, but none of the daughters inherited partial sterility. Comparative studies showed that 3-chromosome doubly translocated males displayed fitness comparable with the wild type males. Among 92 F_1 daughters tested, 8 lines showing regular inheritance of

partial sterility were established. In 5 of these lines the presence of translocations was confirmed cytologically, four involved autosome-autosome translocations and one involved an X-autosome interchange. Fertility of these lines was found to be significantly higher than 50%. An attempt to produce viable autosomal translocation homozygotes was unsuccessful. For all five lines investigated, data obtained from the hatching rates of intercrossed heterozygotes, suggested that translocation homozygotes were lethal.

Among other insects of medical and veterinary importance, attempts have been made to isolate translocations in Musca domestica (Wagoner, 1967, 1969; Wagoner et.al., 1974), Glossina austeni (Curtis, 1969, 1970, 1971; Curtis et.al., 1972), Lucilia cuprina (Foster and Whitten, 1974).

Laboratory experiments conducted with various translocations of C. p. fatigans by Laven (1969) have shown a potential value of translocations for genetic control. The use of male-linked translocations was strongly recommended by Laven (1969) and Laven et.al., (1971a), as only males are released and sterility is propagated through male progeny alone and male-linked translocations can never become fixed as a homozygous fully fertile population. Whitten (1971a) pointed out that the problem following the release of male-linked translocations is a levelling off in the response so that no advantages are obtained comparable with those afforded by the swamping effect that normally develops after successive releases of fully sterile insects. When normal males and male-linked partially-sterile males are mixed together in a population, there is bound to be a rapid reversion to fixation of the normal type

(Whitten, 1971a,Curtis and Hill, 1971).

A field trial in a small isolated wild population of C. pipiens involved releasing male-linked translocation heterozygotes, and was carried out near Montpellier, Southern France by Laven et.al., (1971c, 1971d, 1972). Further observations were made by Cousserans and Guille (1972, 1974) and the experiment has been critically re-examined by Curtis (1975b). He concluded that a steady decline in translocation frequency after termination of releases observed over three years of fertility measurements by Cousserans and Guille (1974) could be fitted to theoretical expectations because of the selective disadvantage of translocation heterozygote males. It was also concluded that there was no valid evidence from this experiment that the sterility introduced caused any suppression of the population. Rai et.al., (1973) conducted field release experiments of heterozygote translocated males of Ae aegypti near Delhi. Five weeks after the last release a high percentage of wild females were found to have been inseminated by translocated males which indicated that the translocation was inherited in the wild population for at least one generation.

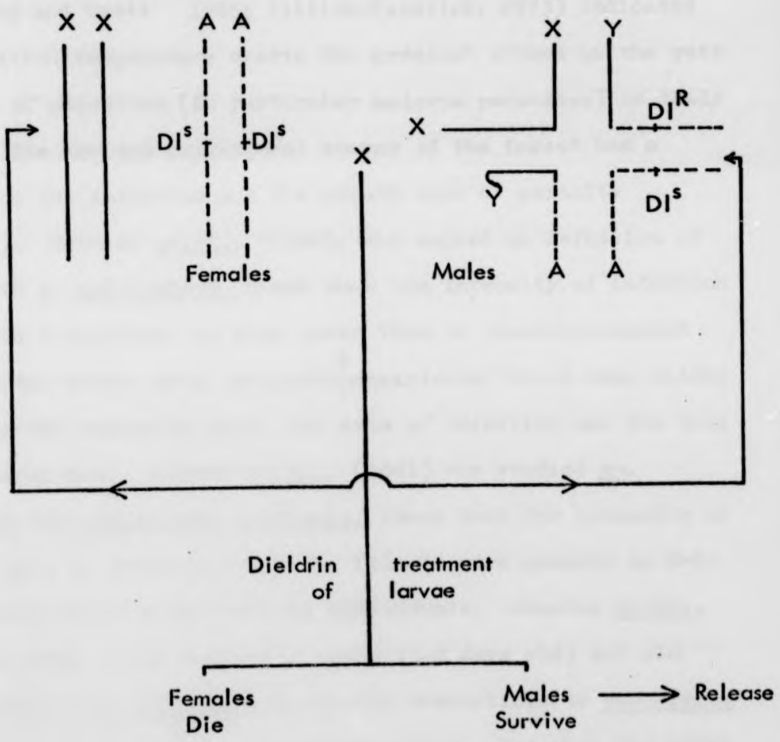
Chromosome translocations may also have an important role in supporting the sterile male technique or other forms of genetic control as part of systems for the elimination of females from batches of insects being prepared for release (Whitten et.al., 1977; Curtis et.al., 1976; 1978 Curtis, 1978a, 1978b, Seawright et.al., 1978). The principle of such genetic sexing systems is based on the translocation of a suitable autosomal gene on to the male sex chromosome. This was first used by Whitten (1969) who linked a pupal colour gene to the male sex

in the Australian sheep blowfly, Lucilia cuprina. In mosquitoes, the systems produced so far have been based on the translocation of autosomal genes for insecticide resistance on to the Y-chromosome. In the case of An. gambiae s.s., the semidominant autosomal gene for dieldrin resistance has been shifted by means of a translocation designated R70 on to the Y-chromosome, so that when R70 males are crossed or backcrossed to homozygote dieldrin susceptible females, the male progeny are heterozygotes and the females are homozygous susceptible (see Figure 2). Such females can be killed at the first larval instar by 24 hours treatment with 0.02 ppm dieldrin. Mating competitiveness experiments showed that the translocated males were virtually equal to normal ones in the conditions of a large laboratory cage (Curtis et.al., 1976). Such genetic sexing systems for elimination of females has been established in at least four species, in Lucilia cuprina (Whitten et.al., 1977) and in three anopheline mosquitoes, An. gambiae s.s. (Curtis et.al., 1976), An. arabiensis (Curtis, 1978b) and An. albimanus (Seawright et.al., 1978).

4. Susceptibility of Insects to Infections

Susceptibility of insects to infection with animal or human parasites for which they serve as vectors may vary widely among different species, among different geographical strains of the same species, and even among different individuals of the same strain. The reasons for these variations are largely unknown. Although the analysis of variation is usually difficult, it has been demonstrated that environmental or physiological factors and inheritance each play an important role.

Figure 2: Showing sexing system in which Y chromosome is linked to the dieldrin resistance gene.



4.1 Environmental or Physiological Factors

Most of the observations are concerned with mosquitoes. There are a variety of environmental and physiological factors which might affect the infectivity and rate of parasite development in the insect intermediate host. Among these are temperature, age, nutrition, humidity and density of the population.

Many investigators (Huff, 1941; Ghosh and Ray, 1957; Garnham, 1964; Vanderberg and Yoeli 1965; Killick-Kendrick, 1973) indicated that the incubation temperature exerts the greatest effect on the rate of development of parasites (in particular malaria parasites) in their insect hosts. The age and nutritional status of the insect has a marked effect on the infection and the growth rate of parasite (Garnham, 1964). Terzian et.al., (1956), who worked on infection of Ae. aegypti with P. gallinaceum found that the intensity of infection in 2-4 weeks old mosquitoes was much lower than in recently-emerged ones, but when the older group was given a non-infected blood meal within 9 days prior to the infective meal, the rate of infection was the same as in young mosquitoes. Duxbury et.al., (1961) who studied An. quadrimaculatus and Dirofilaria uniformis, found that the intensity of infection and rate of development of the filaria were greater in 9-13 day old mosquitoes than in 4-5 day old individuals. Wharton et.al., (1963), on the other hand, found that young (5-8 days old) and old (18-25 days old) C. p. fatigans were equally susceptible to Wuchereria bancrofti when the infective meal was their first. But when the older group was given a prior uninfected meal the infection rate was significantly higher. Terzian and his co-workers (1953, 1960) investigated the susceptibility of Ae. aegypti to P. gallinaceum and the effects

of feeding antibiotics, vitamins, hormones, bases, acids and salts to the mosquitoes (Terzian, 1953; Terzian et.al., 1953; Terzian and Stahler, 1960). They found that in a number of cases the infection was increased, in others the infection rate fell and in a few cases the rate of infection was decreased or increased by the same compound according to the dose applied. Garnham (1964) stated that a blood diet for the mosquitoes is better than glucose for the growth of malaria oocysts. Bhattacharya and Chowdhury (1964) stated that steroid hormones (Prednisolone and hydrocortisone acetate) could abolish the refractoriness of Ae. aegypti to infection with W. bancrofti. With regard to the effects of density, Ward (1963), who worked on Ae. aegypti and P. gallinaceum, stated that there were no significant differences in infectivity among groups of mosquitoes reared at different densities at the larval stage and furthermore no effects were found of the restriction of movement of adults on the infectivity.

4.2 Genetic Factors

Over the past two or three decades, studies on the genetic aspects of the susceptibility of insects to infection by animal and human pathogens have been conducted by numerous investigators. Huff (1927, 1929, 1931) showed that resistance or susceptibility of C. pipiens to P. cathemerium could be increased by selection. Later studies (Huff, 1934, 1935) indicated that infectibility of C. pipiens with P. cathemerium was controlled by a pair of Mendelian genes with susceptibility being recessive. Macdonald (1967) has reviewed Huff's data and stated that the data are not conclusive. At the same period, a genetic effect on the susceptibility of the leafhopper, Cicadulina mbila to maize streak virus was reported by Storey (1932-1933). Roubaud

et.al., (1936) and Roubaud (1937) observed variations in the susceptibility rate between geographic strains of Ae. aegypti to the same strain of D. immitis and suggested that the susceptibility of mosquitoes to this filarial infection was genetically controlled. Trager (1942), working on Ae. aegypti and P. lophurae and Hicks (1949), who worked on C. pipiens with P. elongatum, presented data in respect of the genetic basis of susceptibility, but no conclusions could be drawn about the mode of inheritance or the factors influencing susceptibility. Boyd and Russel (1943) studied the inheritance of susceptibility of An. quadrimaculatus to P. vivax but were not able to provide conclusive data. Jeffery (1944) was also unable to select a more susceptible strain of Ae. albopictus to P. lophurae. Hovanitz (1947) attempted to increase the susceptibility of Ae. aegypti to P. gallinaceum by selection, but he was unable to increase the susceptibility level after six generations. Investigators at the Rockefeller Foundation (1948, 1950,) increased the susceptibility and refractoriness of An. quadrimaculatus to P. gallinaceum by selection and they concluded that multifactorial inheritance controlled susceptibility. Kartman (1953) measured the susceptibility of C. p. pipiens and C. p. fatigans to infection with D. immitis but he was unable to obtain full susceptibility or refractoriness to infection. Taylor (1960) and Hawking and Worms (1961) stated that a stock of Ae. aegypti which was initially susceptible to D. immitis after a time became refractory to it. Macdonald (1962) showed that the susceptibility of Ae. aegypti to subperiodic Brugia malayi was controlled by a sex-linked recessive gene which he called f^m . Ramachandran et.al., (1960) had shown that different stocks of Ae. aegypti varied in their susceptibility, and such variation could be correlated with differences

in the frequency of the gene f^m in the stocks (Macdonald, 1967). The f^m gene also controls susceptibility to other Brugia and Wuchereria species, but not to Dirofilaria (Macdonald and Ramachandran, 1965), or Waltonella (formerly Dolevella) (Terwedow, 1973). Using B. pahangi and Ae. aegypti, Townson (1971) showed that susceptible mosquitoes have higher mortality than refractory ones and that mortality is related to the microfilarial density. Raghavan et.al., (1967a) succeeded in producing refractory and susceptible Ae. aegypti to infection with D. immitus. A single sex-linked recessive gene, was found to control susceptibility to D. immitis. (McGreevy, 1971; Zielke, 1972, 1973; McGreevy et.al., 1974). Coluzzi and Gironi (1971) also produced resistant and susceptible strains of Ae. aegypti with respect to D. repens. On the other hand, Thomas and Ramachandran (1970) and Zielke(1973) were able to increase the susceptibility level of C. p. fatigans to W. bancrofti.

Different strains of Ae. aegypti contain different genes for susceptibility and strains vary markedly in susceptibility to B. pahangi (Rodriguez, 1973; Rodriguez and Craig, 1973). The larval stages of this species have been found to develop if injected into susceptible male mosquitoes (Townson, 1974). Susceptibility to B. pahangi in C. p. fatigans is probably controlled by a single sex-linked gene (Obiamiwe and Macdonald, 1973). C. p. fatigans from West Africa showed rapid response to selection for refractoriness to W. bancrofti but members of the same mosquito species from India which were fed on the same W. bancrofti donors, did not respond to selection (Zielke and Kuhlow, 1977).

With regard to malaria parasites, Ward (1963) stated that a single pair of genes, or a block of closely linked genes, with incomplete dominance controls the susceptibility of Ae. aegypti to P. gallinaceum. Kilama (1969) and Kilama and Craig (1969) showed that refractoriness to infection of Ae. aegypti to P. gallinaceum is controlled by a single autosomal recessive gene, pls. This pls gene was found in eight African Ae. aegypti strains but not strains from other continents (Kilama, 1973). Similarly, a semi-dominant gene on linkage group 3 controls resistance to P. cathemerium in C. pipiens (Dennhofer 1971). In An. stephensi, Corradetti et.al., (1969) were able to select susceptible and refractory strains to P. gallinaceum and Frizzi et.al., (1975) stated that the refractoriness to P. gallinaceum in An. stephensi is controlled by an autosomal dominant gene. It was concluded that there is polygenic control of refractoriness in An. gambiae species A to P. berghei berghei (Al-Mashhadani, 1974; Al-Mashhadani and Davidson, 1976). With An. atroparvus, van der Kaay and Boorsma (1975, 1977) showed that the refractoriness to P. b. berghei is controlled by a gene with intermediate dominance. Differences in the infectivity of Anopheles to human malarial infections have been shown with respect to different geographical strains and species of the parasite and the insect. (Ramsdale and Colluzzi, 1975; Collins, et.al., 1976; Dashkova, 1977). Warren et.al.,(1977) discovered that three distinct phenotypes of An. albimanus from El-Salvador, separated by the pupal morphology but not reproductively isolated, differed both in the proportions of mosquitoes becoming infected with P. vivax and P. falciparum and their levels of infection. The difference between the susceptibility in these phenotypes were greater with respect to P. vivax than P. falciparum.

With regard to viruses, Bruce-Chwatt (1950) made an attempt to select Ae. aegypti for increasing susceptibility to yellow fever virus but the result was not conclusive. As with filaria and malaria, different mosquito species vary in their susceptibility to experimental infection and in their ability to transmit arboviruses. (McIntosh and Jupp, 1970; Mangiafico, 1971; Watt et.al., 1973; Saliba et.al., 1973; Schiefer and Smith, 1974). Variations amongst geographic strains of Ae. albopictus in the susceptibility to infection with dengue and chikungunya viruses were also reported by Gubler and Rosen (1976) and Tesh et.al., (1976). Attempts by these authors to increase the refractoriness or susceptibility in Ae. albopictus to dengue and chikungunya viruses have failed. However, crosses between susceptible and resistant strains produced hybrid progeny with intermediate susceptibility (Gubler and Rosen, 1976; Tesh et.al., 1976). Jones and Foster (1974) were able to establish a resistant and a susceptible colony of the midge Culicoides variipennis with respect to bluetongue virus (a virus disease of wild and domestic ruminants). Recently, Hardy et.al., (1978) have reported selection of a strain of C. tarsalis with greatly reduced susceptibility to Western equine encephalomyelitis virus. Crossing results indicated that refractoriness was controlled by autosomal incompletely recessive genes. The reports of inheritance studies of the susceptibility of vectors to pathogen are summarised in Table 1.

Table 1. Summary of the reports of inheritance studies of susceptibility of vectors to pathogens

Vector species	Pathogens	Inheritance of Susceptibility	References
	A. Helminth		
1) Ae. aegypti	1) Brugia malayi	1) Sex-linked recessive	1) Macdonald (1962)
	2) B. malayi (Periodic form) Wuchereria bancrofti (Periodic and Subperiodic forms) B. pahangi	2) Sex-linked recessive	2) Macdonald and Ramachandran (1965)
	3) Dirofilaria immitis	3) Sex-linked recessive	3) Zielke (1973) McCreevy et. al., (1973)
	4) Waltonella flexicauda	4) Sex-linked recessive	4) Terwedow and Craig (1977)
2) C.p. fatigans	1) B. pahangi	1) Sex-linked recessive	1) Obiamiwe and Macdonald (1973)
	2) W. bancrofti	2) Intermediate	2) Zielke and Kuhlow (1977)
	B. Protozoa		
1) Ae. aegypti	1) Plasmodium gallinaceum	1) Autosomal Incomplete dominant	1) Ward (1963); Kilama (1969); Kilama and Craig (1969) Kilama (1973)
2) C.p. fatigans	1) P. cathemerium	1) Autosomal recessive	1) Dennhofer (1971)
3) An. stephensi	1) P. gallinaceum	1) Autosomal recessive	1) Corradetti et.al., (1969)

Table 1 (continued)

Vector species	Pathogens	Inheritance of Susceptibility	References
4) An. gambiae s.s.	1) P. berghei berghei	1) Polygenic	1) Al-Mashhadani (1974) Al-Mashhadani and Davidson (1976)
5) An. quadrimaculatus	1) P. gallinaceum	1) Polygenic	1) Rockefeller Foundation (1948,1950)
6) An. atroparvus	1) P. berghei	1) Autosomal intermediate dominance	1) van der Kaay and Boorsma (1975, 1977)
	C. Virus		
1) Cicadulina mbila	1) maize streak virus	-	1) Storey (1932-33)
2) Culicoides variipennis	1) blue tongue virus	-	1) Jone and Foster (1974)
3) Ae. albopictus	1) Dengue	-	1) Gubler and Rosen (1976)
	2) Chikungunya virus	-	2) Tesh et.al. (1976)
4) C. tarsalis	1) Western equine oncephalomyelitis	1) Autosomal incomplete dominant	1) Hardy et.al. (1976)

5. Sublethal effects of insecticides

The acute lethal effects of insecticides have been fairly thoroughly investigated as it is these which are of principal importance in the practical use of insecticides. There is an extensive literature on the pathological effects of insecticides on insects (see e.g. Brown, 1963). It is well-known that in addition to their acute lethal effects insecticides can produce chronic effects on insects but there is little information on the mechanisms involved. There are at least five possible types of such chronic effects of insecticides.

5.1 Effects on longevity

The subacute effects of insecticides may reduce the life span of insects. After the acute toxicity phase has passed, additional mortality may be induced over an extended period (Moriarty, 1969). Sublethal treatment of dieldrin to Anopheles larvae was found to have latent toxicity on the emerged adults (Kuhlow, 1957; Gavms , 1961). The larval development of Drosophila melanogaster treated with DDT is slower than that of the untreated control and most of the treated individuals died at the pupal and adult stages. (Kalina, 1950). Reductions in longevity by subacute doses of DDT have been reported in Leptinotarsa decemlineata (Schwartz, 1951), Pediculus humanus (Eddy et.al., 1955), Musca domestica (Hunter et.al., 1958, 1959; Sherman and Sanchez, 1964) and Pectinophora gossypiella (Adkisson and Wellso, 1962). Similar life span reductions have been found due to the action of dieldrin in Ae. aegypti (Duncan, 1963) and diazinon in female Musca domestica resistant to this compound (Hunter et.al.,

(1958, 1959). Sadex et.al., (1974) found that Culex pipiens molestus treated with either DDT, Abate (Temephos) or sevin (Carbaryl) had a life span slightly shorter than the control. Effects of DDT and Abate were also shown on the development of the larvae. Contrasting results have been obtained by Georghiou (1965) who found that a variety of different insecticides applied to adult houseflies at subacute doses did not affect the longevity.

5.2. Effects on reproductive potential

Sublethal doses of insecticide can affect the reproductive potential in several ways. Effects on egg laying, egg-hatchability and metamorphosis have been mentioned, although nothing was then known about the mode of action of the insecticides concerned (Hoskin, 1940). Effects of sublethal doses of insecticides on egg production of various insects have been reported by many investigators (Kalina, 1950; Knutson, 1955; Chadwick, 1962; Kuipers 1962; Duncan, 1963, Beard, 1965; Zaghloul and Brown, 1968). In most cases the effects found were reductions in egg production but sublethal doses of DDT were found to increase the number of follicles in Ae. aegypti (Sutherland et.al., 1967) and C. p. fatigans (Zaghloul and Brown, 1968). Increases in egg production were reported in Drosophila melanogaster with dieldrin (Knutson, 1955) and in Leptinotarsa decemlineata with DDT (Kuipers 1962). In Ae. aegypti, Duncan (1963) attributed the effects of dieldrin on egg laying to inhibition of feeding.

DDT reduced the percentage of fertile females and egg fertility of Musca domestica (Hunter et.al., 1958, 1959) and

Dysdercus koenigii (Singh and Lal, 1966). Similar results were obtained for Musca domestica dosed with γ-BHC (Ramade, 1967). In mosquitoes, some organochlorines appear to affect fecundity, fertility and egg hatch when applied for example to adult Ae. sollicitans (DeCoursey and Webster, 1952) and An. quadrimaculatus (DeCoursey et.al., 1953). DeCoursey et.al., (1953), working with An. quadrimaculatus and Mohan (1955b) working with An. fluviatilis and An. stephensi stated that exposure of the adults to DDT forces them to lay eggs indiscriminately without choosing suitable oviposition sites. Mohan (1955b) also reported that sublethal contact of DDT with An. fluviatilis and An. stephensi did not inhibit the development of eggs, whether the females were exposed before or after blood feeding or when half gravid. Zaghloul and Brown (1968) found that a sublethal treatment of DDT applied to adult C. p. fatigans caused the ovary to degenerate and reducedthe proportion of females that fed and oviposited. On the other hand, the results obtained by Sadex et.al., (1974) showed that sublethal concentrations of DDT, Abate (Temephos) or Sevin (Carbaryl) had a delaying effect on oviposition in C. p. molestus. Exposure of Ae. aegypti and C. p. pipiens to partially lethal or sublethal doses of DDT did not change their fecundity (Blazquez and Maier, 1950; Havertz and Curtin, 1967). Sublethal doses of dieldrin to C. p. fatigans larvae caused a subsequent slight increase in fecundity (Thomas, 1973). Results obtained by Sadex et.al., (1974) have shown that sublethal concentrations of DDT, Abate or Sevin had no effect on the incubation period and the hatchability of the eggs of C. p. molestus.

Sublethal doses of organophosphorus and carbamate insecticides also affect the reproductive potential (Moriarty, 1969). Most of the

reports are concerned with effects on egg production (Ouye and Knutson, 1957; Hunter et.al., 1958, 1959; Georghiou, 1968).

5.3. Effects on behaviour

There are very few studies of the effects of sublethal doses of insecticides on the behaviour of insects. Mosquitoes in contact with DDT first show symptoms of poisoning within a few seconds, and consequently their resting periods are shorter than those of controls (Kennedy, 1947). Sublethal doses of dieldrin interfere with feeding in Ae. aegypti (Duncan, 1963). It has been observed in many malaria campaign programmes that residues from DDT spraying can affect the behaviour of Anopheles species (Pampana, 1963; WHO, 1970, 1976; Brown and Pal, 1971). It has been reported that residual spraying with insecticides, particularly DDT, appears to have changed the habits of Anopheles populations towards greater exophily and zoophily. This was reported in many Anopheles species, e.g. An. gambiae (Hamon and Dufour, 1951; Pampana, 1963; Brown and Pal, 1971), An. minimus (Brown and Pal, 1971; Notanonda, 1973) and An. funestus (Hamon et.al., 1958). This change of habits of the vector population may be explained either by assuming that the original population consisted of two strains, one endo-anthropophilic, which was eventually selectively eliminated by house spraying, and another exo-zoophillic, which persisted as the only strain; alternatively it may be proposed that the excito-repellency of the insecticide drives away the vectors from their indoor habits. A combined genetic and physiological process may also occur in which there is selection for a greater tendency to be repelled by DDT as this would reduce an individual's chances of being killed by the insecticides.

Insecticides differ very much in their excito-repellency, and anopheline species differ in the degree of irritability, and even strains within a species may present different degrees of irritability. DDT has a greater excito-repellency than any other insecticide. In An. albimanus it appears that DDT and diazinon are the most irritant toxicants, followed by BHC and lindane. Baytex and dieldrin are not irritant at all (Mancera Viqueras and Corzo, 1960). On the other hand An. albimanus is more DDT irritable than An. quadrimaculatus or An. aztecus (Hecht et.al., 1960) and An. punctimacula (Vargas,1961). However, An. albimanus is much less irritable than An. gambiae. An. maculipennis (Brown and Pal, 1971) or laboratory strains of An. stephensi and An. atroparvus (Coluzzi,1963). Different strains of An. sacharovi (de Zulueta, 1959), An. albimanus and laboratory strains of An. stephensi (Busvine, 1964) showed different irritant levels to DDT. It has been reported that DDT resistant populations or strains of An. sacharovi, An. stephensi and An. culicifacies, as well as those of Ae. aegypti and C. p. fatigans,are less irritable by DDT than normal strains (WHO, 1970). Recently, contrasting results were obtained from Eshghy and Laarman (1977) who reported that a resistant strain of An. stephensi was more irritated by DDT than a susceptible.

In some cases, insects have the ability to avoid a dose of insecticide which would kill them and the term "behaviouristic resistance" has become commonly used. Populations of An. pseudopunctipennis in Mexico (Martinez and de Zulueta (1964), An. albimanus in Panama (Trapido, 1952), An. punctimacula in Colombia and An. cruzii in Brazil (WHO, 1960) have developed an increased ability to escape from sprayed houses without

detectable change in their physiological susceptibility or irritability to DDT. It is still not proved whether this is a result of a change of habits of the population due to the insecticide pressure or a result of other factors. Gerald and Laarman (1964) demonstrated in the laboratory with An. atroparvus that the increased activity and ability to escape could develop by selective pressure of DDT deposit.

5.1. Effects on physiology and biochemistry

There is no doubt that insecticides, particularly the chlorinated hydrocarbons exert physiological effects on insects (Brown and Pal, 1971). The rate of heart beat of Lucusta migratoria and larvae of Allomyrine dichotomus is decreased by DDT but is unaffected by BHC (Yamasaki and Ishii, 1949, 1950, 1951). In Periplaneta americana, the heart beat is not affected by DDT, BHC and other organochlorine insecticides (Orser and Brown, 1951). Blazejewska (1965) stated that the heart beat of larval Leptinotarsa decemlineata decreases after DDT, methozychlor and BHC treatments. These apparently conflicting results could presumably be due to differences in insecticide distribution or concentration (Moriarty, 1969). Several different cyclodiene insecticides reduced the excretory efficiency (Patton et.al., 1959) and caused dehydration (Roan and Hopkins, 1961) of Periplaneta americana. A sublethal dose of lindane was also shown to affect the growth rate of this species (Marshbarger and Forgash, 1964).

Several studies of the effects of sublethal doses of insecticides on the biochemistry of insects have been reported. The activity of microsomal NAD-kinase in the metabolic pathway for DDT in insects

increases in DDT treated Triatoma infestans (Ilivicky et.al., 1964). DDT was found to stimulate protein synthesis (Agosin et.al., 1965)

5.5. Effects on vector susceptibility

There is relatively little information on the relation between insecticides and the ability of insects to carry pathogens to human and animals. Several workers have shown that certain chemosterilants can affect the development of parasites in vectors, the susceptibility to infection and the vector potential of insects. Altman (1963) demonstrated that the exposure of Ae. aegypti to the chemosterilant tepa prior to or after infection with P. gallinaceum produced a marked inhibition of development of the parasite and hence a decrease in the mosquito infectivity rate. Bertram et.al., (1964), studied the effect of thiotepa and Ward et.al., (1965) studied the effect of tepa, metepa and apholate, both using the same Ae. aegypti and P. gallinaceum host-parasite system. They found that thiotepa, tepa and metepa, but not apholate, interfered with the development of the malaria parasite. Das et.al., (1967) stated that infectivity rates of C. p. fatigans to W. bancrofti were lower in mosquitoes treated with apholate than in the untreated control. Similar results were obtained by Omar et.al., (1974) who determined the effect of pupal treatment with a thioaziridine chemosterilant, which has been designated ENT-61585, on the susceptibility to infection of An. albimanus and An. freeborni by P. vivax. They found that the infection rate of treated An. albimanus was significantly reduced below that of the control, but the effect was less pronounced in An. freeborni They also stated that effects were quantitative and not qualitative, i.e. the infection of sterilized mosquitoes was not necessarily eliminated and the sporogony of P. vivax could be completed in them in a normal manner.

Possible effects of sublethal doses of synethetic insecticides on vector susceptibility have been looked for by several investigators. Mohan (1955a) found that the treatment of adults with low doses of DDT did not affect the vectorial capacity of Ae. aegypti for P. gallinaceum or C. p. fatigans and An. fluviatilis for P. relictum. The same author (1960) stated that susceptible and highly insecticide resistant C. p. fatigans were about equally susceptible to infection by P. relictum and that the sporogonic cycle is completed normally in the resistant strain kept in continuous contact with DDT deposits throughout the extrinsic incubation period. Recently there have been three more publications reporting negative results in a search for effects of insecticides on vector susceptibility. Firstly, Rafaat et.al., (1974) demonstrated the vectorial capacity for P. vivax of An. pharoensis was not affected following treatment in the larval stage with sublethal doses of DDT, Abate (Temephos) or sevin (Carbaryl). The concentration used had no effect either on parasite survival or on the extrinsic cycle of the parasite. Khalil et.al., (1974, 1975) also found that a sublethal dose of DDT, Abate and Sevin applied to the larval stage of C. p. molestus had no effect on the infection rate or on the vectorial capacity of the adults to Wuchereria bancrofti.

Recently, remarkable results were obtained by Gaaboub and Busvine (1975) who studied the effects of sublethal DDT treatments at the larval stage of Ae. aegypti. The treatments were given to strains susceptible or refractory to Brugia sp., that is homozygous for the gene f^{m} or its allele F which were originally identified by Macdonald (1962). Gaaboub and Busvine found that DDT decreased the percentage of females of the susceptible strain in which Brugia pahangi reached

the infective stage; conversely following DDT treatment a proportion of the refractory strain females became susceptible to infection. The same authors (1976) also stated that sublethal treatment with the chitin inhibitor, Dimilin (PH60:40) given to the fourth instar larvae of Ae. aegypti did not have any appreciable effect on the vectorial capacity of the susceptible strain, but caused the appearance of a moderate level of susceptibility in the refractory strain. Their experiment with heat stress to test the hypothesis that the similar effects of two such different insecticides were due to generalized stress showed negligible effects on the vectorial capacity of both strains so that the effects shown did not seem to be due to simple stress.

MATERIALS AND METHODS

MATERIALS

1. Mosquitoes

One strain of Anopheles stephensi and seven strains of A. gambiae s.s. (=A. gambiae species A) were used in these studies as follows:

1.1. BEECH

A strain of An. stephensi which originated from Delhi, India. This was found to be highly susceptible to rodent malarial parasites (Yoeli , 1965; Vanderberg and Yoeli, 1966; Wery, 1968; Al-Mashhadani, 1974). It has been maintained in the Ross Institute, London School of Hygiene and Tropical Medicine since 1947.

1.2. LD5

This An. gambiae s.s. strain carries the white eye marker which is visible in the larva, pupa and adult. The white eye is controlled by a single recessive sex-linked gene (Mason, 1967). It was selected for refractoriness to infection by the rodent malaria parasite Plasmodium berghei berghei in the Ross Institute and is also refractory to P. yoeli nigeriensis (Al-Mashhadani, 1974; Al-Mashhadani and Davidson, 1976; Al-Mashhadani, 1976).

1.3. P.B.

This strain of An. gambiae s.s. was selected by Al-Mashhadani (1974) for high susceptibility to P. b. berghei. It was shown to be highly susceptible to P. y. nigeriensis (Al-Mashhadani, 1976). It has been maintained without further selection in the Ross Institute since then.

1.4. TOG

This An. gambiae s.s. strain originated from Hlande-Agba, Togo in 1969. By mass selection with DDT a nearly homozygous resistant strain was produced. This resistance was stated to be controlled by a single dominant gene (Faridi, 1972). Since then it had been maintained in the laboratory without further selection.

1.5. IAN

This strain of An. gambiae s.s. was caught resting indoors in the daytime at Iworo, Lagos, Nigeria in 1975. The original population proved to be homozygous for dieldrin resistance (no mortality on exposure to 4% dieldrin for 2 hours), and with high resistance to DDT; only 2 out of 26 (7.8%) died when exposed to 4% DDT for 1 hour. (G. Davidson, personal communication).

1.6. PALA

This strain of An. gambiae s.s. was collected at Pala, Upper Volta. The original population was found to be homozygous for dieldrin resistance and not resistant to DDT (G. Davidson, personal communication). It has been maintained in the Ross Institute since 1967.

1.7. 16cSS

This strain of An. gambiae s.s. originated from Lagos, Nigeria. It was collected from eggs obtained from susceptible females in the field in 1951. It is homozygous for dieldrin susceptibility and for the autosomal marker collarless (Mason, 1967). It was found to be susceptible to DDT (C.F. Curtis, personal communication).

1.8. R70

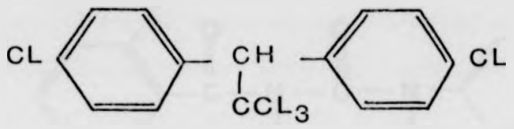
This An. gambiae s.s. strain has an induced translocation which links the gene for dieldrin resistance to the Y chromosome. Females can be killed with dieldrin at the first larval instar or adult stage, but the males survive (Curtis et.al., 1976). The proportion of cross-overs between the resistance gene and the translocation break point was about 0.25% (Curtis et.al., 1976). Radiation sterilized males of this strain were found to be fully competitive for mating in a large laboratory cage and the R70 translocation provides a genetic sexing system which appears to be suitable for the production of males for mass release (Curtis et.al., 1976).

2. Malarial Parasite

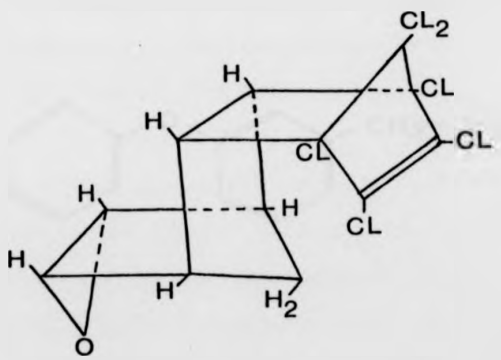
The mosquitoes were fed on mice which were infected with P. y. nigeriensis. The malarial strain was originally isolated in 1967 from a thicket-rat, Thamnomys rutilans, trapped at Ilobi in the Western State of Nigeria (Killick-Kendrick et.al., 1968). A detailed history of this parasite was given by Killick-Kendrick (1970, 1973, 1974a, 1974b). It was provided by Dr D. Wallicker of the Protozoan Genetics Unit, University of Edinburgh to the Ross Institute. P. y. nigeriensis was chosen rather than P. b. berghei because the former requires a higher optimum temperature (24° - 26°C) for development in the mosquito. Hence there is a shorter extrinsic incubation period of 10-12 days (Killick-Kendrick, 1973).

3. Insecticides

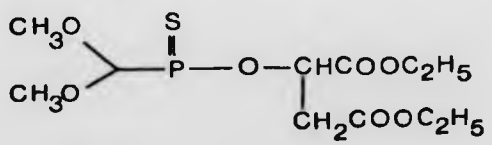
3.1. DDT (1,1,1,-trichloro-2,2-di-(4-chlorophenyl) ethane)



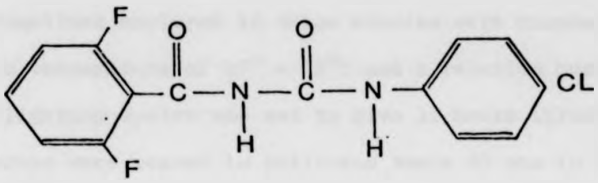
3.2. Dieldrin (HEOD) (1,2,3,4,10,10-hexachloro-6,7, epoxy-1, 4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene)



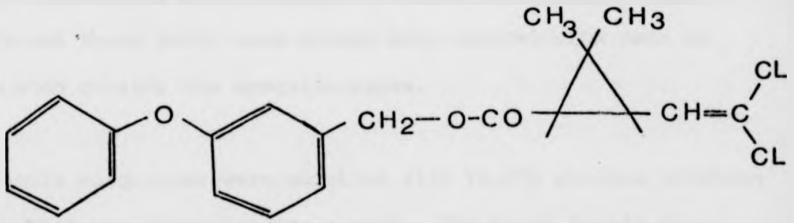
3.3 Malathion S-(1,2-di(ethoxycarbonyl) ethyl) dimethyl phosphordithiote.



3.4. Dimilin (PP-60:40) 1-(4-chlorophenyl)-3-(2,6-difluoro-benzoyl) urea



3.5. Permethrin (NRDC 143) (3-phenoxybenzyl-2,2 dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate)



METHODS

1. Rearing methods for mosquitoes

The mosquitoes employed in these studies were maintained in an insectary at a temperature of 26° - 29°C and a relative humidity of 70-80%. The lighting system was set to give 12 hours illumination per day. The larvae were reared in polythene bowls 30 cms in diameter and 15 cms deep, containing 2-3 litres of tap water. The adults were kept in cages measuring 20 x 20 x 20 cms with a 15 cms circular opening and a 25 cms sleeve attached, which was knotted when not in use. In order to prevent the cross contamination of strains, great care was taken with the labelling of adult cages and corresponding egg, larval and pupal bowls and these bowls were always kept covered with nets or plastic plates when outside the mosquito cages.

The adult mosquitoes were supplied with 10-20% glucose solution on a lint wick which was changed twice a week. The stock female mosquitoes were fed by placing an anaethetised guinea pig on the cage. The feeding period was about 30 minutes twice a week for each cage. Two days after the blood meal, an enamel bowl, 10 cms in diamter, lined with Whatman's No. 1, 15 cms diameter filter paper, was half filled with tap water and placed in the cage. Eggs were laid at night about 60 hours after the blood meal. The enamel bowl with eggs was removed the next day and it was kept in the insectary at 26° - 29°C at which temperature the eggs hatch within 2-3 days. About 200 - 300 newly-emerged larvae were transferred to each larval rearing bowl and a piece of turf was added to the bowl. This provided nutriment, shade and an area of attachment for the larvae. In order to prevent growth of micro-organisms which are

lethal to larvae or delay their development, it is essential that the bowls are thoroughly washed with hot water and allowed to dry before being re-used. Food was not added until the larvae cleared the water surface and subsequently it was added only in a very small amount as required. The first and second instar larvae were fed with "Farex" (a commerical baby food) ground more finely than that obtained commercially. This grinding was not necessary as the larvae grew and the quantity of "Farex" was increased. Careful feeding of anopheline larvae is important as excess will lead to bacterial and fungal growth, thereby killing the larvae. Forced aeration is not necessary for anopheline larvae. The larval development of An. stephensi and An. gambiae s.s. took 7-8 days. When the larvae pupated, they were picked out by pipette or were collected in a sieve and transferred to a plastic bowl and put into a cage for emergence. All adults emerged within two days of pupating. The total time required for development from egg to adult was about 12-13 days.

2. Preparation of Insecticide Test papers

The test of insecticide resistance or susceptibility was made according to the WHO standardised methods for adult and larval mosquitoes. When no papers of a particular required concentration were available from the WHO, an appropriately impregnated paper was prepared as follows. The insecticide was dissolved in the primary solvent to make a stock solution. The appropriate solvents used were Risella oil, olive oil and silicone fluid for DDT, malathion and permethrin respectively. The stock was prepared at the highest concentration required and dilutions were prepared later. All solutions were stored in a refrigerator in darkness to decrease any decomposition.

Whatman's No. 1 filter paper was cut into pieces measuring 12 x 15 cms (180 cm^2) and were used for insecticide impregnations. In order to apply insecticide solution to the filter paper, volatile solvents are required, DDT in Risella oil was dissolved in chloroform, malathion in olive oil and permethrin in silicone fluid were both dissolved in acetone. Papers were labelled with pencil before impregnation. Quantities of each concentration required were prepared from the stock by pipetting 0.7 ml of the insecticide solution into a small glass bottle, followed by 1.8 ml of the appropriate solvent, using a different pipette. The first pipette was used to mix the two and the mixture was applied in parallel lines in 0.5 ml aliquots over the filter paper. To prevent loss of insecticide to the substrate during application, papers were supported on 3 fine points. They were hung to dry for approximately 30 minutes and were then ready to be used.

3. Testing methods

The standard adult and larval tests procedure were carried out by the method recommended by WHO (1970) which was originally described by Busvine and Nash (1953). In addition, Rongsriyam and Busvine (1973) stated that it was possible to calculate concentration-time values (CT) in two ways, from LT50 x concentration or LC50 x time, and their tests showed that the values estimated in these two different ways were not substantially different. In the present study, most of the adult tests were carried out by using a constant concentration of insecticide and by varying the time of exposure. One-day-old male and female mosquitoes were used. All the tests were performed under insectary temperature and humidity conditions. Mosquitoes were examined

after 24 hours and mortality percentages were corrected by Abbott's formula (WHO, 1970).

In the experiments on the effect of sublethal doses of insecticides on susceptibility to Plasmodium the doses used were fractions of the LC50, therefore the adult mosquito tests were carried out by using an appropriate constant time of exposure and varying the insecticide concentrations. In this study, all the tests were performed with a one hour constant exposure period, and 2-4 day-old unfed females were used. The probit-mortality log-dose was plotted on graph paper. The LC50 value was estimated graphically from the regression line.

The standard WHO test procedure for larvae was used to determine the effect of the insect growth inhibitor, Dimilin (PH60:40). Larvae were tested when they were in the early fourth stage. Before the tests were conducted the larvae were sieved, rinsed and transferred into a bowl with clean water. Dimilin was prepared in acetone. Groups of 25 early fourth instar larvae were exposed to a mixture of 249 ml of water and 1 ml of acetone solution of Dimilin at desired concentrations. The larvae were exposed for 24 hours. The effects on the development of the mosquitoes after the treatment were determined and classified as described by Busvine et.al., (1976). Again, the LC50 value was estimated from the probit-mortality log dose regression line.

For DDT resistance studies of the mosquito larvae the method used was similar to those applied for the Dimilin experiment. Instead of using early fourth instar larvae, the first instar larvae were

exposed to a mixture of DDT at the desired concentrations in 50 ml water for 24 hours and then mortality was recorded.

4. Selection of DDT Resistant and Susceptible Colonies

4.1. Selection of DDT resistant colonies

The selection of DDT resistant colonies from the Tog, Ian and Pala strains was carried out as follows.

4.1.1. Mass selection.

This method was used in order to establish a highly resistant population. The general practice used was that of the WHO standard adult test method (WHO, 1970) already referred to. Four percent DDT papers were used throughout and a series of times of exposure were used. At each generation the survivors of the highest exposure period which gave less than 30% survival were transferred into cages and allowed to mate among themselves. A blood meal was offered two days after the treatment and subsequently twice a week in order to obtain eggs. The range of exposure periods were increased as the average resistance of the population rose until the LT50 values became stable. This selection procedure was also applied to select for a permethrin resistance strain.

4.1.2. Single family selection

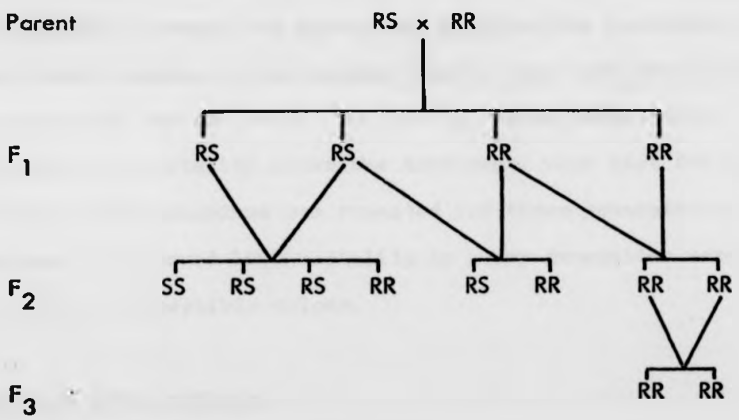
A pure homozygous colony was required for genetical studies and, in the event that resistance was largely or completely dominant, mass selection cannot be relied upon to produce homozygosity since a proportion of heterozygotes would survive the exposure at each generation and continue the propagation of the susceptibility gene. To guard against this possibility, the method of single family selection was

adopted. The mosquito strains were first selected by mass selection for several generations until less than 10% mortality was produced after an exposure of 4% DDT for 1 hour. At this generation, the survivors of DDT exposure for 8 hours of the Tog and Ian strains and 4 hours for the Pala strain were allowed to mate among themselves. Three days after the blood meal, the gravid females were tubed individually in glass vials, 7.5 x 2.5 cms, which were lined with filter paper (see Figure 3). The vials were covered with netting, labelled and a small amount of water added. Only one egg batch was taken from each female and the eggs were allowed 48 hours for hatching. The larvae of single families were reared separately in bowls 30 cms in diamter. The rearing techniques were aimed at producing a high yield of adults from the first instar larvae. The newly emerged adults were exposed to 4% DDT for 1 hour. Only families that had zero or a very low mortality after the treatment were kept for the next generation. The mosquitoes were allowed to mate between brother and sister in the cages and 2-3 days after the blood meal gravid females were tubed individually for egg laying. This inbreeding process was repeated for 3-4 generations, the lines that showed no mortality after the treatment in every generation were chosen as the homozygote resistant colony. In some strains after the third generation, mortality was still found in every family line. These were further selected for another three inbred generations, as the susceptibility gene may remain concealed in a family until the third generation in the case of dominant resistance (see Figure 4).

The single family selection lines for DDT resistance were mostly propagated using mass mating when a family was numerous. However,

Figure 3. Vials containing water and individual gravid female mosquitoes for obtaining eggs.

Figure 4: A diagram to show the effect of inbreeding and selection.



R = resistance allele

S = susceptible allele

(N.B. In the event that resistance is fully dominant the F_1 generation would be fully resistant and susceptibility would only appear in a proportion of the F_2 families).

when the numbers were very low, an artificial mating technique was used. The induced copulation of mosquitoes was originally described by McDaniel and Horsfall (1957). In this study a modified method which has been described by Baker et.al., (1962), Mason (1964) and Haridi (1971) was used.

4.2. Selection for susceptible colony

Because the original susceptible strain did not give a 100% mortality after an exposure to 4% DDT for one hour, it was decided to re-select for DDT susceptibility by the single family selection method as described above, but applying the selection in reverse. Only a sample of the emerged adults from each family were exposed to 4% DDT for one hour. The sibs of these sample adults, which showed 100% mortality after the treatment, were kept for further generations. This procedure was repeated for three generations. Only the families that showed 100% mortality in every generation were considered for the susceptible colony.

5. Genetical study methods

In order to investigate the degree of dominance of resistance, the resistant and the susceptible strains were crossed by mass mating. The reciprocal cross was also made to test for any maternal affect.

Preliminary work confirmed that sperms were not found in the spermathecae of one day old mosquitoes which had been caged with males of the same age. Virgin females could therefore be reliably collected by separating the sexes within 24 hours of emergence. Mass matings were performed by placing 50-100 virgin females of one strain with

at least 100 males of the other strain in a 20 x 20 x 20 cms cage.

Because the single family rearing techniques described by Davidson (1958), Mason (1964) and Haridi (1971) are laborious and time consuming, the following modified mass rearing method was used. Eggs were collected by placing bowls containing filter paper and water in cages containing fully gravid females overnight. The bowls were removed from the cages and the eggs allowed to hatch. About 300 larvae were then reared in the larval bowls as described above in section 1 of the Methods. Feeding and the density of the larvae is very important because the emerged adults may be more susceptible to insecticides if the larvae are underfed or overcrowded (Davidson, 1958). All rearing methods were standardized as much as possible in order to obtain the best yield in the larval stages and ultimately adults in a suitable physiological state. With these standardized rearing methods a yield of emerged adults of about 75% of the larval input was obtained.

One-day-old adults from the resistant and susceptible strains and the progeny of the reciprocal crosses (F1) were tested with 4% DDT by a time response test. This has been used for houseflies (Milani, 1960, 1963), mosquitoes (French and Kitzmiller, 1963; Suzuki, 1968) and cockroaches (Susuki, 1968). The regressions of probit mortality on log exposure times were calculated using an HP25 calculator programmedto provide the slope of the regression line and the LT50 value.

To try to determine whether a single gene is responsible for DDT resistance the following method was used, which is based on a plan proposed by Wright (1952). This involves repeating backcrossing of

of the hybrids to the susceptible parental strain and the selection of the offspring with a dose that will kill all the susceptible homozygotes but is sublethal to the heterozygous population. These crosses are illustrated in Figure 5 on the assumption that only one gene is involved in causing resistance. In this case 50% of susceptibility are expected at each backcross.

The inheritance of dieldrin resistance was studied using two discriminating doses of dieldrin as follows:

a) 0.4% dieldrin for 1 hour's exposure to kill all the homozygote susceptible mosquitoes; the resistant heterozygous and homozygous individuals survived at this dose.

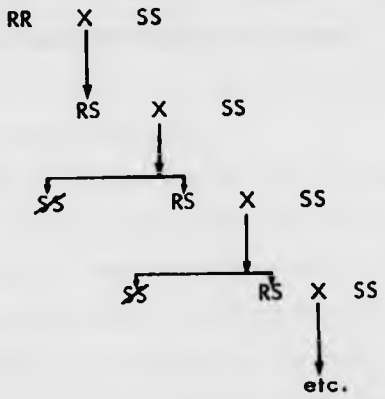
b) 4% dieldrin for 2 hours' exposure to kill all the susceptible homozygous and the resistant heterozygous mosquitoes; only the resistant homozygous individual survived at this dose.

6. Linkage studies

To determine the linkage of the DDT and dieldrin resistance genes in the various resistant colonies, they were each crossed with the susceptible 16c10 stock by mass mating. The F1 progeny were then backcrossed with the susceptible (16c10) strain. The first instar larvae were divided into 3 groups.

Group 1. The larvae were reared as normal. Half of the emerged adults were tested with 4% DDT for 1 hour and the remainder were tested with 0.4% dieldrin for 1 hour.

Figure 5: Demonstration of monofactorial inheritance by repeated backcrosses. The SS genotypes are eliminated by the selective dose of DDT.



R = resistance allele

S = susceptible allele

Group 2. The larvae were treated with 0.01 ppm DDT for 24 hours and the survivors were reared as usual. The emerged adults were tested with 0.4% dieldrin for 1 hour.

Group 3. The larvae were treated with 0.01 ppm dieldrin for 24 hours, the survivors were reared and the emerged adults were tested with 4% DDT for 1 hours.

The results of the larval treatment and adult tests were recorded and analysed statistically to determine the extent of linkage between the dieldrin and DDT resistance genes.

7. The population cage experiment

After mass selection for DDT resistance the Tog strain was used in this study to initiate a cage population. The survival time under the cage condition was investigated by recording the daily mortality and egg production of a cohort of males and females. The mean generation time was calculated as the time from the start of a cage population until the median day of female egg production together with the duration of the immature stages.

On the basis of the adult male and female survival times and the size of the cage, it was considered that a weekly input of male and female pupae would give an equilibrium population of suitable size. The pupae were sexed under the stereomicroscope (see Figure 6 which illustrates the sex difference). The pupae were released into a large laboratory cage (size 45 x 45 x 45 cms) weekly to establish a constant cycling population. In order to evaluate the time when the cage

population had come to equilibrium, the dead female and male mosquitoes were counted and cleared every day. The caged mosquitoes were fed on anaesthetised guinea pigs twice a week. The eggs were collected two days after the blood meal, counted and allowed 48 hours for hatching. Larval counts were made to determine the hatchability rate. The larvae from every egg batch were reared as described in section 1 of the Methods and some pupae were used to produce the required weekly input. The remainders were allowed to emerge and adults were tested with 4% DDT papers at a series of exposure times and the LT50 was calculated as already described above in section 5 of the Methods.

8. Release of males carrying DDT susceptibility genes

One cage population was maintained as a control. Another population was submitted to released males carrying DDT susceptibility genes.

The R70 strain in which the semidominant dieldrin resistant gene has been translocated onto the Y chromosome (Curtis et.al., 1976) was used to provide males for release into the cycling cage populations to attempt to change its composition. In this strain it was found that adult females are susceptible to both dieldrin and DDT, but the males are heterozygous for resistance to dieldrin. To avoid the accumulation in this strain of the products of crossing-over between the dieldrin locus and the translocation, the R70 males were continually backcrossed to susceptible homozygote females of the 16c10 strain. This backcross was set up every two weeks. To ensure that there were enough susceptible pupae or adults to be released, two cages of this backcross were always maintained. Eggs were collected twice a week three days after each blood meal. They were allowed 48 hours for hatching in the

bowls and the larvae were decanted off leaving the eggs adhering to the filter paper. The first stage larvae were treated with 0.02 ppm dieldrin for 24 hours (Curtis et.al., 1976) to kill all the susceptible homozygotes (i.e. females). After 24 hours exposure, instead of diluting the solution tenfold, as was done by Curtis et.al., (1976), the larvae were filtered and washed with clean water several times (see Figure 7). The surviving larvae were set up in the bowls and maintained as described before. Almost all the emerged adults were males and a record was kept of the number of females. Some of the males were used to set up the new backcross to susceptible 16c10 virgin females and from the remainder a counted number of the male pupae (sexed under the microscope) or adults as specified in the Results section were used for weekly release into the cage population. As specified in the Results section, at a certain stage in the experiment the newly emerged females for introduction were treated with 4% DDT for 1 hour and the required input of females was obtained from among the survivors.

9. Stabilation

In order to carry out the quantitative studies required in the present work standardised infection procedures were essential. Wery (1968) described the inoculation into mice of blood infected with rodent Plasmodium leading to a slowly rising parasitaemia and gametocyte formation but he pointed out that a large proportion of the gametocytes were not viable. He obtained good mosquito infections by using parasites which had been stored deep-frozen at -70°C which had not been maintained through more than five or six syringe passages. Susceptible strains of mosquitoes fed on these mice became reliably infected. Wery (1968)

Figure 6. Pupae of An. gambiae s.s., ventral view, to show the differences in the shape of the genital pouch of female and male which was used for sex separation.

a) blunt and small genital pouch of female pupa

b) pointed and large genital pouch of male pupa

Figure 7. Filtration of the R70 first instar larvae following 24 hours treatment with 0.02 ppm dieldrin.

differences

used for

24 hours

Figure 6

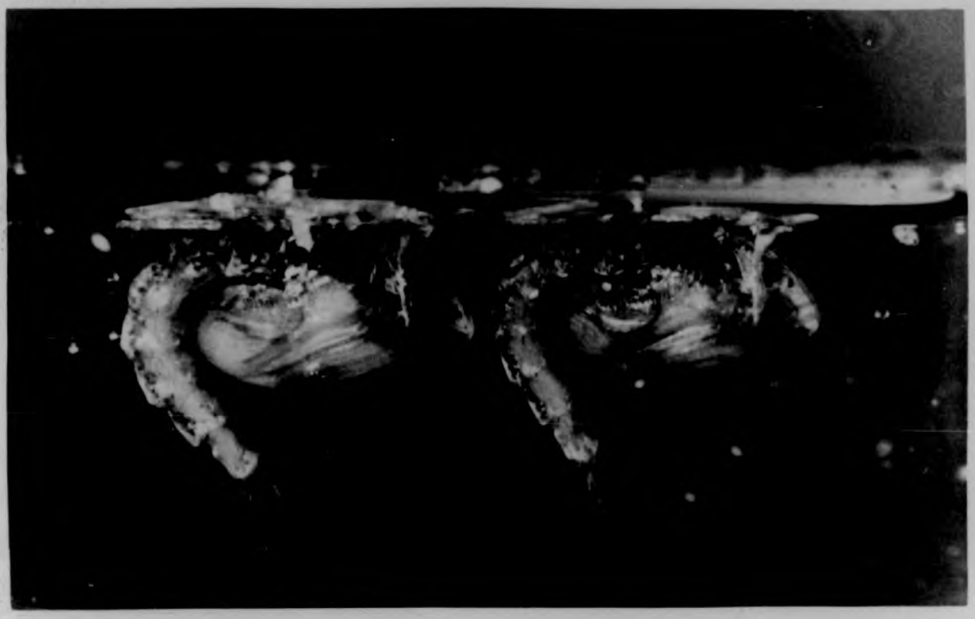


Figure 7

found that from the 7th blood passage onwards the infection rates in the fed mosquitoes dropped quickly with irregular infections and the stomachs were only lightly infected. After ten syringe passages infection became very difficult. However, if a cyclical passage through a mosquito was performed after the 7th blood passage, it restored the viability of the gametocytes and a high rate of infection could again be obtained in mosquitoes for about five blood passages.

Several other workers have confirmed that continuous blood passage of rodent malaria parasites from mouse to mouse results in the loss of their ability to produce gametocytes (Yoeli *et.al.*, 1966; Vanderberg *et.al.*, 1968; Hawking, 1972). In order to avoid this phenomenon in the present work and to standardize the viability of the gametocytes, a large stock of parasites was prepared and preserved so that numerous replicate inocula could be obtained as described below. Blood from one mouse could be used to prepare 50-70 stabilates.

A batch of *A. stephensi* carrying infective sporozoites was allowed to feed on 3 mice aged 3-4 weeks for a few hours. Blood-stage parasites were seen on the fourth day after the mice were infected with sporozoites and the mice developed high parasitaemia in 6-7 days. Stabilates were prepared on day 7, when the parasitaemia was about 20%. The stabilation of a heparinized suspension of the blood of an infected mouse was performed as described by Lumsden *et.al.*, (1973) who found that such stabilates can be kept in liquid nitrogen (-196°C) for at least 2 years without loss of viability.

10. Mosquito infection

For mosquito infection Theiler's original (T.O.) strain of white mice were used. This strain is highly susceptible to all rodent malarias, (Yoeli, 1965; Wery, 1968; Killick-Kendrick, 1974). Only male mice, aged 4-6 weeks, weighing between 12-20 gms, were used.

For each of the malaria infection experiments, a group of three mice were inoculated with stabilate which had been diluted 1:10 with normal saline. Mice Nos 1, 2 and 3 were inoculated with 0.1, 0.2 and 0.3 ml of diluted blood respectively. Thin blood smears were made daily, stained in Giemsa as described by Maryon and Shute (1966) and examined for parasitic stages and parasitaemia. The rise of the parasitaemia took three to six days in these mice. One out of the three mice was chosen for further passage on the basis of a parasitaemia of 10-20% with the parasites mostly at the trophozoite or young schizont stages. 0.05 ml of infected blood were taken from the tail of the selected mouse and mixed with 0.95 ml normal saline and passaged into another group of three mice. The amounts of diluted blood used were 0.1, 0.2 and 0.3 ml and these were injected intraperitoneally into mice Nos 1.1, 1.2 and 1.3 respectively. This group of mice was ready for feeding the mosquitoes three days after inoculation. At this time, thin blood films were made from blood samples from the tails of the mice. The proportion of infected blood cells and number of male and female gametocytes were counted to select the most suitable mouse for mosquito feeding. Preference was given to a mouse with a parasitaemia of about 5-15% and very few gametocytes. Garnham (1966) stated that to get a very good infection, the best time to feed the mosquitoes is when the gametocytes are in the very early

stages of maturation. The stages of the infection procedure are shown in Figure 8.

The general techniques followed those adopted by Very (1968), Killick-Kendrick (1971) and Al-Mashhadani (1976) in their studies of the rodent malarias. The mouse was placed on its back on a wooden board, immobilized by means of adhesive plaster and drawing pins and the abdomen was shaved. It was found that if the infected mouse was placed in the mosquito cage mosquito feeding took a very long time. The following quicker method was therefore usually employed. Starvation of mosquitoes by taking the sugar lint wick out 12 hours before being fed was not necessary. Thirty to fifty unfed female mosquitoes, aged 5-7 days old, were aspirated out of the cage and placed in a plastic drinking cup, covered with netting. This cup of mosquitoes was held against the abdomen of the mouse by means of adhesive plaster attached to the wooden board as shown in Figure 9. It was left for 30 minutes, by which time nearly all the mosquitoes were blood fed. The control groups of mosquitoes were allowed to feed on the mouse first, followed by groups treated with sublethal doses of insecticide. In some experiments, a group of mosquitoes was treated with an insecticide only once before being given an infected blood meal and in the other experiments, groups of mosquitoes were treated with an insecticide once before being given an infected blood meal and then treated 2-3 times after the blood meal. After feeding, the mosquitoes were released into a 20 x 20 x 20 cms cage. All unfed or partially fed female mosquitoes were destroyed leaving only the fully engorged females for future dissection. The mosquitoes were supplied with 10-20% glucose solution

Figure 8: A diagram showing the stages leading to mosquito infection.

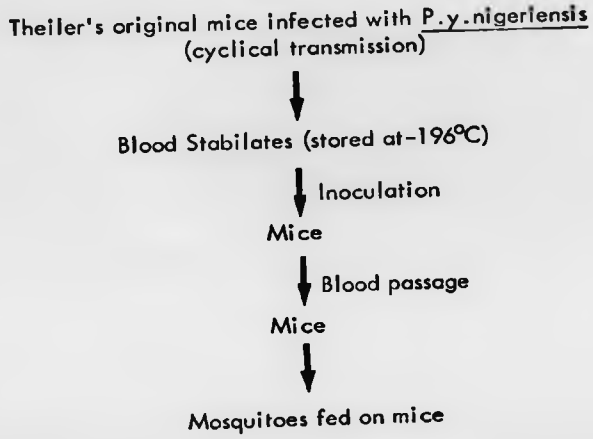


Figure 9. Mosquito feeding:

9.a) The mouse was fixed on a wooden board and the abdomen shaved

9.b) The cup of female mosquitoes was held against the abdomen of the mouse in order to allow them to feed. This feeding procedure was performed in cages to avoid the escape of mosquitoes that are infected.

Figure 9a

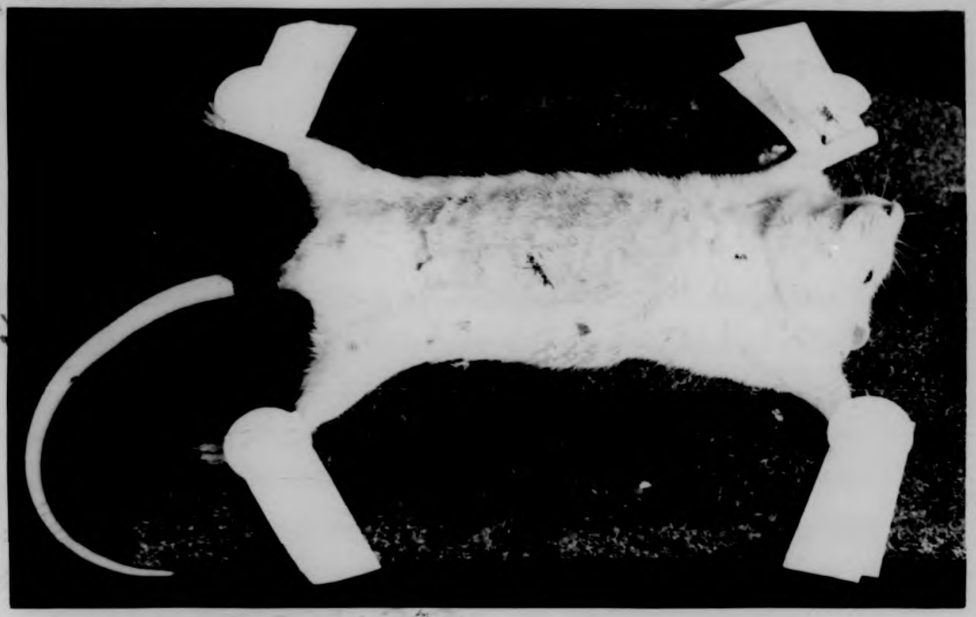
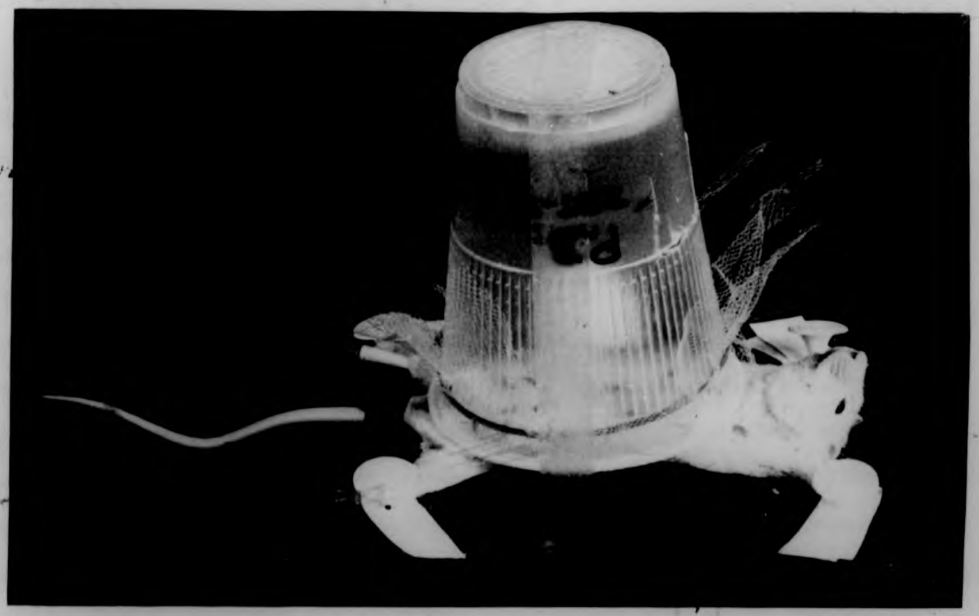


Figure 9b



without any more blood meals and held in a somewhat cooler insectary than usual. Killick-Kendrick (1973) stated that the optimum temperature of sporogony is 24° - 26°C. For the present work these mosquitoes were kept at 24° - 26°C and a relative humidity of about 70-80%.

11. Preservation of mosquitoes for malarial oocyst and sporozoite dissections

In most of the experiments, after the infected blood meal the mosquitoes were allowed to feed only on 10-20% glucose solution without any more blood meals. However, in certain experiments, the mosquitoes were given regular blood meals twice a week. It was found that the sporogonic cycle of P. y. nigeriensis in A. stephensi and A. gambiae s.s. takes 10 days when the infected mosquitoes were maintained at 25-25.5°C and in most experiments all the surviving mosquitoes were killed 10 days after the infecting blood meal.

In determining malaria infection rates in mosquitoes, the number which could be dissected on any one day was very limited. When the sample size was small, all the mosquitoes could be dissected on the day of killing. However, when the sample size was large, a freezing technique based on that which has been described by Ward (1962) and Ungureanu (1971) was used to keep the mosquitoes for dissection when time permitted. The details of the technique were as follows. The mosquitoes were immobilized by exposure to ether and transferred to a glass bottle containing a small piece of filter paper moistened with water, which was added to prevent drying of the mosquitoes. The bottles were capped, labelled and stored in a -10°C to -20°C freezer until dissection (see Figure 10).

To dissect the stored mosquitoes, a bottle was removed from the freezer and was allowed to remain at room temeprature for a few minutes before it was opened. The legs and wings of the mosquitoes were removed and four to five mosquitoes were placed in separate drops of normal saline (0.85% NaCl) - one slide (see Figure 11). The mosquitoes were dissected according to the method described by Maryon and Shute (1966). The presence or absence of the parasite was recorded, the number of oocysts on the stomach were counted and the density of the sporozoites in the salivary gland was recorded on an arbitrary scale as follows. The gland infections were grade 1+ when one to ten sporozoites were found. Grades 2+, 3+ and 4+ when 11 to 100 , 101 to 1,000 and greater than 1,000 sporozoites respectively were judged to be present. A sporozoite mean score was calculated by the following formula.

$$\text{Sporozoite mean score} = \frac{\text{Total of sporozoite grade scores}}{\text{Number mosquitoes dissected}}$$

In view of the wide range in numbers of oocysts per stomach, the oocyst counts per stomach were averaged as Williams' means (Williams, 1937).

Figure 10. Glass bottles containing moistened filter paper and mosquitoes. The bottles were capped, labelled and stored in a -10°C to -20°C freezer.

Figure 11. Dissection of the gut and salivary glands of mosquitoes. Four to five mosquitoes were dissected on the same slide.

Figure 10

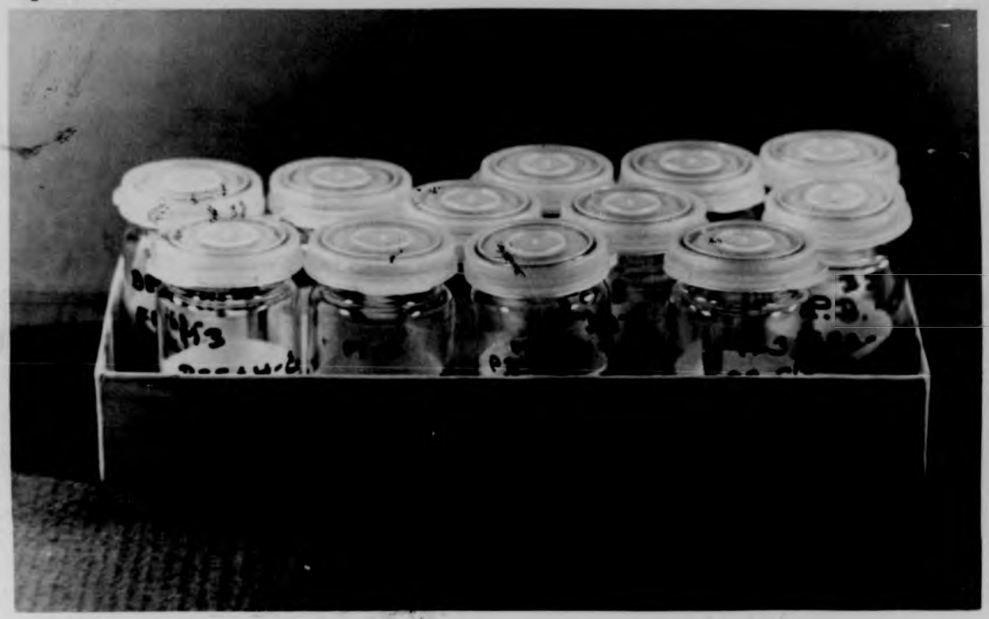
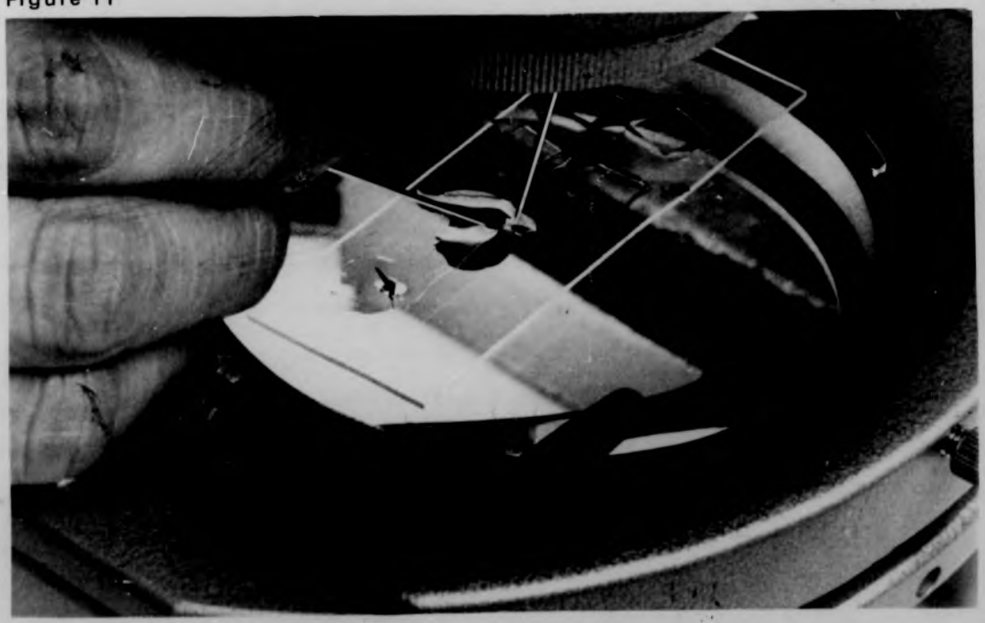


Figure 11



RESULTS

1. Selection of DDT resistant and susceptible colonies

1.1. Selection of DDT resistant colonies

Three strains of An. gambiae s.s., namely Tog, Ian and Pala were submitted to DDT selection by either the mass or the single family selection method. Four percent DDT applied to the adults was used throughout the whole series of selection.

1.1.1. Mass selection

For mass selection the population of males and females was exposed to 4% DDT at a series of exposure periods and the survivors were pooled and used as parents for the next generation. This process started with a short period of exposure, e.g. 1 hour, and was repeated with increased exposure times. Highly DDT resistant colonies were obtained after several successive generations of mass selection.

A. Tog strain. The results of mass selection are shown in Table 2 which includes data on the mortality at each exposure time and the slopes of regression lines of probit mortality against log dose and the calculated LT50 values. The original stock showed 37% mortality following exposure to 4% DDT for one hour. The 63% of survivors were allowed to mate among themselves to give 'Tog 1'. A one hour exposure period was used for a further two generations to select the parents for 'Tog 2' and 'Tog 3'. Adults 'Tog 3' were exposed to 4% DDT for four hours, which gave about 32% survivors. These were again allowed to mate among themselves to give 'Tog 4'. From 'Tog 4' onwards eight hours exposure was used as the dose for selecting the parents of every

Table 2: Mass selection for DDT resistance of adults of the Tog strain of *An. gambiae s.s.* by exposure to 4% DDT

Generations	% Mortality following a series of exposure periods (No. tested)					Slopes* of regression lines	LT50 values (hrs.)
	0.5 hr.	1 hr.	2 hrs.	4 hrs.	8 hrs.		
Parental Tog	10.7 (309)	37.4 (318)+	73.2 (168)	90.0 (100)	-	2.8	1.3
Tog 1	10.7 (56)	19.5 (231)+	69.8 (116)	82.7 (52)	-	2.6	1.6
Tog 2	6.0 (50)	19.0 (100)+	57.3 (75)	70.2 (201)	92.0 (25)	2.4	2.1
Tog 3	-	23.2 (138)	52.4 (194)	67.7 (301)+	71.2 (125)	1.4	2.5
Tog 4	-	13.4 (97)	49.0 (100)	62.9 (116)	68.8 (138)+	1.7	3.1
Tog 5	-	3.4 (207)	7.6 (145)	25.3 (75)	54.3 (140)+	2.2	7.8
Tog 6	-	5.6 (125)	21.0 (100)	40.0 (100)	40.8 (120)+	1.5	8.3
Tog 7	-	4.8 (62)	6.7 (60)	36.0 (100)	42.9 (91)+	1.9	8.9
Tog 8	-	4.7 (128)	20.9 (43)	26.9 (52)	51.2 (127)+	1.8	7.7
Tog 9	-	4.4 (69)	7.2 (111)	34.0 (47)	50.0 (60)+	2.1	7.7
Tog 10	-	4.4 (90)	13.3 (30)	30.0 (40)	48.8 (129)+	1.9	8.0
Tog 11	-	6.3 (16)	0 (72)	2.4 (41)	66.4 (182)+	2.2	6.7
Tog 12	-	0 (12)	-	44.8 (67)	55.0 (191)+	6.0	6.0
Tog 13	-	1.4 (74)	7.2 (69)	29.1 (55)	64.7 (292)+	2.9	6.1
Tog 14	-	5.1 (158)	20.1 (229)	39.8 (83)	55.9 (136)	2.0	6.0

+ Denotes exposure used to select the parents of the next generation

* Regression of probit mortality on log exposure period

successive generation until 'Tog 14'. It will be seen that LT50 values increased fairly steadily in successive generations, particularly at 'Tog 5' when the population was first selected for eight hours. LT50 values remained very similar around 7-8 hours from 'Tog 6' to 'Tog 10' and in the following four generations the LT50 values remained around six hours. The periodic renewal of the DDT papers in the testing tubes is, perhaps, one of the reasons for the minor fluctuations. Selection with eight hours exposure periods has therefore been carried out for ten successive generations without any further appreciable change in the LT50 values. As stated in section 1.1.2 of the Methods, if the resistance was dominant, mass selection would not give full homozygosity. This population was maintained without further selection and designated Tog 14. The DDT resistance level as measured by the LT50 value had made a 4.6 fold increase on the original stock.

B. Ian strain. The selection procedures were similar to those of the Tog strain and the results of the DDT selections are shown in Table 3. The original stock of this strain showed a 19% mortality at one hour exposure to 4% DDT which is about a half that of the Tog strain. Therefore for the Ian stock, 4% DDT for two hours was used as the selection dose from the beginning. The survival rate was about 52%, yielding the parents for 'Ian 1'. 'Ian 1' was exposed to 4% DDT for four hours which gave about 29% of survivors which were the parents for 'Ian 2'. From 'Ian 2' onwards an eight hours exposure period was repeatedly applied until 'Ian 10', and for the last eight generations the LT50 values remained 5-6 hours, i.e. slightly less than in the 'Tog 14' stock. This population is now called 'Ian 10'. The DDT resistance level is approximately 3.1 x that of the original stock.

Table 3: Mass selection for DDT resistance of adults of the Ian strain of *An. gambiae s.s.* by exposure to DDT

Generations	% Mortality following a series of exposure periods (No. tested)					Slopes* of regression lines	LT50 values (hrs.)
	0.5 hr.	1 hr.	2 hrs.	4 hrs.	8 hrs.		
Parental Ian	6.0 (50)	19.0 (100)	48.0 (100)+	69.2 (26)	92.0 (25)	1.8	1.8
Ian 1	-	18.6 (70)	42.0 (50)	71.4 (140)+	85.0 (74)	2.4	2.4
Ian 2	-	16.0 (25)	44.0 (25)	54.0 (50)	76.7 (103)+	1.8	3.1
Ian 3	-	10.8 (167)	13.2 (76)	32.5 (77)	73.8 (107)+	2.1	5.2
Ian 4	-	8.2 (98)	16.7 (84)	35.4 (147)	73.7 (186)+	2.2	4.9
Ian 5	-	9.4 (53)	22.0 (41)	45.2 (62)	64.9 (134)+	1.9	4.9
Ian 6	-	0 (112)	24.5 (110)	44.3 (122)	63.3 (150)+	5.5	5.0
Ian 7	-	10.1 (208)	18.6 (70)	42.9 (133)	73.2 (354)+	2.1	4.5
Ian 8	-	4.4 (159)	8.0 (138)	39.6 (101)	63.9 (255)+	2.1	7.7
Ian 9	-	6.5 (301)	12.0 (159)	20.0 (10)	67.1 (79)+	2.1	6.7
Ian 10	-	4.6 (285)	19.7 (198)	41.8 (91)	59.0 (200)+	2.1	5.6

+ Denotes exposure used to select the parents of the next generation

* Regression of probit mortality on log exposure period

C. Pala strain. Results of DDT selection of this strain are shown in Table 4. The original stock showed little resistance to DDT and the final level of resistance reached was less than those of the first two strains. The original stock was exposed to 4% DDT for one hour which gave about 13% survivors and these were used as the parents of 'Pala 1'. From 'Pala 1' to 'Pala 6' two hours was used as the selection dose and at 'Pala 6' approximately 53% survivors was obtained at this dose. The exposure period for selection was then increased to four hours. About 17% survivors were obtained following four hours treatment of 4% DDT in 'Pala 7' and these survivors were used as the parents of 'Pala 8'. This process was repeated for another two generations when approximately a 3.5 fold increase on the resistance level of the original stock had been achieved. This final product was called 'Pala 10'.

The response of DDT selection in these three strains investigated are compared in Figure 12. This figure re-emphasizes that the Tog strain showed a greater response to DDT selection than either Ian or Pala.

1.1.2. Single family selection

Though mass selection reached plateau levels, it is very difficult to say that the DDT resistance had become homozygous because of the possibility that resistance was dominant and that some susceptibility genes would be left in the populations, hidden in heterozygous form and hardly affected by mass selection when the frequency of the resistance gene became high. Homozygosity for resistance may be produced by single family selection, as described in

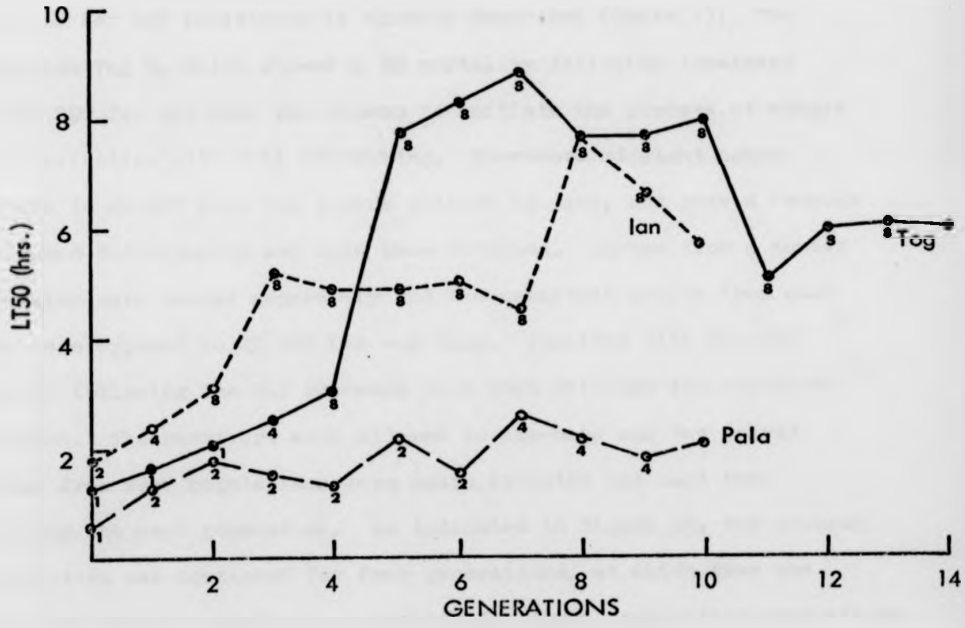
Table 4: Mass selection for DDT resistance of adults of the Pala strain of *An. gambiae s.s.* by exposure to 4% DDT

Generations	% Mortality following a series of exposure periods (No. tested)					Slopes* of regression lines	LT50 values (hrs.)
	0.5 hr.	1 hr.	2 hrs.	4 hrs.	8 hrs.		
Parental Pala	36.0 (50)	87.0 (57)+	98.0 (80)	100 (50)	-	4.4	0.6
Pala 1	-	44.4 (45)	59.7 (72)+	98.3 (50)	-	3.7	1.3
Pala 2	-	24.1 (115)	29.0 (100)+	90.8 (139)	100 (50)	5.0	1.8
Pala 3	-	34.8 (155)	47.7 (55)+	97.7 (170)	100 (150)	4.8	1.5
Pala 4	-	45.6 (114)	54.4 (114)+	99.3 (149)	100 (30)	4.6	1.3
Pala 5	-	5.0 (159)	33.7 (202)+	83.8 (148)	100 (30)	5.8	2.2
Pala 6	-	26.7 (30)	46.8 (154)+	100 (52)	100 (55)	7.2	1.5
Pala 7	-	5.7 (105)	27.0 (100)	83.2 (161)+	97.5 (120)	4.1	2.6
Pala 8	-	19.3 (83)	36.4 (66)	69.3 (368)+	97.5 (120)	3.1	2.2
Pala 9	-	24.1 (83)	57.1 (196)	75.8 (244)+	99.2 (132)	3.3	1.8
Pala 10	-	13.1 (84)	41.9 (43)	88.3 (324)	96.4 (28)	3.4	2.1

+ Denotes exposure used to select the parents of the next generation

* Regression of probit mortality on log exposure period

Figure 12: Response of the Tog, Ian and Pala strains of An. gambiae s.s. to selection for DDT resistance when selected with 4% DDT with steadily increasing exposure periods*.



*The numbers under each line indicate the exposure periods (hrs.) used for selection of the parents of the next generation.

section 1.1.2 of the Methods, in which after mass selection the progeny of single females are inbred and those families are selected in which there is no sign of segregation of homozygous susceptible individuals. Treatment with 4% DDT for one hour was utilized throughout the whole series to test for the production of such homozygous susceptibles (Davidson and Zahar, 1973; WHO, 1976).

A. Tog strain. The original strain was submitted to mass selection for DDT resistance as already described (Table 2). The generation Tog 5, which showed a 3% mortality following treatment with 4% DDT for one hour was chosen to initiate the process of single family selection with full sib-mating. Survivors of eight hours exposure to 4% DDT from Tog 5 were allowed to mate, and gravid females were tubed individually and eggs were obtained. Larvae from a number of females were reared separately and the resultant adults from each batch were exposed to 4% DDT for one hour. Families with 95-100% survival following the DDT exposure were then utilized for continued selection. The survivors were allowed to sib-mate and the gravid females from such populations were again isolated and used for obtaining the next generation. As indicated in Figure 13, the process of selection was continued for four generations, at which time one family had shown virtually no mortality for three consecutive generations. This is strong evidence that this family was homozygous for DDT resistance. This family was now called Tog-RR.

A population of this family was built up and used for genetic studies. Adults of Tog-RR were tested with 4% DDT to determine resistance levels and the results are included in Table 6. The LT50

Figure 13: Single family selection of the Tog strain of An.gambiae s.s. The figures indicate mortality on exposure to 4% DDT for one hour.

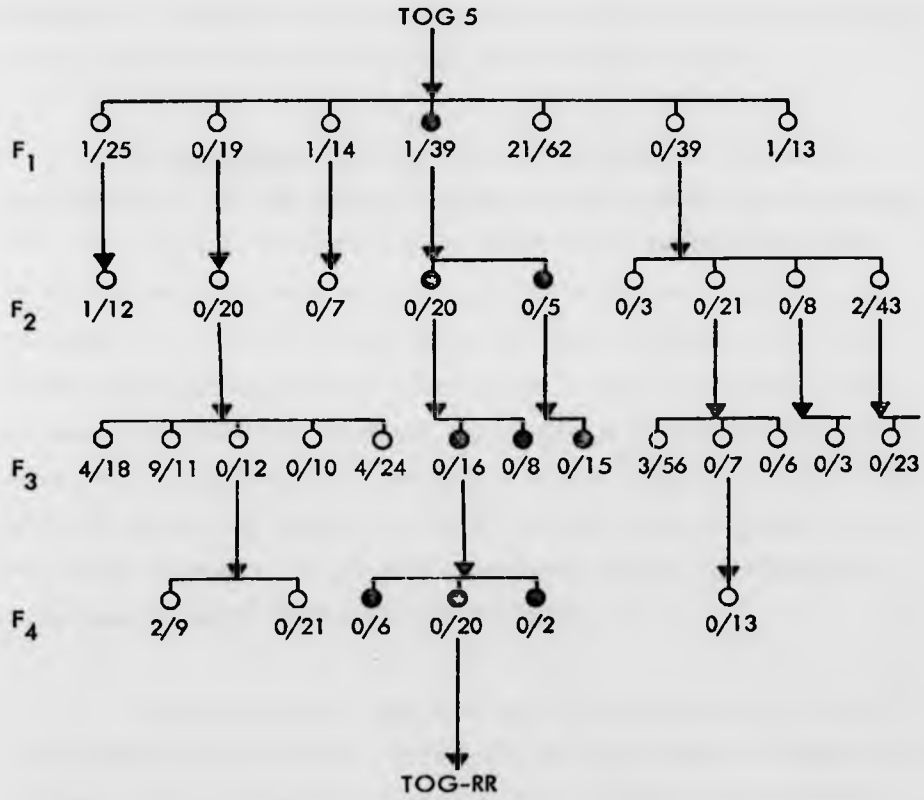
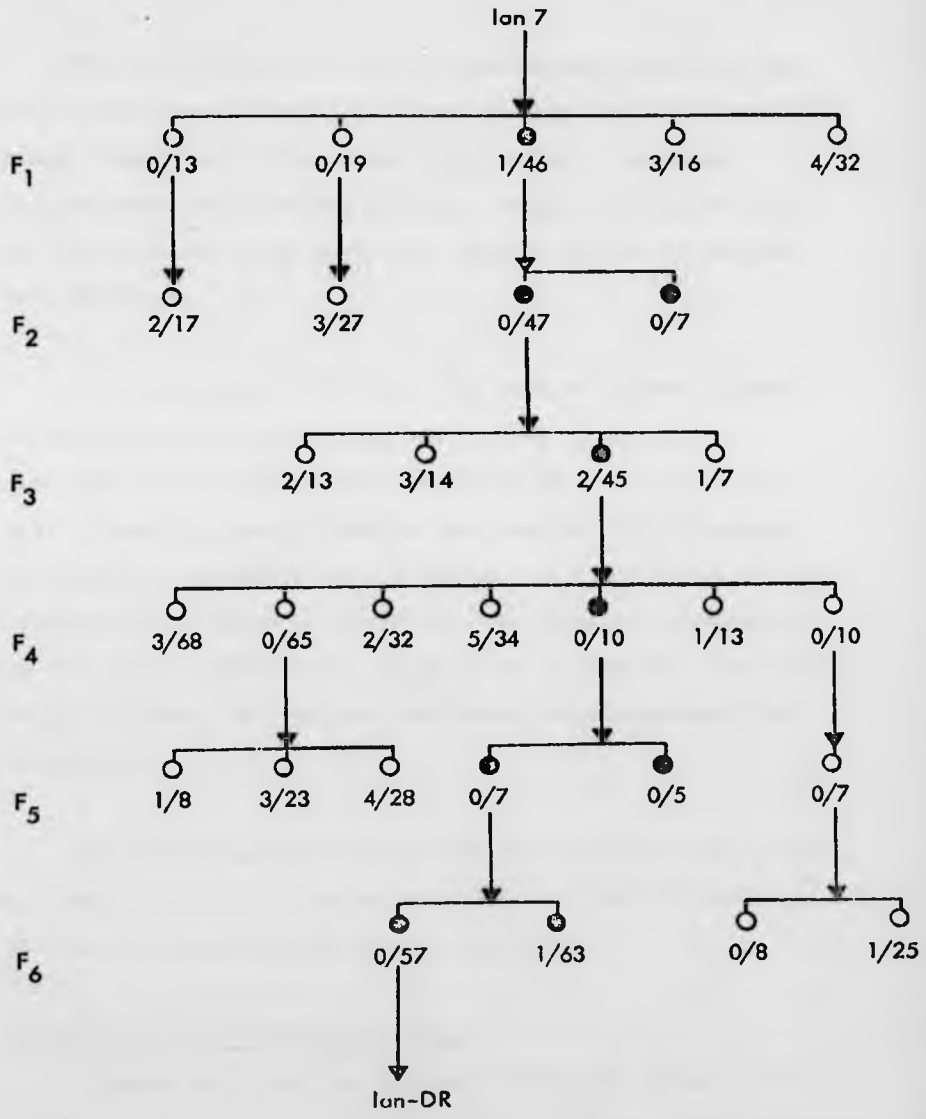


Figure 14: Single family selection for homozygous DDT resistance in the Ian strain of An.gambiae s.s. The figures indicate mortality on exposure to 4% DDT for one hour.



successfully. Because of doubts about its mating capacity, during single family selection of this strain artificial mating was always used.

The Ian-DR strain was built up and tested with 4% DDT and the results are shown in Table 11. Although this strain was derived from single family selections, about 12.5% mortality was found following exposure to 4% DDT for one hour. Moreover, the LT50 value was only 3.1 hours which was about half that of the Ian 10 derived from mass selection.

C. Pala strain. The method was similar to that applied to the Tog and Ian strains and was applied to Pala 7, where a 6% mortality was found following exposure to 4% DDT for one hour (see Table 4). A number of gravid females that survived 4% DDT treatment for two hours were separated for egg laying. An illustration of this selection process is shown in Figure 15. The selection process was carried out for six generations. From one of the families, the Pala-DR was finally obtained, and this was considered to be homozygous for DDT resistance.

The Pala-DR was also tested with 4% DDT and the results are shown in Table 13. The LT50 value was 2.1 hours which is similar to Pala 10 which had been produced by mass selection.

1.2. Selection of DDT susceptible colony

A sample of 139 of the original 16cSS stock showed a 92.1% mortality following treatment with 4% DDT for one hour. This incomplete

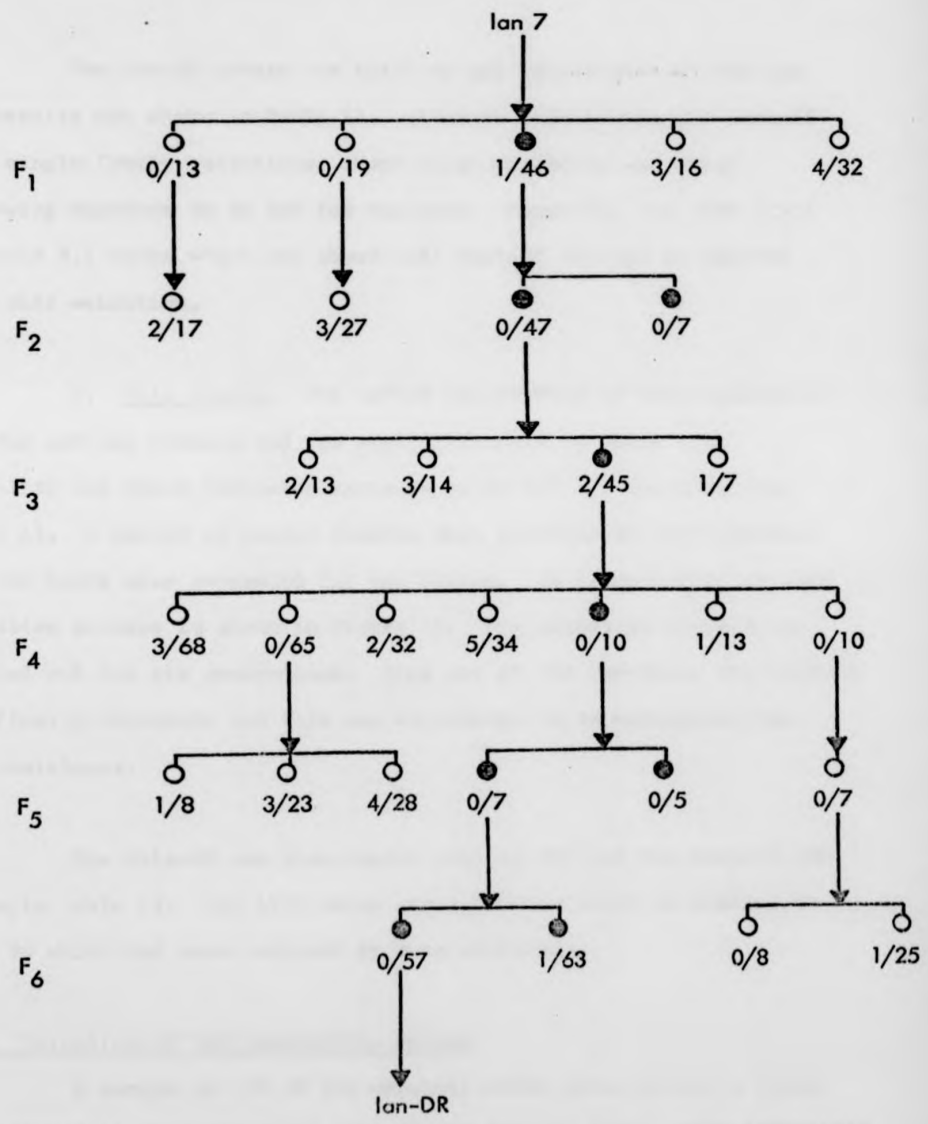
value was 5.6 hours, which is a 4.3 fold increase on the original stock. It is noteworthy that the LT50 was no higher and may have been slightly lower than that of the mass selected Tog 14 stock.

After inbreeding for many generations the data showed that the adult yield was generally poor, presumably due to inbreeding depression. Where the numbers in a family were very small, artificial mating was carried out as mentioned in the Methods section.

B. Ian strain. The generation procedure was similar to that applied to the Tog strain. Single family selection was initiated from Ian 7 where a 10% mortality was found following treatment with 4% DDT for one hour (see Table 3). The whole process is illustrated in Figure 14. It will be seen that after three generations of full sib-mating, some mortality was still found in all the families. The procedure was therefore continued for another three generations. The final product was designated Ian-DR. Although there was one death out of 63 on testing the progeny of the sib of the parent of Ian-DR (i.e. its "first cousins") at the last generation, Ian-DR was considered to be most probably homozygous DDT resistant.

It was found that some families, although present in fairly large numbers (approximately 30-65), did not mate under the cage conditions. This is thought to be due to the proportion of males being too low. This is suggested by results obtained during the mass selection programme when it was found that even when males and females of this strain were kept together at high densities, the number of males had to be about twice that of the females for mating to occur

Figure 14: Single family selection for homozygous DDT resistance in the Ian strain of An.gambiae s.s. The figures indicate mortality on exposure to 4% DDT for one hour.



successfully. Because of doubts about its mating capacity, during single family selection of this strain artificial mating was always used.

The Ian-DR strain was built up and tested with 4% DDT and the results are shown in Table 11. Although this strain was derived from single family selections, about 12.5% mortality was found following exposure to 4% DDT for one hour. Moreover, the LT50 value was only 3.1 hours which was about half that of the Ian 10 derived from mass selection.

C. Pala strain. The method was similar to that applied to the Tog and Ian strains and was applied to Pala 7, where a 6% mortality was found following exposure to 4% DDT for one hour (see Table 4). A number of gravid females that survived 4% DDT treatment for two hours were separated for egg laying. An illustration of this selection process is shown in Figure 15. The selection process was carried out for six generations. From one of the families, the Pala-DR was finally obtained, and this was considered to be homozygous for DDT resistance.

The Pala-DR was also tested with 4% DDT and the results are shown in Table 13. The LT50 value was 2.1 hours which is similar to Pala 10 which had been produced by mass selection.

1.2. Selection of DDT susceptible colony

A sample of 139 of the original 16cSS stock showed a 92.1% mortality following treatment with 4% DDT for one hour. This incomplete

Figure 15 Single family selection for homozygous DDT resistance in the Pala strain of An. gambiae s.s. The figures indicate mortality on exposure to 4% DDT for one hour.

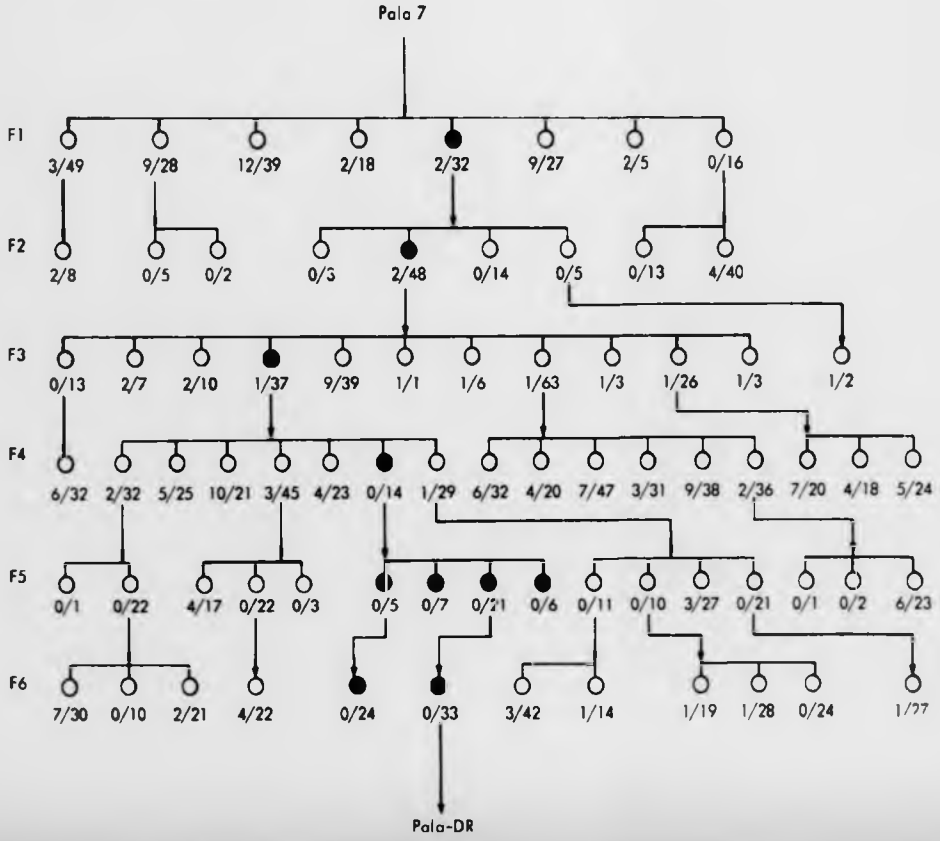
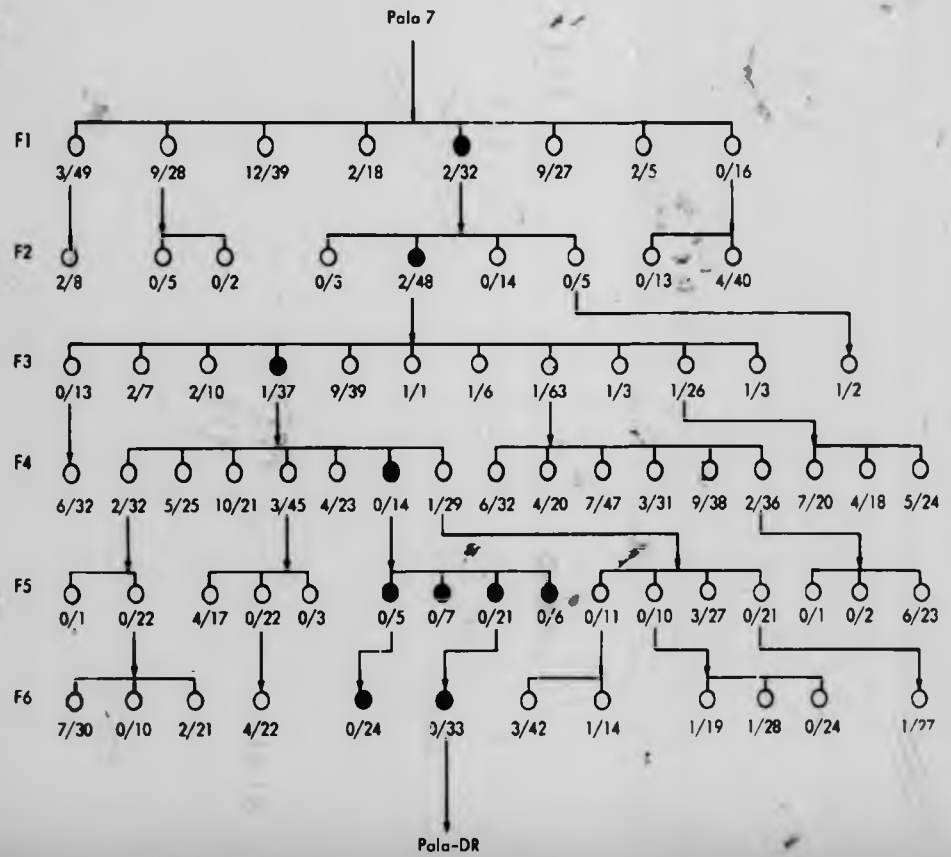
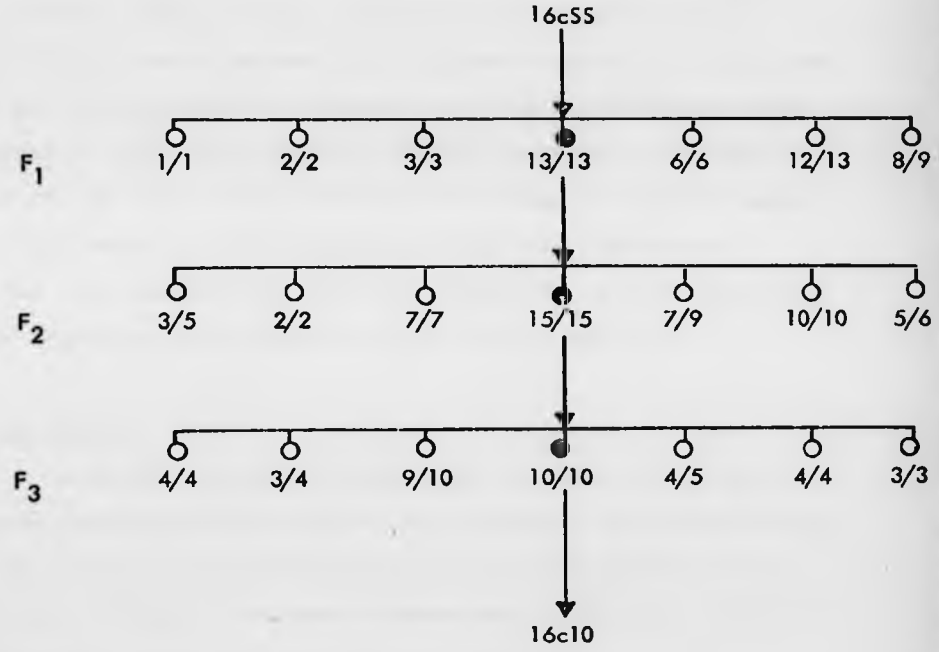


Figure 15: Single family selection for homozygous DDT resistance in the Pala strain of An.gambiae s.s. The figures indicate mortality on exposure to 4% DDT for one hour.



mortality on the discriminating dose for susceptible homozygotes indicated that this stock may not have been fully homozygous susceptible to DDT. It was decided to select for homozygosity by a single family selection procedure. The method is similar to that applied to the resistant strain, but it was applied in reverse. Only a sample of the emerged adults from each family were used for testing. Where the sample showed 100% mortality after treatment, the remainders of the family were kept for continuing selection. Only a family which showed a 100% mortality in each of three consecutive generations was chosen to form the fully susceptible colony. Figure 16 shows an illustration of DDT susceptibility selection process on 16cSS. Four families showed 100% mortality, but all the sample sizes were small and the one with the largest sample (10 mosquitoes) was chosen and designated as 16c10. This was considered to be homozygous susceptible to DDT. This 16c10 stock showed only a 96.9% mortality out of 225 mosquitoes tested following treatment with 4% DDT for one hour (see Table 5).

Figure 16: Single family selection for a DDT susceptible homozygote colony of the 16cSS strain An.gambiae s.s. The figures show mortality on exposure to 4% DDT for one hour.



2. Genetic studies of DDT resistance

Though the susceptible 16cSS strain was subjected to DDT selection for susceptibility, the selected 16c10 strain still showed only 96.9% mortality after one hour exposure to 4% DDT. Thus even after taking precautions to ensure homozygosity of the susceptible strain the supposed discriminating dose did not kill 100% of the susceptible strain. However, 100% mortality was obtained after two hours exposure. Results of DDT testing for a series of times are shown in Table 5, which includes the calculated slope of the regression line of probit mortality on log dose and the LT50 value obtained from the regression line. The LT50 value was 0.34 hours and the regression line was plotted and is shown in Figures 17, 19 and 21. Larval tests on the 16c10 strain to DDT were also performed with first instar larvae and these results are also shown in Table 5. The LC50 was 0.006 ppm; the regression line is shown in Figures 18, 20 and 22.

2.1. Tog strain

Comparisons of the resistance data of adults of the Tog 14 and Tog-RR resistant colonies with that of the 16c10 susceptible colony are shown in Table 6. The LT50 values of Tog 14 and Tog-RR were 6.0 and 5.6 hours, hence the resistance factors were respectively 17.7 and 16.5 times that of the susceptible 16c10 strain.

Resistant mosquitoes of the Tog 14 and Tog-RR strains were reciprocally crossed with the susceptible 16c10 strain and the F1 progeny of the crosses were tested with 4% DDT. These results are shown in Table 6. The regression lines obtained from parents and their hybrids are plotted in Figure 17. The LT50 of both reciprocal hybrids was

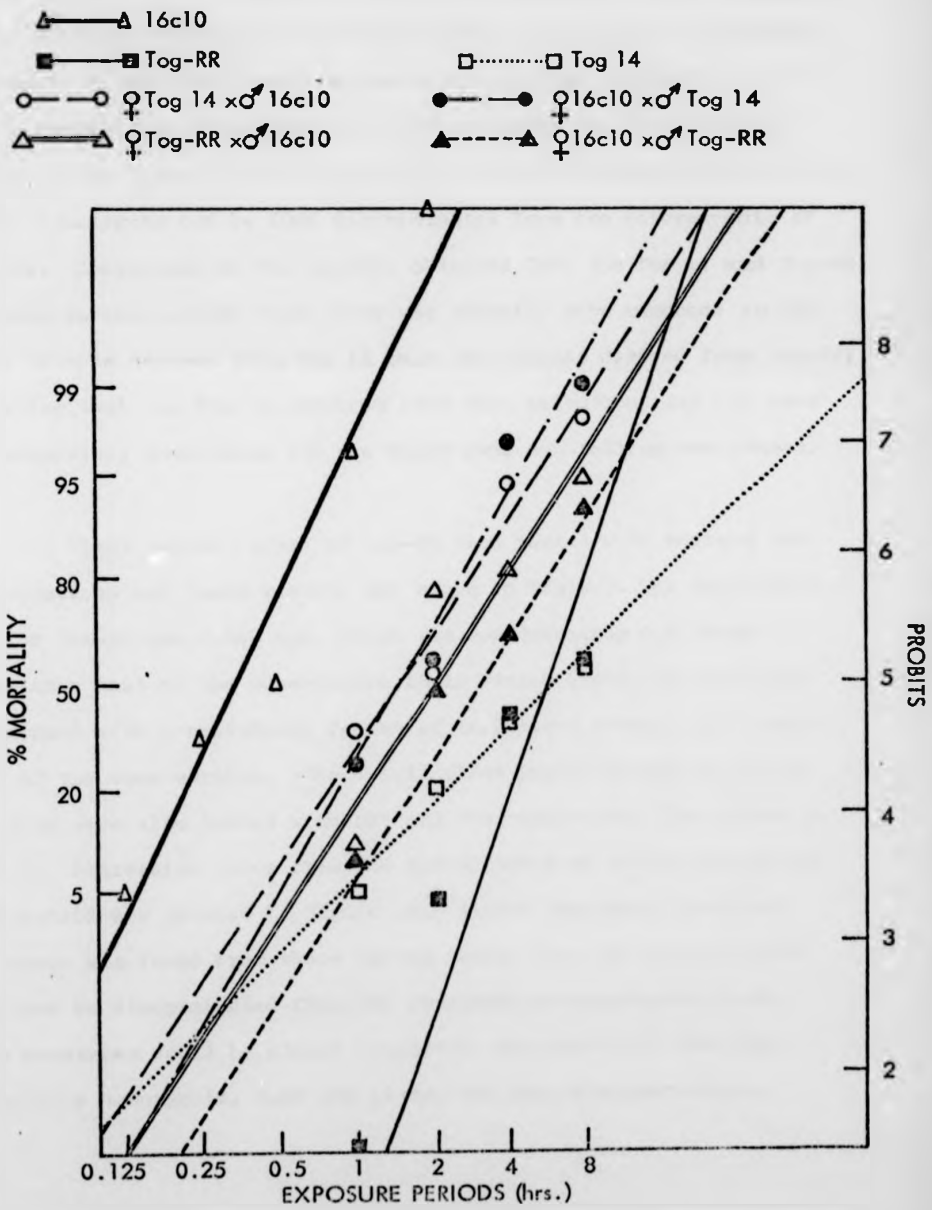
Table 5. Data on the DDT susceptibility of the selected homozygous susceptible strain 16c10

Adult test			First instar larval test (24 hrs exposure)		
4% DDT exposure periods (hrs.)	No. tested	% Mortality	DDT con-centration (ppm)	No. tested	% Mortality
0.125	113	5.3	0.001	222	0.9
0.25	168	33.3	0.005	238	51.5
0.5	162	49.3	0.01	504	77.2
1	225	96.9	0.015	295	86.1
2	55	100.0	0.02	364	97.0
Slope of regression line	4.3		Slope of regression line	3.2	
LT50 value (hrs.)	0.34		LC50 value (ppm)	0.006	

Table 6: Comparison of the resistance of the Tog 14 and Tog-RR resistant strains and the 16c10 susceptible strain and the hybrids from resistant and susceptible stocks. The adults were exposed to 4% DDT

Stocks or hybrids	% Mortality following a series of exposure periods (No. tested)				Slopes of regression lines	LT50 values (hrs.)
	1 hr.	2 hrs.	4 hrs.	8 hrs.		
Susceptible 16c10	96.9 (225)	100 (55)	-	-	4.3	0.34
Resistant Tog 14	5.1 (158)	20.1 (229)	39.8 (83)	55.9 (136)	2.0	6.0
Hybrid (♀ Tog 14 x ♂ 16c10)	35.1 (271)	52.5 (177)	94.9 (255)	97.8 (180)	2.9	1.5
Hybrid (♀16c10 x ♂ Tog 14)	26.3 (236)	57.3 (253)	97.2 (180)	99.2 (240)	3.6	1.5
Resistant Tog-RR	0 (298)	4.7 (256)	40.0 (315)	57.1 (259)	5.6	5.6
Hybrid(♀Tog-RR x ♂16c10)	10.3 (165)	74.9 (243)	80.9 (429)	94.7 (360)	2.9	2.0
Hybrid (♀16c10 x ♂ Tog-RR	8.2 (717)	49.3 (586)	64.3 (765)	91.0 (702)	2.8	2.7

Figure 17: Log time-probit regression lines for DDT-resistant stocks Tog 14 and Tog-RR, susceptible strain 16c10 and for the hybrids from these resistant and susceptible stocks. The adults were exposed to 4% DDT.



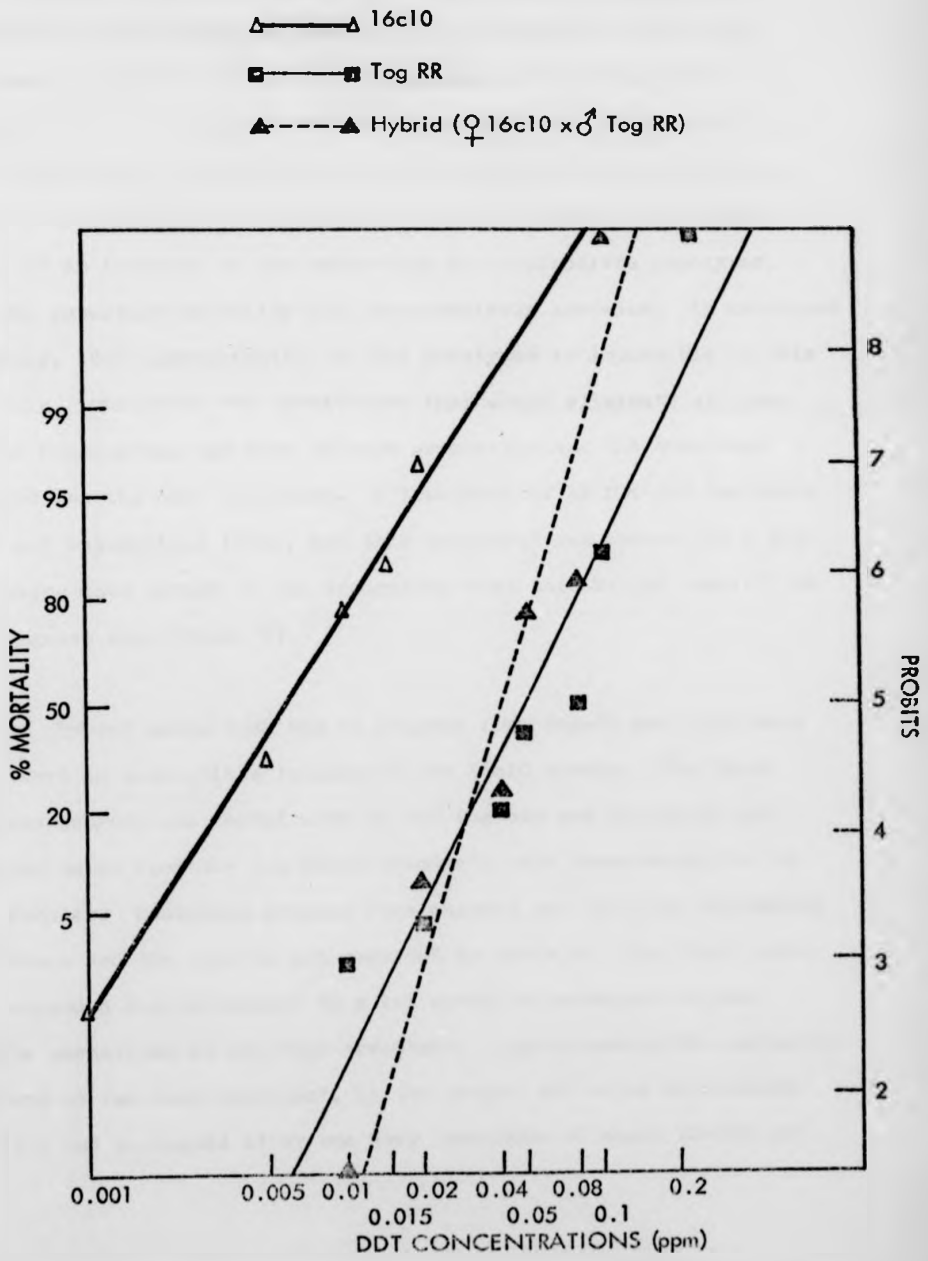
greater than the geometric mean of parents and, by the definition of Georghiou (1969), this indicates that the resistance is incompletely dominant. The hybrids from each pair of reciprocal crosses (derived from Tog 14 and Tog-RR) showed very similar responses to DDT suggesting that no maternal effects or sex-linked genes were involved. Moreover, the results showed that there was an overlap of the regression lines for the susceptible homozygotes and heterozygotes and there was also overlap of the lines for resistant homozygotes and heterozygotes. Thus neither homozygote can be 100% discriminated from the heterozygote at any dose. Comparison of the hybrids obtained from the Tog 14 and Tog-RR resistant parents showed that there was slightly more response to DDT in the hybrids derived from Tog 14 than the hybrids derived from Tog-RR; suggesting that the Tog 14 (derived from mass selection) may not have been completely homozygous for the major gene controlling resistance.

First instar larvae of Tog-RR were tested with various DDT concentrations and these results are shown in Table 7. The calculated LC50 for Tog-RR was 0.049 ppm, which was approximately 8.2 times higher than that of the susceptible 16c10 strain (Table 5) which may be compared with a resistance factor of 16.2 times found in the adult tests of the same strains. The hybrid first instar larvae of Tog-RR and 16c10 were also tested with DDT and the results are also shown in Table 7. Regression lines from the larval tests of 16c10, Tog-RR and these hybrid are plotted in Figure 18. Almost completely dominant resistance was found from these larval tests, i.e. the heterozygote could not be discriminated from the resistant homozygote but both these genotypes could be almost completely discriminated from the susceptible homozygote, 0.02 ppm giving the best discrimination.

Table 7: Data from 24 hours exposure of DDT of the first instar larvae of Tog-RR and its hybrid with 16c10

DDT Concentrations (ppm)	Tog-RR		Hybrid (♀16c10 x ♂Tog-RR)	
	No. Tested	% Mortality	No. Tested	% Mortality
0.005	-	-	108	0
0.01	880	1.8	214	0
0.02	200	4.0	223	7.6
0.04	254	19.7	119	25.2
0.05	496	41.1	78	75.6
0.08	582	49.8	49	83.7
0.1	523	87.8	175	100.0
0.2	313	100	-	-
Slopes of regression lines	4.2		7.4	
LC50 values (ppm)	0.049		0.042	

Figure 18: Log concentration-probit regression lines for DDT-resistant Tog-RR strain, susceptible 16c10 strain and their hybrid. First instar larvae were exposed to various concentrations of DDT for 24 hours.



2.1.1. Backcross studies

Evidence on the mode of inheritance of resistance can be obtained by repeated backcrosses of the survivors of insecticide treatment to the susceptible parent. If resistance is determined by a single major factor, half of the progeny of each backcross generation will be resistant and the other half will be susceptible. On the other hand if several major factors determine the resistance, the level of resistance will decrease in each successive backcross, because of an increase of the proportion of intermediate genotypes, hence the resultant mortality will progressively increase. As mentioned previously, 100% discrimination of the genotypes is impossible in this Tog strain. Therefore the lowest dose that would eliminate all susceptible homozygotes was used at each generation and the survivors were used for the next backcross. A treatment of 4% DDT for two hours killed all susceptible 16c10, and this treatment was chosen for a discriminating dose though it was recognized that this killed some of the heterozygotes also (Table 6).

Hybrid males from the F1 progeny from Tog-RR and 16c10 were backcrossed to susceptible females of the 16c10 strain. The first backcross progeny was tested with 4% DDT for one and two hours and surviving males from the two hours treatment were backcrossed to the 16c10 females. Backcross studies were carried out for four successive generations and the results are recorded in Table 8. The first backcross showed a fair agreement to a 1:1 ratio of resistant to susceptible phenotypes at one hour treatment. Approximately 80% mortality was found at two hour treatment. In the second and third backcrosses mortality had increased after one hour treatment to about 70-74% but

Table 8: Data on the resistance of successive backcrosses to 16c10 following a cross of this stock to Tog-RR

Back-crosses	% Mortality following treatment with 4% DDT for one hour (No. tested)			% Mortality following treatment with 4% DDT for two hours (No. tested)		
	Female	Male	Total	Female	Male	Total
1	61.8 (353)	50.0 (372)	55.7 (725)	82.9 (497)	76.6 (530)	80.7 (1027)
2	71.0 (648)	69.7 (538)	70.4 (1186)	89.2 (342)	79.9 (344)	84.6 (686)
3	79.6 (627)	65.5 (441)	73.8 (1068)	89.3 (308)	79.8 (426)	83.8 (734)
4	59.3 (496)	45.4 (436)	52.8 (932)	81.7 (371)	79.1 (321)	80.5 (692)

the mortality at two hours remained very similar in the consecutive three generations. In the fourth backcross, similar results to the first backcross were obtained. It is difficult to draw a firm conclusion, since the results from one hour exposure rose at backcrosses 2 and 3 but then declined. However, the evidence of similar mortalities in four consecutive generations after a two hour treatment and the lack of an upward trend of mortality, suggests that DDT resistance in Tog-RR was controlled by a single major gene; but the possibility of some polygenic influence is not excluded, especially as the two hours selection at each backcross would have removed those segregants with a large proportion of polygenes for susceptibility.

Data on homozygosity and also on whether polygenic influence is important may also be obtained by observing whether there are changes in the resistance level of the supposedly homozygous population over several generations. When there was no DDT selection pressure, if the population was not homozygous for the major gene for resistance or there were modifying genes involved, after several generations the resistance levels may decrease if natural selection favours the susceptible type. Conversely, renewed DDT selection pressure may increase the resistance level if the supposedly homozygous resistant population is in fact heterogeneous for the major gene or other factors involved in controlling resistance.

DDT resistance levels of the unselected Tog-RR stock were observed for five successive generations and the results are shown in Table 9. LT50 values were 5.6 hours in every generation, except in the second generation where it rose to 7.6 hours. The Tog-RR (produced as

Table 9: Data on resistance of the Tog-RR stock maintained without artificial selection

Generations	% Mortality following 4% DDT at a series of exposure periods (No. tested)				Slopes of regression lines	LT50 values (hrs.)
	1 hr.	2 hrs.	4 hrs.	8 hrs.		
1	0 (126)	4.6 (151)	35.0 (120)	55.4 (168)	5.6	5.8
2	0.7 (149)	11.6 (86)	26.5 (153)	46.2 (143)	2.5	7.6
3	4.2 (48)	8.9 (123)	41.3 (172)	66.0 (285)	2.5	5.5
4	1.5 (274)	8.7 (185)	46.8 (124)	57.8 (204)	2.7	5.9
5	1.7 (118)	11.4 (132)	42.8 (138)	59.8 (164)	3.4	5.6

already described by inbreeding and family selection) was submitted to selection with 4% DDT for eight hours for five generations and the results are shown in Table 10. Although the stock was intensively selected by eight hours treatment, the LT50 values remained similar to that of the parental stocks and to the unselected population of Tog-RR (Table 9). A low level of mortality following 4% DDT exposure for one hour was found in most of the generations, although the original Tog-RR stock showed no mortality at this dose (Table 6). Thus the evidence obtained supported the supposition of homozygosity for resistance in the Tog-RR stock and also supported the hypothesis that DDT resistance of Tog-RR is controlled by single major genes.

2.2. Ian strain

Results of tests on Ian 10 and Ian-DR which were produced by mass and single family selections are shown in Table 11 and it appears that the DDT resistance factors of Ian 10 and Ian-DR were respectively, 16.5x and 9.1x that of the susceptible 16c10 strain. Progeny of the reciprocal crosses of Ian 10 and Ian-DR to 16c10 were tested with DDT and the results are shown in Table 11. Regression lines of probit mortality on log exposure period of 16c10, Ian 10, Ian-DR and their hybrids are plotted in Figure 19. The resistance expressed in the hybrids of Ian 10 showed incomplete dominance, but the hybrids of Ian-DR were very close in their resistance level to the resistant parental stock. However, this is still considered to be a case of incomplete dominance. It is once again impossible to obtain discriminating doses for the three genotypes.

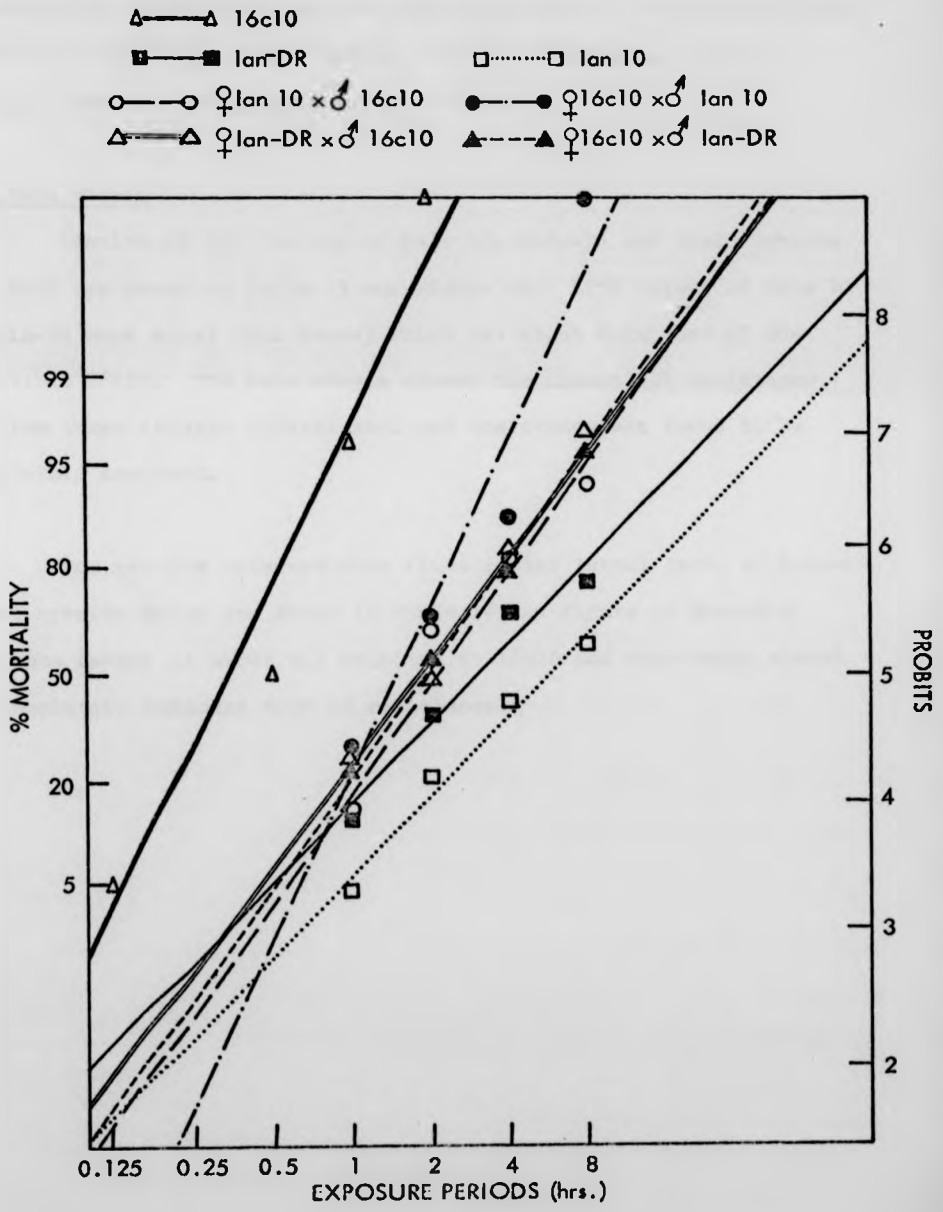
Table 10: Effect of renewed selections on the Tog-RR stocks. The selection exposure was to 4% DDT for eight hours.+

Generations	% Mortality following 4% DDT at a series of exposure periods (No. tested)				Slopes of regression lines	LT50 values (hrs.)
	1 hr.	2 hrs.	4 hrs.	8 hrs.		
1	0 (65)	10.6 (66)	49.5 (95)	51.1 (180)+	5.4	5.5
2	3.3 (121)	8.6 (58)	25.0 (48)	62.6 (267)+	2.4	6.7
3	0 (58)	8.8 (80)	-	61.6 (315)+	5.4	6.0
4	3.9 (103)	6.0 (84)	37.8 (127)	73.9 (188)+	2.8	5.2
5	3.1 (129)	18.2 (198)	34.3 (99)	72.6 (131)	2.6	5.0

Table 11: Comparison of the adult DDT resistance of the selected resistant Ian 10 and Ian DR strains with the 16c10 strain and the F_1 hybrids

Stocks and hybrids	% Mortality following 4% DDT for a series of exposure periods (No. tested)				Slopes of regression lines	LT50 values (hrs.)
	1 hr.	2 hrs.	4 hrs.	8 hrs.		
Susceptible 16c10	96.9 (225)	100 (55)	-	-	4.3	0.34
Resistant Ian 10	4.6 (285)	19.7 (198)	41.8 (91)	59.0 (200)	2.1	5.6
Hybrid (♀Ian 10 x ♂16c10)	12.6 (127)	63.1 (222)	83.2(173)	93.3 (239)	2.8	2.0
Hybrid (♀16x 10 x ♂Ian 10)	27.6 (105)	67.9 (134)	89.3(103)	100 (105)	4.4	1.5
Resistant Ian-DR	12.6 (247)	36.9 (244)	67.8(248)	76.4 (123)	2.1	3.1
Hybrid (♀Ian-DR x ♂16c10)	26.0 (319)	48.8 (248)	84.3(223)	95.8 (239)	2.7	1.8
Hybrid (♀16c 10 x ♂Ian-DR)	23.2 (254)	55.2 (270)	78.4(291)	96.8 (278)	2.8	1.9

Figure 19: Log time-probit regression lines for DDT-resistant stocks Ian 10 and Ian-DR, susceptible strain 16c10 and for the hybrids from these resistant and susceptible stock. The adults were exposed to 4% DDT.



The first instar larval test was also performed in Ian-DR and its hybrids with 16c10. Results are shown in Table 12 and Figure 20. The resistance factor was 8.7x compared with 16c10 which was similar to the factor obtained from the adult tests. The DDT resistance of Ian-DR at the first instar larval stage was dominant, which is different from the result at the adult stage.

2.3. Pala strain

Results of DDT testing of Pala 10, Pala-DR and their hybrids with 16c10 are shown in Table 13 and Figure 21. LT50 values of Pala 10 and Pala-DR were equal (2.1 hours) which was about 6.2x that of the susceptible 16c10. The Pala strain showed the lowest DDT resistance among the three strains investigated and resistance was found to be incompletely dominant.

The results obtained from first instar larval tests of Pala-DR and the hybrids which are shown in Table 14 and Figure 22 showed a resistance factor of about 5.7 relative to 16c10 and once again showed an incompletely dominant type of resistance.

Table 12: Results of first instar larval test (24 hours exposure) of Ian-DR and its hybrids with 16c10

DDT concentrations (ppm)	Ian-DR		Hybrid (♀Ian-DR x ♂16c10)		Hybrid (♀16c10 x ♂Ian-DR)	
	No. tested	% Mortality	No. tested	% Mortality	No. tested	% Mortality
0.01	321	0.3	478	1.9	154	0
0.02	-	-	-	-	133	9.8
0.04	-	-	79	22.8	-	-
0.05	585	40.7	40	47.5	86	48.8
0.08	452	47.4	123	56.9	63	68.3
0.01	716	79.5	151	100.0	291	93.8
0.2	355	100.0	203	100.0	59	100.0
Slopes of regression lines	4.5		5.5		5.9	
LT50 values (ppm)	0.052		0.044		0.047	

Figure 20: Log concentration-probit regression lines for DDT-resistant Ian-DR strain, susceptible 16c10 strain and their hybrids. First instar larvae were exposed to various concentrations of DDT for 24 hours.

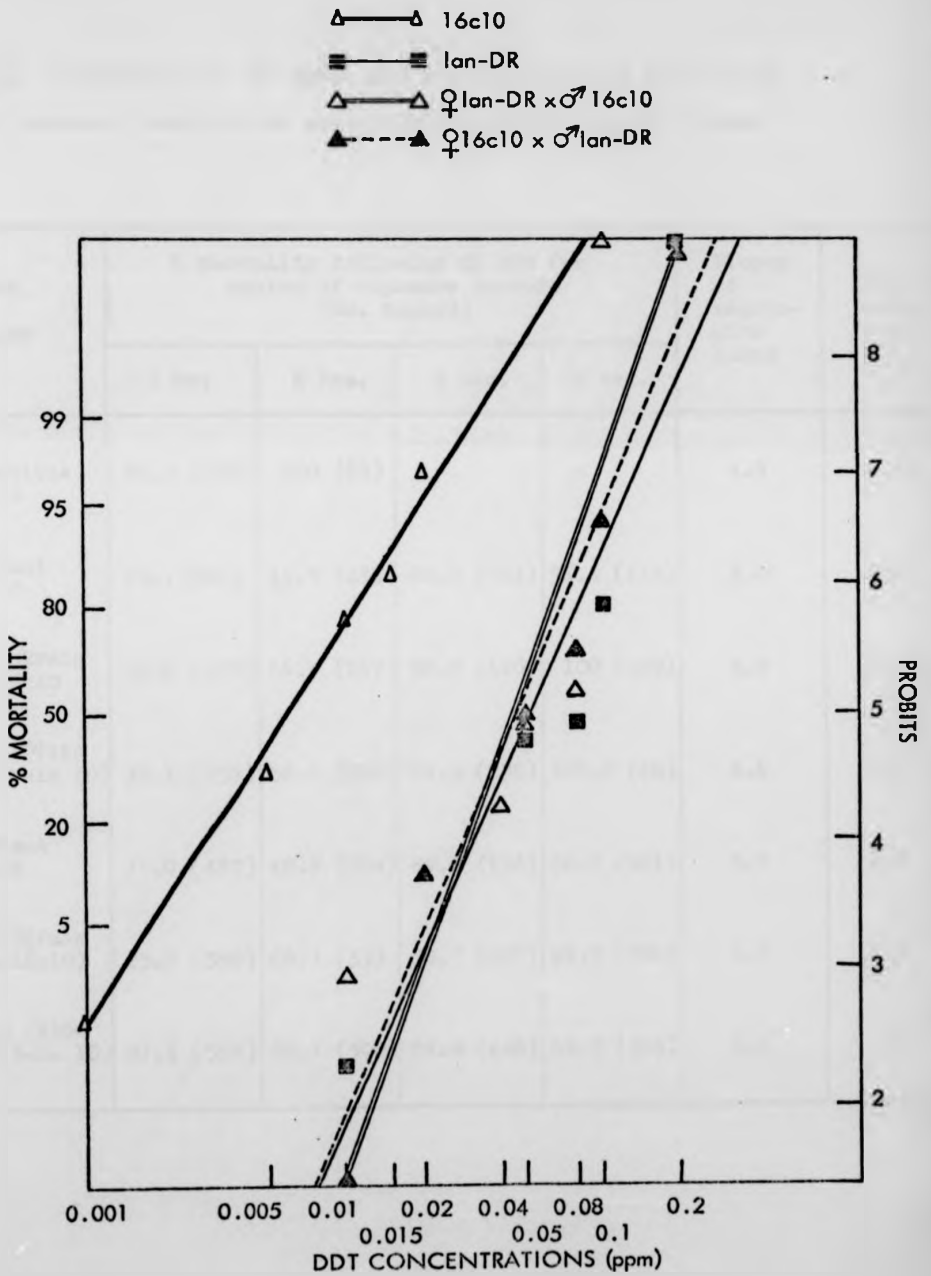


Table 13: Comparison of the adult DDT resistance of the Pala 10 and Pala-DR resistant strain with susceptible 16c10 and the F_1 hybrids

Stocks and hybrids	% Mortality following 4% DDT for series of exposure periods (No. tested)				Slopes of regression lines	LT50 values (hrs)
	1 hr.	2 hrs.	4 hrs.	8 hrs.		
Susceptible 16 c 10	96.9 (225)	100 (55)	-	-	4.3	0.34
Resistant Pala 10	13.1 (84)	41.9 (43)	88.3 (324)	96.4 (112)	3.4	2.1
Hybrid (♀Pala 10 x♂16c10	58.8 (170)	66.8 (217)	90.0 (120)	100 (109)	3.8	1.2
Hybrid (♀16c 10 x♂Pala 10)	35.1 (151)	56.6 (288)	94.9 (236)	100.0 (60)	4.6	1.5
Resistant Pala-DR	11.0 (482)	48.9 (354)	89.7 (156)	96.7 (181)	3.5	2.1
Hybrid(♀Pala 10 x♂16c10)	25.8 (399)	69.1 (33)	88.7 (467)	99.0 (200)	3.2	1.5
Hybrid (♀16c 10 x♂Pala 10)	27.3 (506)	68.7 (300)	92.0 (448)	99.7 (324)	3.6	1.5

Figure 21: Log time-probit regression lines for DDT-resistant stocks Pala 10 and Pala-DR, susceptible strain 16c10 and for the hybrids from these resistant and susceptible stocks. The adults were exposed to 4% DDT.

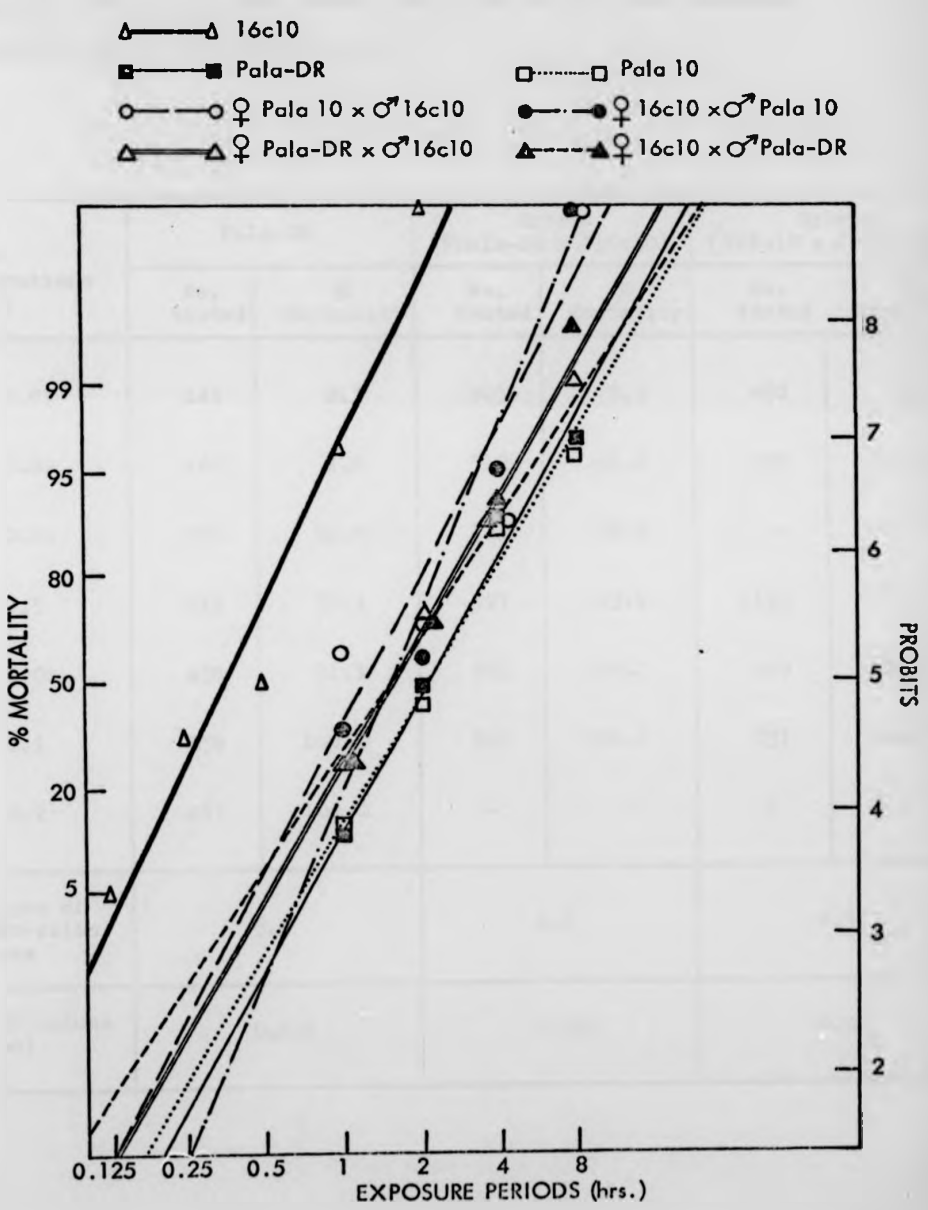
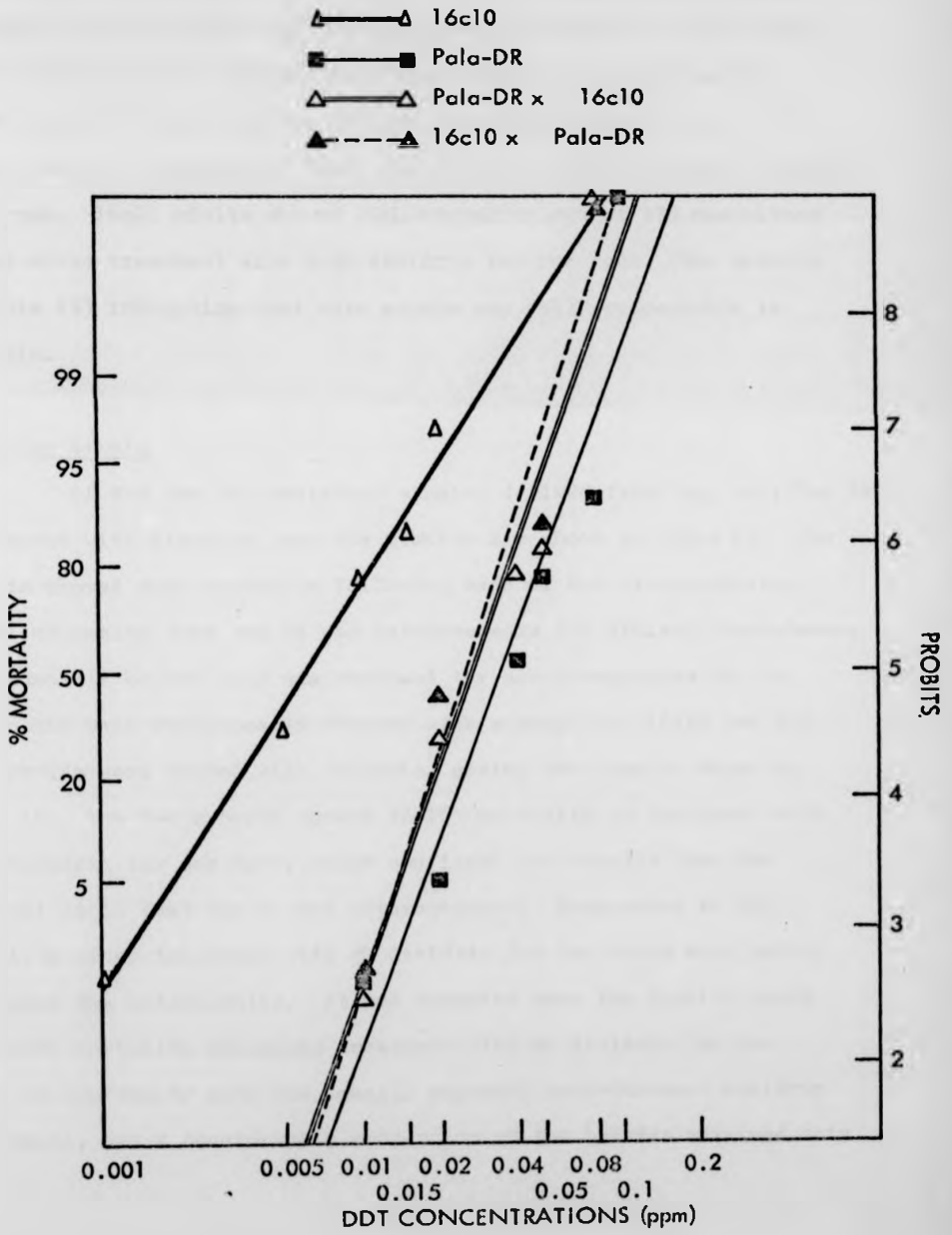


Table 14: Results of first instar larval tests (24 hours exposure) of Pala-DR and its hybrids with 16c10

DDT concentrations (ppm)	Pala-DR		Hybrid (♀Pala-DR x ♂16c10)		Hybrid (♀16c10 x ♂Pala-DR)	
	No. tested	% Mortality	No. tested	% Mortality	No. tested	% Mortality
0.01	143	0.7	403	0.5	852	0.9
0.02	462	4.6	512	41.6	529	30.3
0.04	372	52.8	169	76.9	-	-
0.5	419	77.1	397	83.1	1195	87.0
0.08	458	91.1	606	100.0	589	100.0
0.1	358	100.0	316	100.0	231	100.0
0.2	417	100.0	-	-	-	-
Slopes of regression lines	5.6		6.2		6.3	
LC50 values (ppm)	0.034		0.026		0.025	

Figure 22: Log concentration-probit regression lines for DDT-resistant Pala-DR strain, susceptible 16c10 strain and their hybrids. First instar larvae were exposed to various concentrations of DDT for 24 hours.



3. Genetic studies of dieldrin resistance

The selected DDT resistant Tog, Ian and Pala strains were investigated for dieldrin resistance using the techniques described in section 5 of the Methods. Adult males and females of these three stocks and the hybrid progeny from crossing to 16c10 were tested separately with 0.4% dieldrin for one hour and 4% dieldrin for two hours, the well established doses for discriminating the three dieldrin genotypes. 16c10 adults showed 100% mortality out of 178 mosquitoes tested after treatment with 0.4% dieldrin for one hour. (See details in Table 15) indicating that this strain was fully susceptible to dieldrin.

3.1. Tog strain

Of the two DDT resistant strains derived from Tog, only Tog 14 was tested with dieldrin, and the results are shown in Table 15. The results showed some mortality following each of the discriminating doses indicating that Tog 14 was heterogeneous for dieldrin resistance. Unfortunately before this was realised the non-homogeneous Tog 14 strain had been reciprocally crossed with susceptible 16c10 and the two hybrids were tested with dieldrin, giving the results shown in Table 15. The two hybrids showed 22-26% mortality on treatment with 0.4% dieldrin for one hour, which confirmed the results from the parental stock that Tog 14 was heterogeneous. Forty-nine to 53% mortality after treatment with 4% dieldrin for two hours also partly confirmed the heterogenity. It was expected that the hybrids would show 100% mortality following treatment with 4% dieldrin for two hours in conformity with the usually reported semi-dominant dieldrin resistance, but a considerable proportion of the hybrids survived this

Table 15: Results of dieldrin tests of adults of 16c10, Tog 14 strains and their hybrids

Stocks and hybrids	% Mortality (No. tested)					
	0.4% dieldrin for one hour			4% dieldrin for two hours		
	Female	Male	Total	Female	Male	Total
16c10	100 (54)	100 (124)	100 (178)	-	-	-
Tog 14	8.3 (36)	6.1 (32)	7.1 (68)	29.0 (62)	24.1 (58)	26.7 (120)
Hybrid (♀Tog 14 x ♂16c10)	25.5 (98)	15.4 (52)	22.0 (15)	52.4 (82)	55.0 (91)	53.8 (173)
Hybrid (♀16c10 x ♂ Tog 14)	27.8 (36)	22.7 (22)	25.9 (58)	51.3 (117)	48.4 (126)	49.8 (243)

dose and it was concluded that the Tog stock carried a dominant allele for dieldrin resistance. Under these circumstance the production of a homozygous dieldrin resistant stock would have required inbreeding and family selection and since there was no time for this, no further studies were carried out on dieldrin resistance in this stock.

3.2. Ian strain

Both Ian 10 and Ian-DR were studied. The results of dieldrin tests on Ian 10, Ian-DR and their hybrids with 16c10 are shown in Table 16. These two strains were found to be resistant to dieldrin. The hybrids showed 1% mortality following treatment with 0.4% dieldrin for one hour, indicating the expected homozygosity for dieldrin resistance, but, although the parental stock was homozygous, the hybrids showed 68-80% mortality following treatment with 4% dieldrin for two hours. This result did not fall into either of the previously recognized categories of dieldrin resistance but it fell between the semi-dominant and dominant type. It may be called incompletely dominant.

Although the Tog 14 strain was heterogeneous, only approximately 50% mortality was found in the hybrids after treatment with 4% dieldrin for two hours, which was much lower than in the hybrids derived from Ian 10 and Ian-DR. If Tog 14 had the same type of resistance as the Ian strain, we would expect more mortality following 4% dieldrin treatment than that found in the hybrids from the Ian 10 and Ian-DR because of the non-homogeneity of the Tog strain. Hence it was concluded that the dieldrin resistance allele in Tog 14 was more dominant than in Ian though it was not fully dominant, since the mortality of the Tog 14 hybrids was higher on 4% dieldrin than on 0.4%.

Table 16: Results of dieldrin tests of the adults of the Ian 10, Ian-DR strains and their hybrids with 16c10

Stocks and hybrids	% Mortality (No. tested)					
	0.4% dieldrin for one hour			4% dieldrin for two hours		
	Female	Male	Total	Female	Male	Total
Ian 10	0 (73)	2.6 (39)	0.9 (112)	0 (33)	1.5 (66)	1.0 (99)
Hybrid (♀Ian 10 x ♂16c10)	0.9 (108)	1.8 (111)	1.4 (219)	77.4 (164)	53.5(116)	67.5 (280)
Hybrid (♀16c 10 x ♂Ian 10)	1.1 (98)	0 (81)	0.6 (179)	83.3 (114)	75.7(103)	79.7 (217)
Ian-DR	1.7 (60)	5.2 (58)	3.4 (118)	1.7 (117)	2.7(75)	2.1 (192)
Hybrid (♀Ian-DR x ♂16c10)	1.2 (165)	0.7 (138)	1.0 (303)	74.0 (150)	65.6(180)	69.4 (330)
Hybrid (♀16c 10 x ♂Ian-DR)	1.2 (169)	0.7 (145)	1.0 (314)	71.0 (138)	65.8(111)	68.7 (249)
F_2 (F_1 x F_1) (F_1 = ♀16c10 x ♂Ian 10)	22.2 (108)	26.2(122)	24.4 (230)	71.6 (95)	69.2(143)	70.2 (238)

Some information about the mode of inheritance of resistance may be obtained by testing the F2 progeny. Therefore, the F1 hybrids which derived from the cross of Ian 10x 16c10 were allowed to interbreed to give an F2 generation and the progeny were exposed to 0.4% and 4% dieldrin. The results are shown in Table 16. If dieldrin resistance in Ian 10 was controlled by a single gene, the expected ratio of susceptible (SS): heterozygous (RS): homozygous resistant (RR) would be 1:2:1. In the F2 progeny of the cross of Ian 10 and 16c10, an expected mortality of 25% following exposure to 0.4% dieldrin for one hour would be found. The result observed agreed closely with this expectation (SED= 0.21, $P > 0.05$) which supports the supposition that dieldrin resistance in the Ian strain is controlled by a single gene. On the other hand, the dieldrin resistance of Ian is not semi-dominant, therefore 75% mortality on 4% dieldrin would not be expected. The mortality of the heterozygotes on 4% dieldrin was 70.2% on average, therefore we would expect 60.1 mortality of the F_2 following this dose, which does not agree well with the observations (SED= 3.19, $P < 0.01$).

3.3. Pala strain

Pala 10 and Pala-DR were also tested with dieldrin and the results are shown in Table 17. Almost all adults of the hybrid progeny survived 0.4% dieldrin; however, when the hybrid adults were exposed to 4% dieldrin for two hours, 100% mortality was found. This indicated that dieldrin resistance in Pala 10 and Pala-DR is semi-dominant.

Tests on the F2 progeny were carried out as for those on Ian 10. Results are shown in Table 17 and they agreed closely with the expected mortality of 25% on 0.4% dieldrin and 75% on 4% dieldrin

Table 17: Results of dieldrin tests of the adults of the Pala 10, Pala-DR strains and their hybrids with 16c10

Stocks and hybrids	% Mortality (No. tested)					
	0.4% dieldrin for one hours			4% dieldrin for two hours		
	Female	Male	Total	Female	Male	Total
Pala 10	0 (75)	0 (81)	0 (156)	1.8 (55)	4.2 (48)	2.9(103)
Hybrid (♀Pala 10 x♂ 16c10)	0 (95)	1.1 (95)	0.5 (190)	98.0 (102)	100(110)	99.1(212)
Hybrid (♀16c10 x♂ Pala 10)	0.6 (157)	0 (133)	0.3 (290)	100 (111)	100(105)	100(206)
Pala-DR	2.4 (82)	1.4 (73)	1.9 (155)	0 (61)	2.6 (78)	1.4(139)
Hybrid (♀Pala-DR x♂ 16c10)	1.1 (191)	1.8 (113)	1.3 (304)	100 (95)	100 (113)	100(208)
Hybrid (♀16c10 x♂ Pala-DR)	2.6 (265)	1.3 (320)	1.9 (585)	100 (234)	100 (273)	100(507)
F_2 (F_1 x F_1) (F_1= ♀16c10 x♂Pala 10)	22.2 (189)	23.7 (156)	22.9 (345)	74.0 (104)	68.5(108)	71.2(212)

(SRD= 0.93, P> 0.05; SRD= 1.28, P>0.05). This result supports the supposition that dieldrin resistance in the Pala strain is due to a single semi-dominant gene and supports previous evidence that the Pala strain show single gene controlled dieldrin resistance. (G. Davidson, personal communication).

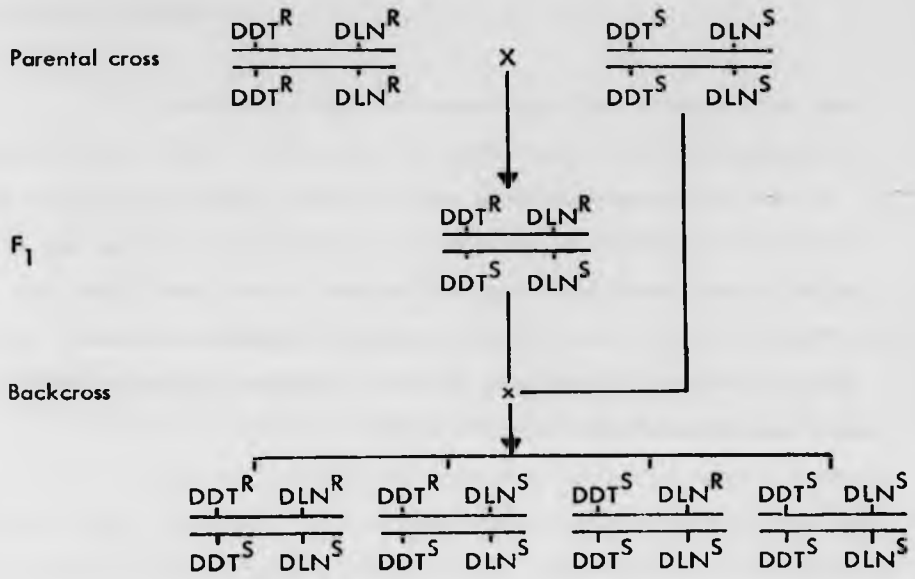
It may be concluded that the three strains of An. gambiae s.s., Tog, Ian and Pala, showed three types of dieldrin resistance, almost fully dominant resistance in the Tog strain, incompletely dominant in the Ian strain and semi-dominant in the Pala strain.

4. Linkage studies between the DDT and dieldrin resistance genes

The extensive studies of DDT resistance in houseflies have shown that several biochemical genetic resistance mechanisms exist. As discussed above, the resistance selected in the three stocks Tog, Ian and Pala (which were of different geographical origin) differed in several respects. To attempt to determine whether resistance alleles at the same locus had been selected in each case, linkage studies were carried out. Haridi (1974) presented evidence that a gene for DDT resistance was linked to a gene for dieldrin resistance in certain An. gambiae strains. The method used in his studies was the double exposure of adult backcross progeny to DDT and dieldrin and comparison of the mortality at the second treatment to the expected 50% which would occur if there was no linkage. This method is questionable because there is much evidence that the age of the mosquito has an effect on the insecticide response and with the double adult exposure the second exposure must be made on the second day of life and not, as in conventional tests, on the first day. In addition, with two treatments made so close together in time one could envisage interactions between the effects of the two chemicals. Therefore for the present studies the following method involving both larval and adult treatments was used. The doubly resistant strains were crossed to the susceptible 16c10 strain and F1 males were backcrossed to susceptible 16c10 females. The first instar larvae of the backcross progeny were divided into three groups for insecticide treatment as described in section 6 of the Methods.

The series of crosses and tests are shown in Figure 23. From the diagram, if there is no linkage between DDT^R and DLD^R the backcross heterozygous survivors of 0.01 ppm DDT or 0.01 ppm dieldrin treatment should show 50% mortality after 0.4% dieldrin or 4% DDT adult treatment.

Figure 23: Series of crosses and tests made to investigate linkage between the DDT and dieldrin resistance genes.



Expected frequency	(1-C)/2	C/2	C/2	(1-C)/2	Expected Mortality
Group A					
1) Adult DDT	L	L	D	D	50%
2) Adult DLN	L	D	L	D	50%
Group B					
1) Larval DDT	L	L	D	D	50%
2) Adult DLN	L	D	-	-	C
Group C					
1) Larval DLN	L	D	L	D	50%
2) Adult DDT	L	-	D	-	C

(L = Lives, D= Dies, C= cross over value between DDT^R and DLN^R)

A significant reduction in mortality compared to the corresponding untreated control group would indicate linkage between the two resistance factors.

The results of the backcrosses with the three strains are shown in Table 18. Results of the first instar larval treatment in most cases showed less mortality than expected because the dose of 0.01 ppm is too low to kill all susceptible genotypes (see Table 5). It was found later that a dose of 0.02 ppm would have given a better kill. Results obtained from group B (Pre-treated with DDT) showed that the mortalities on treatment with 0.4% dieldrin for one hour of the emerged adults of all three backcrosses were significantly lower than in those of dieldrin treatment of group A (Untreated control) ($p < 0.05$ for all three strains). On the other hand, mortalities obtained from DDT treatment of emerged adults of group C (Pre-treated with dieldrin) of all three backcrosses showed similar results to group A (Untreated control). If the two resistants are truly linked, significantly lower mortality should be found in both group B and group C compared with group A. Evidence of linkage was found only in group B from this study.

It is difficult to draw any conclusion from the apparently conflicting evidence. The results obtained in group B might be due to some factor other than linkage, such as partial cross resistance between DDT and dieldrin. It is further noted that the similar pattern of the response to the various treatments among the investigated strains provides some evidence of similarity of the resistance alleles in the three strains.

Table 18: Results of DDT and dieldrin testing backcross progeny to detect linkage between DDT and dieldrin resistance genes.

Backcross	Type of larval treatment	% Mortality of first instar larvae after 24 hours treatment (No. tested	% Mortality of emerged adults (No. tested)					
			4% DDT for one hour			0.4% dieldrin for one hour		
			Female	Male	Total	Female	Male	Total
(Tog RR x 16c 10) x 16c10	Untreated	3.2 (220)	54.2 (24)	45.2 (42)	48.5 (66)	33.3 (30)	45.3 (30)	38.3 (60)
	0.01ppm DDT	47.2 (688)	-	-	-	24.8 (121)	23.9 (117)	24.4 (238)
	0.01ppm dieldrin	38.2 (474)	56.7 (120)	40.0 (125)	48.2 (245)	-	-	-
(Ian 10 x 16c 10) x 16c10	Untreated	3.2 (603)	49.5 (95)	40.0 (75)	45.3 (170)	50.0 (164)	47.6 (147)	48.9 (311)
	0.01ppm DDT	30.6 (663)	-	-	-	42.5 (181)	38.0 (166)	40.4 (347)
	0.01ppm dieldrin	51.5 (404)	65.0 (140)	43.3 (127)	54.7 (267)	-	-	-
(Pala 10 x 16c 10) x 16c10	Untreated	0.7 (388)	79.3 (92)	55.4 (56)	70.3 (148)	49.0 (155)	49.6 (133)	49.3 (288)
	0.01ppm DDT	38.2 (406)	-	-	-	26.9 (134)	22.9 (131)	24.9 (265)
	0.01ppm dieldrin	45.5 (501)	73.0 (74)	70.3 (91)	71.5 (165)	-	-	-

5. Cage experiments

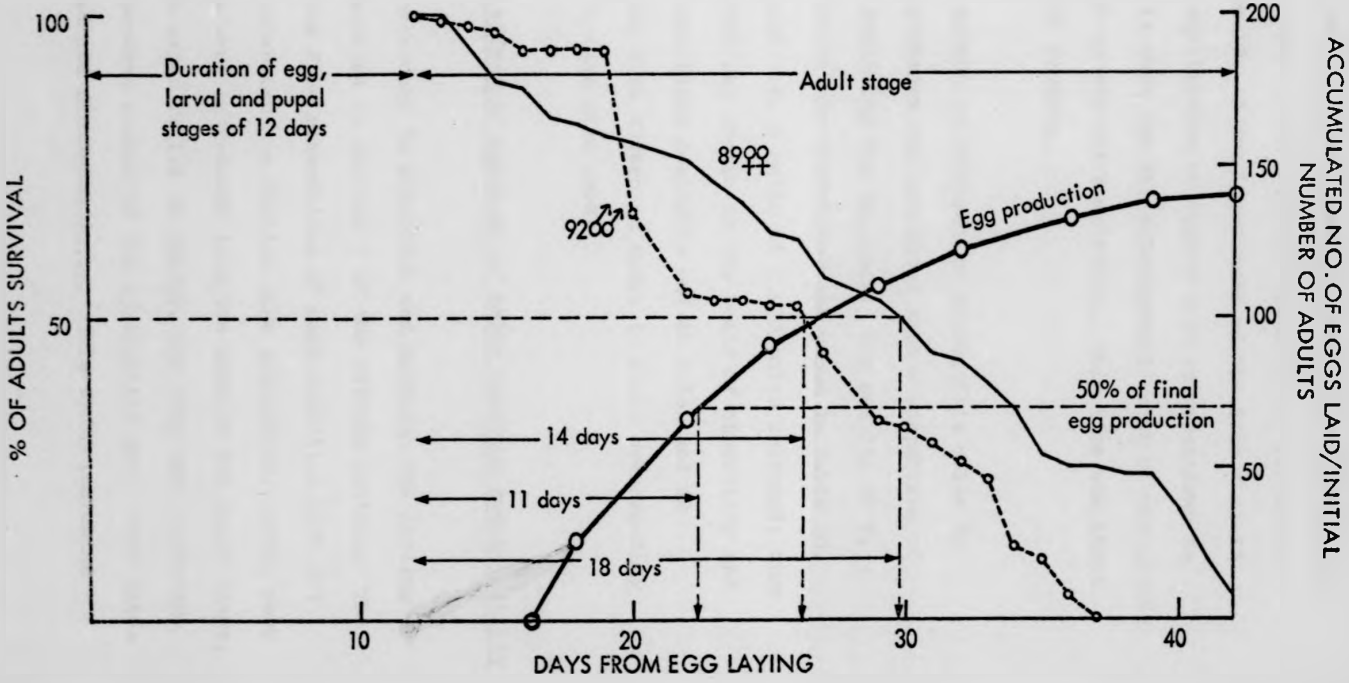
The purpose of this study was to investigate the feasibility of a method for causing delay or reversal of the evolution of DDT resistance by release of males carrying DDT susceptibility genes into a population. A genetic sex separation method was utilized to produce the susceptible males for release into a cage population. Thus the reliability and stability of the genetic sexing system were also investigated.

The resistant Tog strain was used to initiate a cycling cage population with overlapping generations and the R70 strain of the same species, which was considered to be susceptible to DDT, was used for male releases.

The survival and generation times were investigated in order to obtain information for the establishment of the cage population and for evaluation of the effects of the released males on the population. Daily mortality of a batch of males and females of Tog 5 and the egg production of the females were observed. The results are plotted in Figure 24. Median survival time is estimated from the graphs and the time at which females had laid half of their eggs plus the time of the aquatic stage provided an estimate of the mean generation time. Median survival time was 14 days in males and 18 days in females. Half of the total egg production of a batch of females had occurred by day 11 of adult life. Therefore the estimated mean generation time of the Tog 5 strain is 23 days.

The level at which a cycling population would equilibrate for different numbers introduced each week were calculated from the data on

Figure 24: Data on survival and egg production of the Tog 5 strain from a batch of newly emerged mosquitoes all introduced into a cage on the same day.



adult survival time. This was done by multiplying the mean survival in weeks by the weekly input. The expected equilibrated population sizes are shown in Table 19. A rough estimate of the time that it would take to reach an equilibrium of deaths with introductions is given by the time that it took for the experimental batch of mosquitoes illustrated in Figure 24 to die out completely. This time was about 30 days of adult life for females.

The expected effect of releases of susceptible males to replace the gene for resistance was estimated from a comparison of subsequent generations following the releases. The results of 2, 4 and 6 units of male releases are calculated and shown in Table 20. Four units of male release i.e. a ratio of 4 R70 males released: cage population males re-cycled was chosen on the basis of capability and feasibility under the conditions available and 40 males and 40 females re-cycled per week were chosen in order to avoid overcrowding of the mosquitoes in the cage size used.

5.1. Cage test of the effect of releases of males carrying susceptibility genes

The general procedure to establish and maintain the cycling cage population was described in section 7 of the Methods section. The strain Tog 5 derived from five generations of mass selection with DDT (Table 2) was used to establish the cycling cage population. Every week 40 males and 40 females were introduced into the cage at the pupal stage. On many occasions, some pupae failed to emerge, and they were therefore replaced with a corresponding number of the appropriate sex. Dead males and females were counted and discarded daily. This system indicated

Table 19: The expected stable population sizes following different weekly introductions into experimental cage populations of resistant males and females and 4:1 ratio of susceptible R70 males

Number of mosquitoes added to the cage every week	Equilibrated population of mosquitoes in cage		
	Female	Male	Total
30 cage males, 30 females and 120 R70 males	77	300	377
40 cage males, 40 females and 160 R70 males	102	400	502
50 cage males, 50 females and 200 R70 males	128	500	628

Table 20: Expected reduction of frequency of a DDT resistance gene following the release of susceptible males*

Generations	Gene frequency in progeny**		
	2:1 ratio of male releases	4:1 ratio of male releases	6:1 ratio of male releases
0	1	1	1
1	0.67	0.60	0.57
2	0.45	0.36	0.31
3	0.31	0.216	0.177
4	0.207	0.130	0.101
5	0.139	0.078	0.058
6	0.093	0.047	0.033

* DDT resistance gene frequency of releases males = 0

** Initial DDT resistance gene frequency in wild population = 1

when the cage population had come to equilibrium, i.e. when the rate of input is the same as the rate at which mosquitoes died.

The initial population in this experimental cage was called Tog X. The females were blood fed twice a week and eggs were collected three days after each feed. The first batch of eggs, which was collected in the first week of the population establishment, was called X1 and the second batch collected in the same week was called X2. This egg batch counting system was used throughout this study, e.g. X3 and X4 were obtained in the second week, X5 and X6 were obtained in the third week, and so on. The egg hatching rates were observed at intervals for evaluation of the effect of the translocated male releases inducing partial sterility into the cage population. Larvae from each egg batch were reared separately. Only 40 male and 40 female pupae per week were re-cycled into the cage; the remainders were allowed to emerge and the adults were used for testing with DDT to determine the degree of resistance. The testing method used was the WHO test method, males and females being tested separately.

At week 12 where the introduction rate and death rate in the population had nearly equilibrated releases of male pupae of the R70 strain were commenced. Although the genetic sexing system of R70 was applied for killing females at the first instar larval stage with dieldrin treatment, the sex of the R70 pupae was also periodically checked prior to releasing. This was done to observe the reliability and stability of the genetic sexing system. To achieve the desired ratio of 4 R70 males released: 1 re-cycled male: 1 re-cycled female of the cage population, 160 R70 males were released per week.

Detailed results of monitoring the DDT resistance of the cage population are shown in Appendices1 and 6. The results were averaged for each generation (i.e. 3 week period) and LT50 values are plotted in Figure 25 for females and Figure 26 for males. Releases of R70 males were carried out for four generations. The reduction of the LT50 values from about eight hours to 3.4 hours in females and 3.5 hours in males following the first generation of release showed that the cycling cage population had been much influenced by the release. Furthermore, after a second generation of releases had been performed the LT50 value decreased to about one hour in females and 1.6 hours in males. The releases of R70 were continued for a further two generations but they showed little further effect as the population had nearly reached full susceptibility. The results showed clearly that the release of males carrying susceptibility genes could force a resistant population to move towards suceptibility. It should further be pointed out that, although released males were partially resistant to DDT, (see section 5.4) the resistance level of males of the cage population showed a great deal of reduction, to almost the same extent as that of the females of the cage population.

5.2. The control cage

A control cage was established in order to check whether the decline in resistance seen in the Tog X experimental release cage might not have been due to the releases but to a possible reversion towards susceptibility when artifical selection for resistance was relaxed due to reduced fitness of the resistant type.

Figure 25: Effects of release of DDT susceptible males on the resistance level of the cage population females.

*From Table 2.

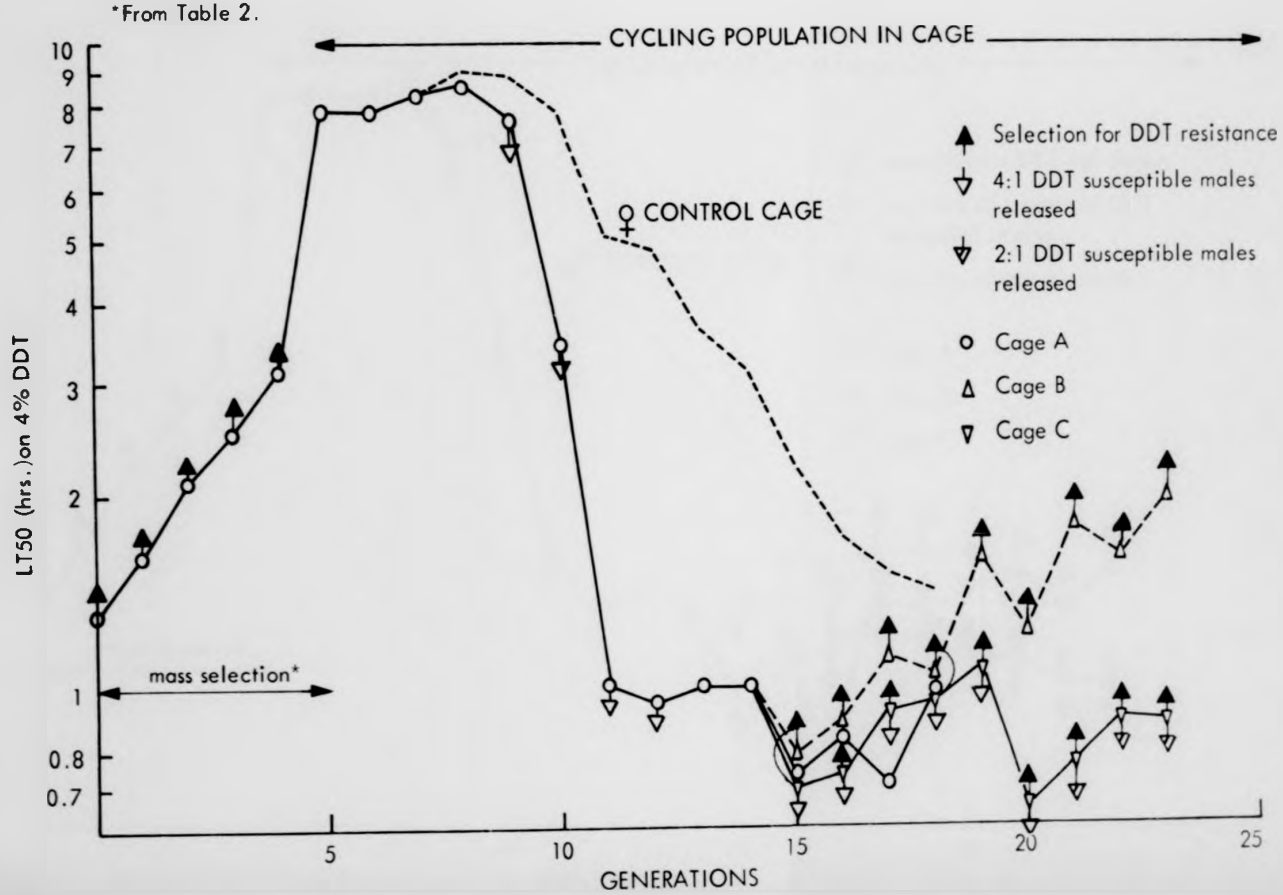
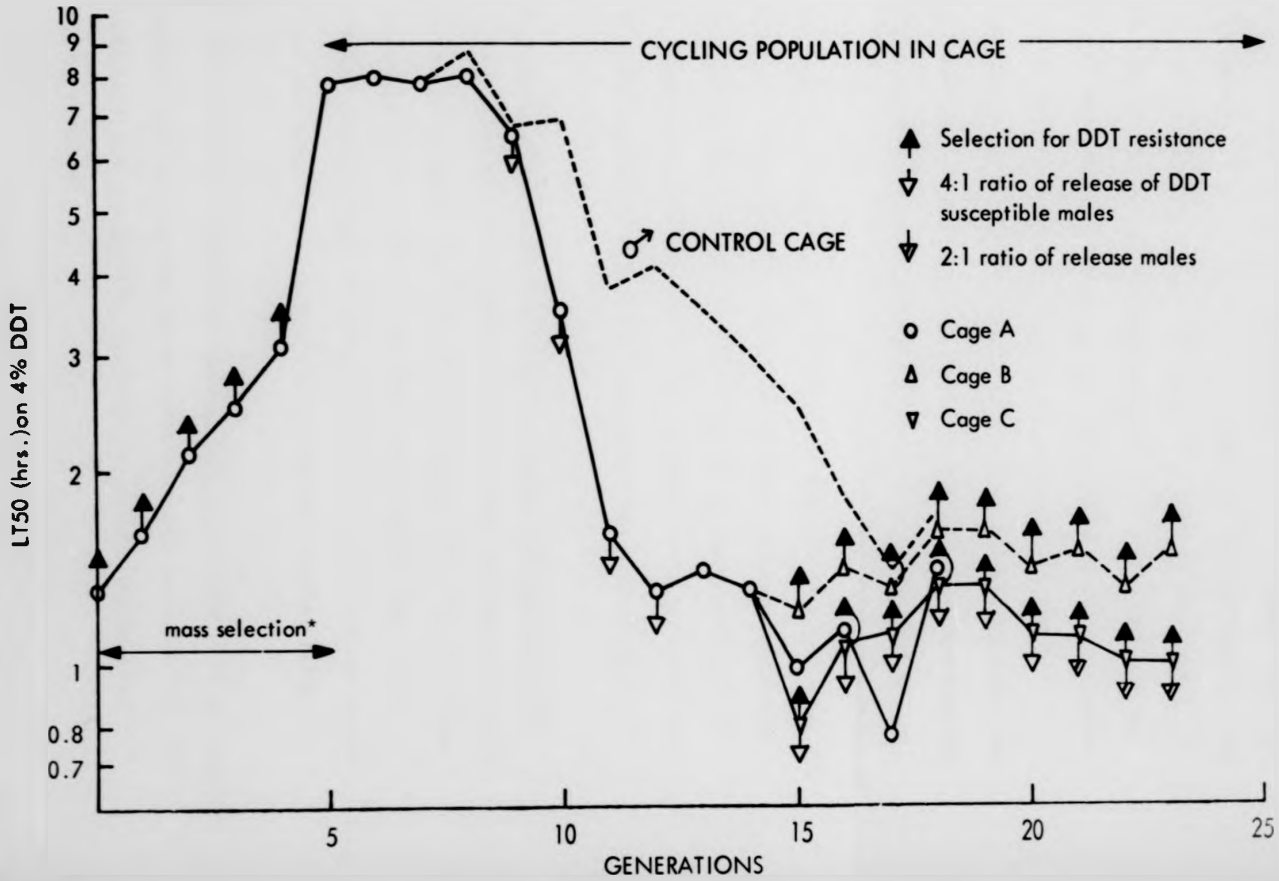


Figure 26: Effects of release of DDT susceptible males on the resistance level of the cage population males.

*From Table 2



This control cage population was derived from the experimental population, Tog X, at week seven before the introduction of the R70 males began. It was allowed to cycle without either any DDT treatment or release of R70 males. This cage was called Tog C. The first egg batch was called C15, the second C16 and so on. The results are shown in detail in Appendices 2 and 7 and in summary in Figures 25 and 26. The control cage showed a decline of resistance levels but the rate was relatively low compared to the Tog X experimental cage, where R70 releases were made, especially when the population reached an intermediate level of resistance. The decline of resistance in the control population indicates that, when a population is not homozygous for resistance, natural selection favours the susceptibility allele because it confers greater fitness than the resistance allele when there is no insecticide selection pressure. This is presumably the reason for the decline of resistance level frequently observed in laboratory stock colonies and sometimes reported in field situations where spraying programmes have been suspended.

5.3. Cage test of the combined effects of selection and releases

The experiment was continued with a test of the ability of released males to prevent the build up of resistance when selection for DDT resistance was applied. In order to simulate the field situation where females tend to come into contact with DDT in houses more than males, the selection was applied to the females only.

Following the fourth release of R70 males described in section 5.1 the population was maintained without releases or selection for two generations. The population was then split into three sub-populations as follows:

Cage A: This population continued to be maintained without any release of susceptible males and without DDT selection pressure. It was planned to observe persistence of the susceptibility of this population under the influence of natural selection alone. This cage was first established at 28 weeks after the original establishment of the Tox X cage. Therefore, the first batch of eggs would correspond with batch number 55, and the first batch from cage A was called XA55 and the counting system used in Tog X was then followed. The results of DDT tests are shown in detail in Appendices 3 and 8 and average LT50 values for females and males are plotted in Figures 25 and 26 respectively.

Cage B: This cage population represents the situation where there was DDT selection pressure on the females because of the recommencement of DDT spraying against a population in which susceptibility had been restored. In the maintenance of this population, each week females were treated with 4% DDT for one hour and 40 survivors were returned to the cage and 40 males were returned without any treatment. The first egg batch was called XB55 and the numbering system continued as in Cage A. The population was maintained without any release of R70 males. The results of DDT testing are shown in detail in Appendices 4 and 9 and the average LT50 values of females and males are plotted in Figures 25 and 26 respectively.

Cage C: It was planned to investigate the capability of the susceptible male release to prevent or delay the evolution of DDT resistance of the population under DDT selection pressure. The cycling population was maintained exactly as in cage B with DDT treatment of

the females, and in addition 160 R70 males were relased each week. Egg batches from this cage were called XC starting from 55. The DDT testing results are shown in detail in Appendices 5 and 10 and average LT50 values are plotted in Figure 25 and 26.

Cage A showed no sign of change in the response to DDT in females and males for five generations after the last release, indicating that when the resistant population was forced by susceptible male release to a low level of resistance, this level remained stable in females and was only slightly variable in males in the absence of insecticidal selection or releases. This cage was discarded after five generations. Cage B showed an increase of DDT resistance in both females and males but the rate of increase was very slow, particularly in males, when compared to that seen when DDT selection pressure was first applied to the Tog population (Table 2 and Figure 12). This difference is partly due to the fact that the original selection pressure was applied to both sexes and not to females only as in cage B. Also it may be that in cage B the frequency of resistance genes had been made so low by the releases that there was little for selection to act on. On the other hand, in cage C, where there was both DDT selection pressure and the release of R70 males, the susceptibility level remained very similar in both females and males to that in cage A. This showed clearly in that the release of R70 males in a 4:1 ratio can hold the susceptibility level constant despite selection for DDT resistance. However, one may argue that the 4:1 ratio of male releases under the conditions of field application would require a great deal of money to produce enough males for release. Therefore, from generation 21 onwards only a 2:1

release ratio was used (i.e. 80 R70 males per week). There was no sign of change in susceptibility after two generations of this release procedure. This indicated that a 2:1 release ratio can hold the susceptibility level of a population constant.

5.4. Properties and stability of the R70 sexing system

The R70 strain was tested with 4% DDT and 0.4% dieldrin for one hour and 4% dieldrin for two hours. Males and females were tested separately and the results are shown in Table 21. Data are shown from February 1977 when stock was first received from the Ross Insitute insectary and from July 1978 after it had been maintained by the author for 18 months. During this period the stock had been maintained by continually outcrossing R70 males to females of the dieldrin and DDT susceptible strain 16c10. Comparison of the data at the beginning and end of the 18 month period of rearing shows complete stability of the properties of the strain. These properties include the fact that females of R70 are fully susceptible to both DDT and dieldrin. The males survived 0.4% dieldrin for one hour but died on 4% dieldrin for two hours, confirming the results of Curtis et.al., (1976) that the males are heterozygous for a semi-dominant gene for resistance to dieldrin derived from the Pala strain (see Table 17). The R70 males were unexpectedly found to be partially resistant to DDT and further investigation of this fact is reported below. Whatever the explanation, it was clearly important that the males and females of the experimental cage were tested separately as the R70 males could be relied upon to transmit DDT susceptibility to their female progeny but not necessarily to their male progeny.

Table 21: Results of DDT and dieldrin testing of the R70 strain.
Data are shown for tests carried out in February, 1977 and July, 1978

Treatment	Year	% Mortality (No. tested)	
		Female	Male
4% DDT for one hour	1977	99.4 (157)	66.7 (135)
	1978	99.4 (166)	47.5 (223)
0.4% dieldrin for one hour	1977	100 (104)	1.8 (111)
	1978	100 (74)	2.3 (89)
4% dieldrin for two hours	1977	100 (126)	100 (153)
	1978	-	100 (129)

First instar larvae of R70 were treated with 0.02 ppm dieldrin for 24 hours to eliminate females and leave only males for release. Following dieldrin treatment, the number of surviving males and females were recorded at the pupal or adult stage. A total of 6,037 males and 27 females were found, i.e. a proportion 0.45% of females, which is only slightly different from the proportion found during 1976 with this stock (Curtis et.al., 1976). This indicated that the R70 genetic sexing system still worked well and could be relied upon to remain stable over a period of years.

To try to find an explanation of the partial DDT resistance of R70 males, the males and females that survived the larval dieldrin treatment were investigated by testing with DDT and dieldrin, and the results are shown in Table 22. Males showed very similar results to those previously mentioned (see Table 21) as would be expected since the larval dieldrin treatment is not intended to kill any males. Among the few females that survived the larval dieldrin treatment almost all were dieldrin resistant at the adult stage. This indicates that they had not "escaped" larval killing by chance but were genuinely dieldrin resistant as a result of a crossover transferrin; the dieldrin resistance into a gamete carrying an X chromosome. These crossover females were found to be DDT susceptible (Table 22) like the normal females of the R70 strains (Table 21). This was a surprising result as it seems to rule out an explanation of the partial DDT resistance of the R70 males as being due to a cross resistance effect of the dieldrin resistance gene or to a DDT resistance gene closely linked to the dieldrin resistance genes. The only remaining explanation seems to be that there is a DDT resistance gene in R70 males even more closely linked to the Y chromosome than is the dieldrin resistance gene.

Table 22: Data on adult insecticide tests on males and females of the R70 strain which survived treatment with 0.02 ppm dieldrin at the first instar larval stage

Treatment	% Mortality (No. tested)	
	Female	Male
4% DDT for one hour	96.7 (30)	41.0 (208)
0.4% dieldrin for one hour	11.1 (9)	2.9 (35)
4% dieldrin for two hours	-	100 (93)

During the release programme, egg batches of R70 strains were periodically counted to determine the hatching rate. A total of 10,686 eggs showed 53.2% hatching. This indicates the persistence of the semi-sterility due to the Y chromosome translocation. During the cage release experiment, further information was obtained about the ability of R70 males to compete for mates by observing the fertility of the eggs of the cage population. The results are shown in Table 23. The Tog 5 population showed a hatching rate of 81% and a decrease to about 63% following the first generation of release of R70 males. The hatching rate declined following further releases to about 57-58%, indicating successful introduction of semi-sterility into the population by the R70 males. The fertility was not reduced quite as low as in the R70 stock itself (53.2%) but it showed little sign of increase when R70 males releases were suspended indicating that by that time very nearly all the males in the population had inherited the translocation and elimination of it by natural selection was therefore a very slow process.

Table 23: Effect of the R70 male releases on the egg hatching rate of the Tog 5 cage populations

Cage population	Number of eggs	Number of larvae	Hatchability %
Original Tog 5 stock	5520	4464	80.9
Generations after commencement of releases of R70 males			
1	5348	3414	63.8
2	1173	724	61.7
3	3647	2298	63.7
4	5014	3000	59.8
5 *	7706	4462	57.0
6 *	10788	6291	58.3

* suspension of R70 male releases

6. Experiments on the duration of infection of mosquitoes

It has been assumed that once mosquitoes become infected with malaria parasites they remain infective for the rest of their life, but few direct observations on this point have previously been made. An investigation was therefore carried out using highly susceptible strains of An. gambiae s.s. (P.B.) and An. stephensi (BEECH), and P. y. nigeriensis. The infection processes followed the techniques described in section 10 of the Methods section. One quarter of the mosquitoes were dissected on day 10 after the infecting blood feed, leaving the majority to be dissected on day 17. The infection rate and intensity of infection were compared between the two days of dissection. Result for the P.B. strain of An. gambiae s.s. are shown in Table 24. The three experiments with the P.B. strain showed that the infection rates and intensity of infection of the mosquitoes dissected on day 17 were highly statistically significantly lower than those on day 10, except in experiment 2 the gland infection rate was not significantly different. This evidence suggested that the mosquitoes lose their infection if they survive beyond day 10. One might argue, however, that the infected mosquitoes had been selectively killed between day 10 and day 17. In order to test this hypothesis, a very highly susceptible mosquito strain was required. Further investigations in An. gambiae s.s. were carried out with the PYN strain. This strain had been obtained by further selection of the P.B. strain for P. y. nigeriensis susceptibility and was kindly provided by P.M. Graves of the Ross Institute. This PYN strain showed approximately 93% guts and glands positive for P. y. nigeriensis infection on day 10. The BEECH strain

Table 24: Comparison of *P. v. nigeriensis* infection in the P. B. strain *An. gambiae s.s.* dissected on day 10 and day 17

Experiment nos.	No Mosq. died between days 10 and 17	Gut dissections for oocysts (no. dissected)						Salivary glands dissection for sporozoites (No. dissected)					
		% of guts positive		SND values	Williams' mean of nos. of occysts		t values	% of glands positive		SND values	Mean sporozoite score		t values
		Day 10	Day 17		Day 10	Day 17		Day 10	Day 17		Day 10	Day 17	
1	-	87.5 (16)	0 (10)	4.25 ($p<0.001$)	9.7 (16)	0 (10)	5.47 ($p<0.001$)	80.0 (15)	11.1 (9)	3.28 ($p<0.001$)	1.47 (15)	0.11 (9)	3.04 ($p<0.01$)
2	26	57.1 (21)	13.2 (38)	3.57 ($p<0.001$)	5.0 (21)	1.2 (38)	5.12 ($p<0.001$)	52.6 (19)	29.7 (37)	1.8 ($p>0.05$)	1.16 (19)	0.49 (37)	2.63 ($p<0.05$)
3	51	73.0 (37)	32.4 (37)	3.49 ($p<0.001$)	5.5 (37)	1.8 (37)	3.49 ($p<0.001$)	73.0 (37)	29.7 (37)	3.72 ($p<0.001$)	1.41 (37)	0.51 (37)	3.39 ($p<0.01$)

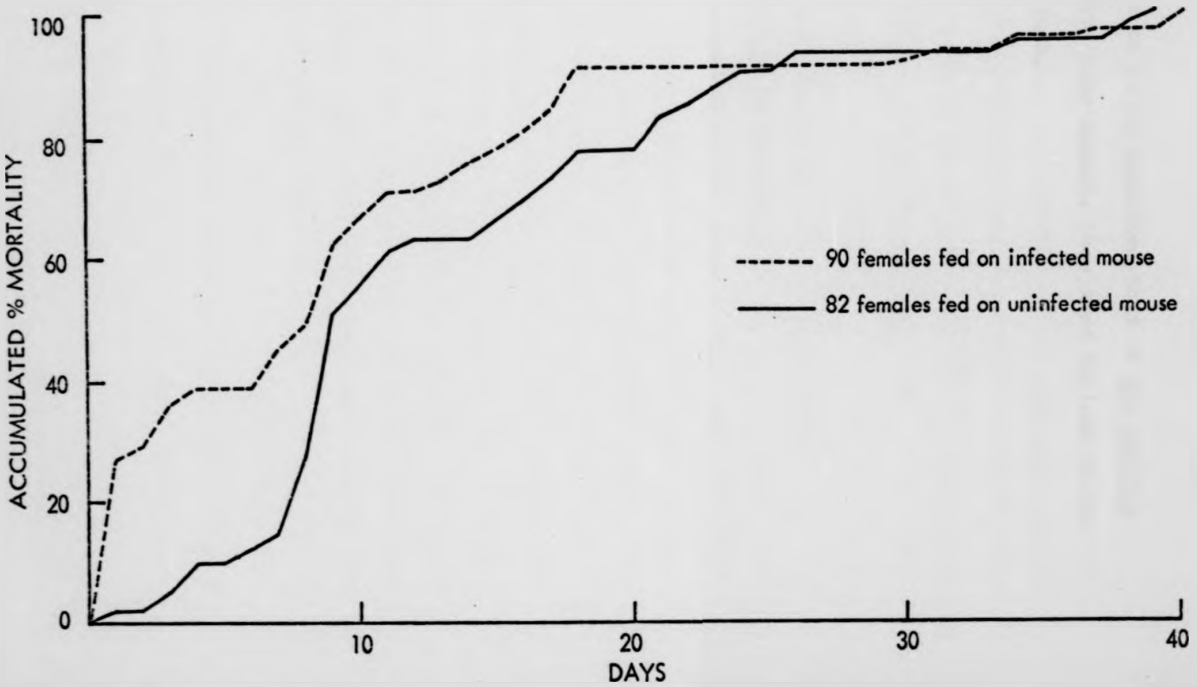
of An. stephensi, which showed very high susceptibility, was also used. The result of these studies are shown in Table 25. Two experiments on the FYN and three experiments on the BEECH strain showed clearly that the infection rate and intensity of infection were significantly reduced at day 17 compared with day 10.

Experiments 4 and 5 (Table 25) showed the highest infections on day 10 and the weighted mean proportion infected on day 10 was 89.9%. The total number which died between days 10 and 17 was 214 and the number which lived until day 17 was 31 giving a grand total of 245. Applying the known day 10 infection rate of 89.9% to the total indicates that at day 10, 220 were infected and 25 were not. If all 214 of those that died were infected this would leave 6 infected and 25 uninfected. In fact, however, 9 were found infected and 22 uninfected. Thus the hypothesis of selective mortality of the infected mosquitoes could explain the facts. Another approach to testing the hypothesis can be made by comparing the rate of mortality in a batch of mosquitoes that fed on an infected mouse and a batch fed simultaneously on an uninfected mouse. This study was carried out with the P.B. strain and the P. y. nigeriensis. Dead mosquitoes were counted and cleared from both cages every day. Mortality rates are plotted in Figure 27. The infected group showed higher mortality rates than the uninfected group during the first eight days after being blood fed, indicating that the mosquito is adversely affected by the parasite during its development. From day eight onwards, however, mortalities were very similar. The samples were relatively small but it appears that once the parasite reaches the infective stage it has little or no effect on the host, so that both infected

Table 25: Comparison of *P. y. nigeriensis* infection in the PYN strain of *An. gambiae s.s.* and the BEECH strain of *An. stephensi* dissected on day 10 and day 17

Experiment nos.	Strains	No mosq. died between days 10 and 17	Gut dissection for oocyst (No. dissected)						Salivary glands dissection for sporozoites (No dissected)					
			% of guts positive		SND values	Williams' mean of nos. of occysts		t values	% of glands positive		SND values	Mean sporozoite score		t values
			Day 10	Day 17		Day 10	Day 17		Day 10	Day 17		Day 10	Day 17	
4	PYN	43	82.2 (29)	33.3 (9)	3.15 ($p<0.01$)	36.2 (29)	1.7 (9)	4.31 ($p<0.001$)	81.5 (27)	33.3 (9)	2.55 ($p<0.05$)	2.37 (27)	0.67 (9)	2.96 ($p<0.01$)
	BEECH	41	79.0 (19)	0 (4)	3.01 ($p<0.01$)	20.8 (19)	0 (4)	2.62 ($p<0.05$)	79.0 (19)	0 (4)	3.01 ($p<0.01$)	2.16 (19)	0 (4)	3.01 ($p<0.01$)
5	PYN	73	93.3 (30)	33.3 (6)	3.6 ($p<0.001$)	17.0 (30)	1.8 (6)	3.12 ($p<0.01$)	89.7 (29)	33.3 (6)	3.14 ($p<0.01$)	2.35 (29)	0.67 (6)	2.83 ($p<0.01$)
	BEECH	57	100 (21)	33.3 (12)	4.3 ($p<0.001$)	23.5 (21)	1.7 (12)	7.18 ($p<0.001$)	100 (21)	8.3 (12)	5.37 ($p<0.001$)	2.29 (21)	0.08 (12)	7.73 ($p<0.001$)
6	BEECH	52	61.1 (18)	20.1 (15)	2.38 ($p<0.05$)	9.6 (18)	1.3 (15)	3.23 ($p<0.01$)	55.6 (18)	13.3 (15)	2.51 ($p<0.05$)	1.17 (18)	0.02 (15)	2.55 ($p<0.05$)

Figure 27: Mortality of batches of female mosquitoes of the P.B.strain fed on a mouse infected with P.y.nigeriensis and on an uninfected mouse



and uninfected mosquitoes died at the same rate.

From this evidence it is concluded that if An. gambiae s.s. and An. stephensi live long enough, they tend to lose their P. v. nigeriensis infections.

7. Experiments on the effects of sublethal doses of insecticide on vectorial capacity

Strains of An. gambiae s.s. which were susceptible and refractory to P. b. berghei were available, and these were designated PB and LD5. In addition, there was a strain of An. stephensi (BEECH) which was highly susceptible to rodent malaria. The sublethal effect of DDT, malathion and Dimilin on vector potential of the three Anopheles strains was investigated using P. y. nigeriensis.

7.1. Determination of tolerance levels in larval and adult anophelines to DDT, malathion and Dimilin

The mortality results of the tests of P.B., LD5 and BEECH with DDT, malathion and Dimilin were plotted against doses to obtain regression lines and LC50 values were estimated from the lines. The LC50 values for larvae and adults for the three insecticides are shown in Table 26. All three strains are partially resistant to DDT and are susceptible to malathion (based on the criteria of Davidson and Zahar, 1973). The LD5 strain showed higher tolerance to both DDT and malathion than the other two strains. LC50 values of the larvae with respect to Dimilin were very low, ranging from 0.002 ppm in the P.B. strain to 0.005 ppm in the BEECH strain.

The concentrations chosen for treating larvae or adults of the three strains were one-tenth and one half of their respective LC50 values.

7.2. Determination of the effect of the various treatments on the gonotrophic cycle and vectorial capacity

To simulate most field situations, where malaria control or eradication programmes rely on residual spraying to control adult

Table 26: LC50 values from the regression lines of Probit mortality against log concentration of insecticide for BEECH strain of An. stephensi and P.B. and LD5 strains of An. gambiae s.s.

Strains	Adults		Larvae
	DDT (%)	Malathion (%)	Dimilin (PH60:40) (ppm)
BEECH	2.4	0.9	0.005
P.B.	2.3	1.1	0.0022
LD5	3.0	1.3	0.003

populations, experiments were performed with the treatment of adults. Initially, one-tenth of the LC50 values were used, which were 0.24%, 0.23% and 0.3% of DDT and 0.09% , 0.11% and 0.13% of malathion for the BEECH, P.B. and LD5 strains respectively. Later, half of the LC50 values were used, i.e. 0.45% and 0.56% of malathion for the BEECH and P.B. respectively.

7.2.1. Experiments with the refractory strain

The results for the LD5 refractory strain are set out in Table 27. The first two experiments were performed with one-tenth of the LC50 values of DDT. There was absolutely no infection in either guts or glands in either experiment 1 or 2 in the untreated control and after the sublethal DDT treatment. Thus there was no evidence that this concentration of DDT interfered with the refractoriness mechanism and the results also confirm the stability of the refractory genotype selected by Al-Mashhadani in 1974 and subsequently reared without any selection in the Ross Institute insectary. The experiment with malathion was performed on a new stock derived from the Ross Institute one year after the experiment with DDT. One-tenth of the LC50 value was used and the results are shown in experiment 11 , Table 27. Unfortunately both untreated control and the sublethal treatment group showed a proportion of infections, suggesting that during the intervening year the stock had become contaminated. Though it had been re-selected for the white eye gene, there had been no attempt to re-select it for refractoriness. Despite this problem the results show that the treatment with malathion had no effects on susceptibility to infection.

Table 27: Data showing response to infecting feeds of the LD5 refractory strain of An. gambiae s.s. in the untreated controls and after treatment with one-tenth of LC50 values of DDT and malathion

Experiment No.	Types of treatment	No. blood fed	No. died during incubation period	Days from feeding to dissection	% Positive (No. dissected)	
					Guts	Glands
1	Control	39	8	10	0 (31)	0 (31)
	DDT-treatment	36	12	10	0 (24)	0 (24)
2	Control	37	9	10	0 (28)	0 (28)
	DDT-treatment	42	12	10	0 (30)	0 (29)
11	Control	50	27	10	26.1 (23)	26.1 (23)
	Malathion-treatment	92	50	10	19.1 (42)	20.0 (40)

7.2.2. Experiments with the susceptible strains

The results of the DDT experiments are shown in Table 28 for BEECH strain and Table 29 for PB strain. The first two experiments on the effect of DDT on BEECH and PB were performed at the same time as experiments 1 and 2 on LD5 reported in Table 27. In the first experiment, the BEECH stock (which was fed on the same mouse as LD5) showed about 50% guts positive for oocysts and about 40% of glands positive for sporozoites and there was no statistically significant difference between the levels of infection in the control and DDT treated mosquitoes, but with the susceptible P.B. strain it was found that the proportion and intensity of infection in the treated group was significantly higher than those of the control group. In experiment 2, different results were obtained. The intensity of infection of the gut in the treated group of the BEECH stock was significantly higher than that of the control, but the proportion of guts and glands positive for infection and the intensity of gland infections were not different. On the other hand, the P.B. strain showed no differences between the treated and control groups.

In order to clarify this situation, experiments 3 and 4 were carried out. These two experiments were performed only with the BEECH and the P.B. strains. The results are shown in Tables 28 and 29. They showed no effect on vectorial capacity of DDT treatment at one-tenth of the LC50 values. Moreover, all four experiments also showed that sublethal treatment with DDT had no effect on the duration of the extrinsic cycle of the parasite.

In experiments 5 and 6, the BEECH and P.B. strains were treated with malathion at one-tenth of their LC50 values. The results are shown in Table 30. There was no difference in the proportion and the

Table 28: Susceptibility to infection with P. y. nigeriensis of females of the BEECH of An. stephensi in the untreated controls and after treatment with one-tenth of LC50 value of DDT

Experiment No.	Types of treatment	No. blood fed	No. died during incubation period	Days from feeding to dissection	Gut dissection (No. dissected)				Gland dissection (No. dissected)			
					% of guts positive	SND values	Williams' mean of nos. of oocysts	t values	% of glands positive	SND values	Sporozoite mean score	t values
1	Control	38	8	10-11	50.0 (30)	0.12	2.8 (30)	0.72	40.0 (30)	0.20	0.7 (30)	0.25
	Treated	44	11	10-11	51.5 (33)	(P> 0.05)	2.2 (33)	(P> 0.05)	42.4 (33)	(P> 0.05)	0.64 (33)	(P> 0.05)
2	Control	26	12	10	78.6 (14)	0.93	14.5 (14)	2.48	72.7 (11)	0.30	1.37 (11)	0.20
	Treated	27	15	10	91.7 (12)	(P> 0.05)	93.0 (12)	(P< 0.05)	66.7 (12)	(P> 0.05)	1.25 (12)	(P> 0.05)
3	Control	27	15	10	58.3 (12)	0.57	6.6 (12)	0.20	50.0 (12)	0.00	0.83 (12)	0.36
	Treated	26	16	10	70.0 (10)	(P> 0.05)	5.6 (10)	(P> 0.05)	50.0 (10)	(P> 0.05)	1.00 (10)	(P> 0.05)
4	Control	31	10	10	52.4 (21)	0.12	2.6 (21)	1.11	41.2 (17)	0.06	0.88 (17)	0.38
	Treated	26	15	10	54.5 (11)	(P> 0.05)	4.6 (11)	(P> 0.05)	40.0 (10)	(P> 0.05)	1.1 (10)	(P> 0.05)

Table 29: Susceptibility to infection with P. y. nigeriensis of females of the P.B. strain of An. gambiae s.s. in untreated control and after treatment with one-tenth of LC50 value of DDT

Experiment	Types of treatment	No. blood fed	No. died during incubation period	Days from feeding to dissection	Gut dissection (No. dissected)				Gland dissection (No. dissected)			
					% of guts positive	SND values	Williams' mean of nos. of oocysts	t values	% of glands positive	SND values	Sporozoite mean score	t values
1	Control	49	9	10-11	38.9 (36)	2.75	1.7 (36)	3.7	33.3 (36)	2.02	0.75 (36)	2.72
	Treated	46	11	10-11	71.4 (35)	(P< 0.01)	5.2 (35)	(P< 0.001)	57.1 (35)	(P< 0.01)	1.71 (35)	(P< 0.01)
2	Control	35	5	10	96.7 (30)	0.19	58.3 (30)	1.156	87.5 (24)	0.68	1.54 (24)	0.12
	Treated	31	8	10	95.7 (22)	(P> 0.05)	49.6 (22)	(P> 0.05)	80.0 (20)	(P> 0.05)	1.50 (20)	(P> 0.05)
3	Control	26	8	10	50.0 (18)	0.14	3.1 (18)	0.63	27.8 (18)	0.76	0.777 (18)	0.01
	Treated	30	7	10	77.8 (23)	(P> 0.05)	2.4 (23)	(P> 0.05)	39.1 (23)	(P> 0.05)	0.783 (23)	(P> 0.05)
4	Control	36	2	10-11	70.6 (34)	1.27	5.6 (34)	0.16	79.4 (34)	0.29	1.77 (34)	0.17
	Treated	55	14	10-11	82.9 (41)	(P> 0.05)	6.0 (41)	(P> 0.05)	82.1 (39)	(P> 0.05)	1.82 (39)	(P> 0.05)

Table 30: Susceptibility to infection with P. v. nigeriensis of females of the BEECH and P.B. strains in untreated control and after treatment with one-tenth of LC50 value of malathion

Strains	Experiment No.	Type of treatment	No. blood fed	No. died during incubation period	Days from feeding to dissection	Gut dissection (No. dissected)				Gland dissection (No. tested)			
						% of guts positive	SND values	Williams' mean of nos. of oocysts	t values	% of glands positive	SND values	Sporozoite mean score	t values
BEECH	5	Control	53	32	10	81.0 (21)	0.82	22.5 (21)	0.30	71.4 (21)	0.26	1.81 (21)	0.28
		Treated	44	24	10	90.0 (20)	(P > 0.05)	18.5 (20)	(P > 0.05)	75.0 (20)	(P > 0.05)	2.0 (20)	(P > 0.05)
	6	Control	50	35	10	93.3 (15)	0.44	65.8 (15)	0.69	93.3 (15)	1.25	2.53 (15)	0.99
		Treated	58	30	10	89.3 (28)	(P > 0.05)	42.8 (28)	(P > 0.05)	78.6 (28)	(P > 0.05)	2.11 (28)	(P > 0.05)
P.B.	5	Control	61	39	10	77.3 (22)	0.30	41.6 (22)	0.03	68.2 (22)	0.43	1.72 (22)	0.23
		Treated	62	41	10	81.0 (21)	(P > 0.05)	40.6 (21)	(P > 0.05)	61.9 (21)	(P > 0.05)	1.62 (21)	(P > 0.05)
	6	Control	64	30	10	85.3 (34)	0.66	22.3 (34)	0.22	61.8 (34)	0.41	1.21 (34)	0.48
		Treated	65	21	10	79.6 (44)	(P > 0.05)	24.7 (44)	(P > 0.05)	57.1 (42)	(P > 0.05)	1.36 (42)	(P > 0.05)

intensity of infection between the control and treated group for each strain. In guts and glands, which were dissected on day 10 after the infecting blood meals, oocyst and sporozoite infections were found in both treated and control mosquitoes, which indicated that treatment with one-tenth of LC50 value of malathion had no effect on the rate of development of the parasite.

In experiment 7, 50% of the LC50 values of malathion was applied to the BEECH and P.B. strains i.e. doses of 0.45% and 0.55% respectively. The results are shown in Tables 31 and 32. Again, there were no differences between the infections in the control and treated mosquitoes in each strain. In experiment 8, the treated BEECH showed an intensity of oocysts and sporozoites significantly lower than those of the control, but there was no difference between the control and treated batches of the P.B. strain. Experiment 9 showed no difference in the vectorial capacity between control and treated mosquitoes in each strain. Despite the concentrations of malathion being increased to half of the LC50 value, the parasites were found in guts and glands on the tenth day after blood feeding to the same extent in the treated group as in the control. This again showed that the malathion treatment had no effect on the development of the parasite.

In experiment 10, BEECH and P.B. were treated with DDT before an infecting blood meal followed by repeated DDT treatment after successive uninfecting blood meals. The intention was to simulate the situation in which females make repeated visits to houses for blood meals at each gonotrophic cycle and in which they encounter weak residual deposits when resting on the walls. The doses used were one-tenth of the LC50

Table 31: Susceptibility to infection with *P. y. nigeriensis* of females of BEECH strain of *An. stephensi* in untreated control and after treatment with half of LC50 value of malathion

Experiment No.	Types of treatment	No. blood fed	No. died during incubation period	Days from feeding to dissection	Gut dissections (No. dissected)				Gland dissection (No. dissected)			
					% of guts positive	SND values	Williams' means of nos. of oocysts	t values	% of glands positive	SND values	Sporozoite mean score	t values
7	Control	33	26	10	87.7 (7)	0.65	16.5 (7)	0.49	71.4 (7)	0.00	1.43 (7)	0.40
	Treated	28	21	10	71.4 (7)	(P > 0.05)	10.5 (7)	(P > 0.05)	71.4 (7)	(P > 0.05)	1.71 (7)	(P > 0.05)
8	Control	58	19	10	100 (39)	2.33	99.5 (39)	4.4	97.4 (39)	1.81	2.54 (39)	2.78
	Treated	75	29	10	87.0 (46)	(P < 0.05)	20.1 (46)	(P < 0.001)	86.4 (44)	(P > 0.05)	1.82 (44)	(P < 0.01)
9	Control	56	42	10	78.6 (14)	0.27	16.6 (14)	1.05	71.4 (14)	0.32	1.86 (14)	0.22
	Treated	74	57	10	82.4 (17)	(P > 0.05)	22.8 (17)	(P > 0.05)	76.5 (17)	(P > 0.05)	1.88 (17)	(P > 0.05)

Table 32: Susceptibility to infection with P. y. nigeriensis of females of the P.B. strain of An. gambiae s.s. in untreated control and after treatment with half of LC50 value of malathion

Experiment No.	Types of treatment	No. blood fed	No. died during incubation period	Days from feeding to dissection	Gut dissections (No. dissected)				Gland dissections (No. dissected)			
					% of guts positive	SND values	Williams' mean of nos. of oocysts	t values	% of glands positive	SND values	Sporozoite mean score	t values
7	Control	30	5	10	72.0 (25)	0.21	12.8 (25)	0.42	66.7 (24)	0.00	1.71 (24)	0.33
	Treated	31	15	10	75.0 (16)	(P> 0.05)	17.1 (16)	(P> 0.05)	66.7 (15)	(P> 0.05)	1.53 (15)	(P> 0.05)
8	Control	37	16	10	76.2 (21)	0.24	10.9 (21)	0.99	76.2 (21)	0.24	1.57 (21)	0.14
	Treated	45	21	10	79.2 (24)	(P> 0.05)	19.0 (24)	(P> 0.05)	79.2 (24)	(P> 0.05)	1.63 (24)	(P> 0.05)
9	Control	55	19	10	94.4 (36)	1.12	49.? (36)	0.67	85.7 (35)	0.05	1.86 (35)	0.08
	Treated	67	29	10	86.8 (38)	(P> 0.05)	37.7 (38)	(P> 0.05)	86.1 (36)	(P> 0.05)	1.83 (36)	(P> 0.05)

values for each strain. Female mosquitoes from the treated and control group of both strains were fed on the same infected mouse. The treated groups were retreated with DDT for one hour every three days after being blood fed. Bowls were provided for egg laying two days after each successive blood meal. The mosquitoes were dissected and examined ten days after the infecting feed and the results are shown in Table 33. Although the samples are relatively small because of the effect of repeated treatments, there was no difference in the infection rate in the control and treated groups in either strain.

Experiment 12 was performed with the insect growth inhibitor Dimilin, and one-tenth of the LC50 values were used, i.e. 0.0005 ppm for BEECH and 0.0002 ppm for P.B.. Dimilin was applied to early fourth instar larvae for 24 hours, then the larvae were rinsed with tap water and reared to obtain adults. The effects of Dimilin were seen during pupation and emergence and in the form of abnormalities of the adult. Emerged females were fed on infected mice when they were approximately 5-6 days old and mosquitoes were dissected on day 10 after being fed on infected blood. Results are shown in Table 34. Unfortunately, both BEECH and P.B. showed relatively low infections and the samples were small, but both the infection rate and the intensity of infection were very similar among the two groups of both strains.

The results obtained from these experiments with P. y. nigeriensis showed in most cases that a single exposure of one-tenth of the LC50 values of DDT and malathion had no effect on vectorial capacity in An. gbehensi or in a highly susceptible strain of An.

Table 33: Susceptibility to infection with P. v. nigeriensis of females of the BEECH and P.B. strains in untreated control and after several treatments with one-tenth of LC50 values of DDT

Experiment No.	Strains	Types of treatment	No. blood fed	No. died during incubation period	Days from feeding to dissection	Gut dissection (No. dissected)				Gland dissection (No. dissected)			
						% of guts positive	SND values	Willaims' mean of nos. of oocysts	t values	% of glands positive	SND values	Sporozoite mean score	t values
10	BEECH	Control	32	22	10	100 (10)	0.99	72.6 (10)	0.22	100 (10)	0.99	2.2 (10)	0.24
		Treated	57	46	10	90.9 (11)	(P> 0.05)	61.6 (11)	(P> 0.05)	90.9 (11)	(P> 0.05)	2.09 (11)	(P> 0.05)
	P.B.	Control	36	16	10	80.0 (20)	0.22	23.8 (20)	0.21	80.0 (20)	0.47	1.8 (20)	0.27
		Treated	62	39	10	82.6 (23)	(P> 0.05)	27.1 (23)	(P> 0.05)	73.9 (23)	(P> 0.05)	1.7 (23)	(P> 0.05)

Table 34: Susceptibility to infection with P. y. nigeriensis of females of the BEECH and P.B. strains in untreated control and after larval treatment with one-tenth of LC50 values of Dimilin

Experiment No.	Strains	Types of treatment	No. blood fed	No. died during incubation period	Days from feeding to dissection	Gut dissection (No. dissected)				Gland dissection (No. dissected)			
						% of guts positive	SND values	Williams' mean of nos. of oocysts	t values	% of glands positive	SND values	Sporozoite mean score	t values
12	BEECH	Control	41	28	10	46.2 (13)	0.16	3.0 (13)	0.34	33.3 (12)	0	0.58 (12)	0.18
		Treated	47	41	10	50.0 (6)	(P> 0.05)	3.8 (6)	(P> 0.05)	33.3 (6)	(P> 0.05)	0.5 (6)	(P> 0.05)
	P.B.	Control	37	27	10	50.0 (10)	0	3.3 (10)	0.28	40.0 (10)	0.42	0.5 (10)	0.36
		Treated	58	50	10	50.0 (8)	(P> 0.05)	3.2 (8)	(P> 0.05)	50.0 (8)	(P> 0.05)	0.63 (8)	(P> 0.05)

gambiae s.s., and the treatment did not provoke the ability to carry *P. y. nigeriensis* in the refractory strain of *An. gambiae s.s.* When the dose of malathion was increased to half of the LC50 value, none of the experiments showed an effect of the high malathion dose, except in one experiment with the BEBCII strain, which showed a significant reduction of the intensity of infection, but, this result was perhaps due to an unusually high infection rate (100%) in the control group. Although a low infection rate was obtained in the Dimilin experiment and the samples used for Dimilin and repeated DDT treatment were relatively small, the results indicated that these treatments had no effect on the susceptibility of the mosquitoes. It is also noteworthy that the three insecticides investigated also had no effect on the rate of development of the parasite, i.e. sporozoite formation occurred as usual on day 10 after the infecting feed.

8. Experiments on the effect of DDT selection - pressure on susceptibility to infection and on susceptibility to the insecticide permethrin

It is often found that selection applied to one character has an effect on other characters. Studies were made of possible effects of DDT selection pressure on susceptibility to infection by P. y. nigeriensis and susceptibility to the killing effect of permethrin (a synthetic pyrethrin compound or pyrethroid) which has been considered to be one of the promising alternative insecticides to replace DDT.

8.1. Test for correlation of DDT resistance and the susceptibility to infection

To investigate a possible correlation between DDT resistance and the susceptibility to infection, use was made of the generations designated Tog, Tog 1, Tog 2, Tog 3, Tog 4 and Tog 5 from the experiment on mass selection with DDT as described in section 1.1.1 of the Results and Table 2 of the Results. Infection experiments were performed by feeding on infected mice. In each experiment, each strain was fed on the same infected mouse. BEECH was used as a control. The results are summarized in Table 35. As already reported, the DDT resistance levels increased at each generation with LT50 values rising from 1.3 hours in the Tog strain to 7.8 hours in Tog 5. On the other hand, the infection rates hardly varied and showed no trend, indicating no correlation between these two characters.

8.2. Correlation of DDT resistance and permethrin tolerance level

Tog and Ian strains were used in this study to investigate the possible effects of DDT selection pressure on the permethrin

Table 35: Data showing P. v. nigeriensis infection rates in females of five generations of the Tog strain of An. gambiae s.s. following mass selection for DDT resistance

Strains	No. infected/No. dissected						% Positive* (No. dissected)		LT50 values for DDT ** (hrs.)
	Gut dissection			Gland dissections					
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Guts	Glands	
Tog	12/34	6/14	9/23	2/34	3/14	9/23	38.0 (71)	19.7 (71)	1.3
Tog 1	13/24	4/10	3/6	4/24	1/10	3/6	50.0 (40)	20.0 (40)	1.6
Tog 2	11/23	8/19	16/32	2/20	3/19	14/32	47.3 (74)	26.8 (71)	2.1
Tog 3	9/18	8/21	6/13	5/17	5/21	5/16	41.8 (55)	27.8 (54)	2.5
Tog 4	-	-	14/20	-	-	12/20	70.0 (20)	60.0 (20)	3.1
Tog 5	-	-	12/27	-	-	11/27	44.4 (27)	40.7 (27)	7.8
Control BEECH (An. stephensi)	12/15	22/25	6/7	9/15	19/24	6/7	85.1 (47)	73.9 (46)	-

* Data from all three experiments pooled together

** Data from Table 2.

tolerance levels. Test papers at a concentration of 0.033% permethrin were found to be suitable and were used throughout this study. The unselected Tog and Ian strains were tested with permethrin for a series of exposure periods. These two strains were then selected for DDT resistance as already described in section 1 of the Results. There was no selection with permethrin on these stocks. Tog RR which was derived from single family selection for homozygous DDT resistance (as described in section 1.1.2 of the Results and Figure 17) was one of the selected strains tested with permethrin and the other was Ian 7 which was derived from Ian by mass selection as described in section 1.1.1 of the Results and Table 3. The results are shown in Table 36. There is clear evidence of a correlated increase in permethrin tolerance in Tog RR. The resistance factor to permethrin in Tog RR relative to Tog was 2.1 fold compared with a resistance factor to DDT of 4.5 fold. In the case of Ian 7 there was much less, if any, correlated increase in permethrin tolerance due to the DDT selection.

The Tog and Ian strains were submitted to permethrin selection for seven generations with increases of the dose in later generations. Each generation was tested in order to evaluate the increase in tolerance level. The results of the tests are shown in Table 37 for the Tog and Table 38 for the Ian strains. The strains finally obtained from this selection were called Tog P7 and Ian P7. The permethrin resistance factors were 2.4 fold for Tog P7 compared with Tog and 4.8 fold for Ian P7 compared with Ian. These resistance factors resulting from deliberate selection are considerably greater than those arising as correlated responses to DDT selection (see Table 36).

Table 36: Data on permethrin tolerance in the original stocks of the Tog and Ian strains of An. gambiae s.s. and in the stocks after selection for DDT resistance

Strains	LT50 values for DDT (hrs.)	% Mortality following 0.03% permethrin treatment (No. tested)				LT50 values for permethrin (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.	
Tog	1.3*	34.2 (152)	71.5 (137)	100.0 (25)	-	1.3
Tog-RR	5.8**	7.2 (97)	33.6 (155)	82.6 (155)	88.0 (117)	2.7
Ian	1.8***	23.2 (82)	80.0 (50)	96.0 (50)	100.0 (31)	1.4
Ian 7	4.5***	17.7 (34)	-	61.7 (60)	100.0 (80)	1.9

* From Table 2

** From Table 6

*** From Table 3

Table 37: Effects of selection of the Tog strain of An. gambiae s.s. with permethrin

Generations	% Mortality following 0.033% Permethrin treatment (No. tested)				Slopes of regression lines	LT50 values (hrs.)
	1 hr.	2 hrs.	4 hrs.	8 hrs.		
Parental Tog	34.2 (152)+	71.5 (137)	100.0 (25)	-	6.9	1.3
Tog P1	46.1 (128)	80.1 (237)+	100.0 (25)	-	6.3	1.2
Tog P2	20.9 (110)	59.3 (119)+	94.0 (50)	-	3.9	1.7
Tog P3	45.8 (94)	68.3 (60)	96.9 (222)+	-	3.3	1.2
Tog P4	31.4 (105)	49.6 (115)	72.8 (206)+	92.0 (137)	2.1	1.9
Tog P5	30.0 (60)	37.5 (104)	78.3 (60)	92.3 (220)+	2.3	2.0
Tog P6	5.1 (39)	45.5 (44)	66.6 (314)	74.0 (104)+	2.5	3.3
Tog P7	1.8 (55)	-	67.5 (166)	-	4.2	3.1

+ Denotes treatment used to select the parent of the next generation

Table 38: Effects of selection of the Ian strain of An. gambiae s.s. with permethrin

Generations	% Mortality following 0.033% Permethrin treatment (No. tested)				Slopes of regression lines	LT50 values (hrs.)
	1 hr.	2 hrs.	4 hrs.	8 hrs.		
Parental Ian	23.2 (82)+	80.0 (50)	96.0 (50)	100.0 (31)	4.7	1.4
Ian P1	20.0 (100)	81.7 (159)+	93.1 (116)	-	3.9	1.5
Ian P2	7.9 (216)	18.4 (245)	65.6 (154)+	93.8 (64)	3.4	3.0
Ian P3	13.7 (102)	21.0 (295)	69.5 (269)+	91.2 (205)	2.9	2.9
Ian P4	0.9 (113)	6.7 (60)	61.7 (60)	71.2 (285)+	3.5	4.6
Ian P5	8.0 (25)	16.9 (184)	34.4 (151)	61.1 (334)+	1.9	6.1
Ian P6	6.0 (83)	18.8 (80)	33.3 (60)	75.3 (348)+	2.4	5.0
Ian P7	1.0 (98)	5.9 (101)	33.3 (60)	54.3 (164)	2.8	6.7

+ Denotes treatment used to select the parents of the next generation

Tog P7 and Ian P7 were tested with DDT in order to investigate whether permethrin selection pressure would confer cross-resistance to DDT. Results of the tests are shown in comparison with the original data on the Tog and Ian strains in Table 39. The results showed no increase in DDT resistance levels in these permethrin selected strains, thus indicating that selection with permethrin did not confer cross-resistance to DDT. The correlated response obtained from DDT selection is presumably due to a rather non-specific mechanism associated with DDT resistance while the permethrin selection apparently built up a specific mechanism concerned with pyrethroid detoxification which has no resistance effect with respect to DDT.

Table 39: Comparison of DDT resistance in the permethrin selected stocks and in the original populations from which they come

Strains	% Mortality following 4% DDT treatment (No. tested)					Slopes of regression lines	LT50 values (hrs)
	0.5 hr	1 hr	2 hrs	4 hrs	8 hrs		
Tog *	10.7(309)	37.4(318)	73.2(168)	90.0(100)	-	2.8	1.3
Tog P7	-	22.8(92)	70.4(81)	94.2(121)	100 (71)	4.8	1.5
Ian **	6.0(50)	19.0(100)	48.0(100)	69.2(26)	92.0(25)	1.8	1.8
Ian P7	-	37.6(133)	55.3(103)	70.0(100)	97.5(203)	2.4	1.6

* From Table 2

** From Table 3

DISCUSSION

Malaria eradication from most areas in which malaria is still endemic is considered improbable in the foreseeable future; therefore, long term control must be planned. Insecticide resistance is one of the most serious problems and has become increasingly serious. It is considered that the concept of "dilution" of the frequency of resistance genes in wild populations by release of males carrying susceptibility genes seems to be a possible method to cope with the resistance problem. This may allow a programme of indefinitely prolonged insecticide application to retain its effectiveness (Curtis et.al., 1978). Male mosquitoes do not bite and hence cannot transmit disease or cause any nuisance to humans. It is generally found that almost all mature wild females are naturally inseminated so that there are almost no remaining virgin females to be mated by the addition of extra males. Thus the release of males would not add to the reproductive potential of the population. Moreover, male mosquitoes tend to enter houses less than females, thus males would have little contact with residual insecticide deposits sprayed in houses. It should therefore be possible to make a release while continuing a spraying programme. An additional safety factor for the males could be arranged if they carried a gene for resistance to the insecticide concerned linked to the Y chromosome so that it could not be passed to their female progeny. The R70 stock satisfies this requirement with respect to dieldrin and partially satisfies it with respect to DDT (Table 21). A high level of DDT resistance in the male could probably be achieved by a deliberate attempt to translocate the major gene for DDT resistance on to the Y chromosome.

The laboratory cage experiments of "dilution" by repeated releases of males carrying the DDT susceptibility gene and a genetic sexing system were carried out in An. gambiae s.s. and they showed that four generations of release at 4:1 ratio of susceptible R70 males: cage males could force the resistance level of a resistant population down to an acceptable level (see Figure 25 and 26). This process was found to be assisted by natural selection against the resistance gene in the absence of treatment, but the releases ensured that the reversion to susceptibility was much faster and more complete than it otherwise would have been. It was also shown that a 2:1 release ratio was capable of holding the susceptibility level despite DDT selective pressure. Theoretically it is expected that a programme of intermittent releases would be sufficient but this has not been tested in the laboratory cage yet. Moreover, although R70 released males were partially resistant to DDT, it was found that the releases also force the resistance level of males of the cage population down to a certain extent from the high level of resistance initially in the population.

The feasibility of the release of males carrying genes for susceptibility under field conditions may depend on the following factors:

1) The effective dominance or recessiveness of the resistance genes under field condition (Comin, 1977; Curtis et.al., 1978), recessive resistance being more easily controlled. As discussed below, the laboratory studies indicated intermediate dominance for the examples of DDT resistance which were studied. However, extrapolation of these results to the field situation are questionable

and field studies of effective dominance are required.

2) Selective pressures on mosquito populations as side-effects of the agriculturalusage of insecticides. It is generally believed that such selection acts by drainage of insecticide into breeding places where it would contribute part of the selection pressure for resistance which has to be countered by the releases. However, a direct adverse effect would arise if agricultural sprays contaminated the vegetation where males rest. The released males could thus be destroyed by the agricultural insecticide deposit with an obvious adverse affect on the male release programme.

A genetic sexing system has been investigated in the R70 strain of An. gambiae s.s. It was shown that the system based on a translocation of semi-dominant dieldrin resistance on to the Y chromosome is very reliable and stable, at least so long as R70 males were continually outcrossed to susceptible homozygote females. The consequences of the less laborious procedure of maintaining the R70 strain by mating within the strain have been described and discussed by Curtis et.al., (1976). Such a genetic sexing system could be applied to other anophelines or insect species and Curtis (1978a) stated that to produce this system, the following points should be considered:

1) The availability of suitable genes by which the heterozygotes could be accurately discriminated from the susceptible homozygote.

2) The availability of a suitable male determining chromosome so that it is feasible to produce male-linked translocations.

3) Crossing-over between the translocation break point and the resistance gene. In the case of the T70 strain crossing-over had been minimized by screening for an optimally positioned translocation. (Curtis et.al., 1976). In An. albimanus, crossing-over was suppressed by using an inversion (Seawright et.al., 1978).

4) The acceptibility of releasing males carrying a resistance gene; where such a resistance is already widespread and/or where the linkage to a semi-sterilising translocation is completed so that the gene could not establish itself in the population, there need be no objection to such releases.

The evidence obtained from these laboratory cage studies have given some hope that the "dilution" method may be feasible. A small scale field trial may now be carried out to investigate the feasibility under more realistic conditions. One of the arguments against this concept is that the release of males would require the setting up of extensive mosquito rearing facilities, but when compared to the method of introduction of sterility into the population by sterile male releases, the former method is expected to require lesser numbers of males and a lesser frequency of releases (Curtis et.al., 1978). Mass rearing of hundreds of thousands of males per day has already been reported in at least three mosquito species (Singh et.al., 1974; Dame et.al., 1974). With a combination of a genetic sexing system, which has been realised in at least three anopheline species as mentioned previously, it would be physically possible to produce a vast number of males for release if the necessary finance can be made available. With knowledge of seasonal changes in density of the wild population, one could choose the right time for release, which is

usually when the population density is very low. Thus the numbers required may be greatly reduced. It would allow great saving of money, manpower and transport etc. if house visitors who go to villages each month for the purpose of case detection could take with them the susceptible males to be released.

With regard to the inheritance studies of DDT resistance, homozygosity of the population is not necessarily proved by finding constancy of the resistance levels over several generations of mass selection. This is particularly so where there is a high degree of dominance of resistance. Under these conditions once a low frequency of the susceptible gene in the population has been reached further progress under mass selection will be very slow. Single family selection should be a more reliable method to obtain homozygous resistant colonies, but the disadvantage is that modifier genes favouring resistance may be lost in the inbreeding process and inbreeding depression may reduce the resistance level. In the case of Ian strain, Ian DR which was derived by single family selection showed about half the DDT resistance level of the Ian 10 which was produced by mass selection (see Table 11). Pooling of single families that showed homozygosity for the major gene for resistance would be the best method to get a colony with maximum resistance. However, this would require more labour and space than was available and therefore in these studies only one inbred single family of each strain was isolated. Crossing of a susceptible strain to all three resistant strains (Tog, Ian and Pala) showed that the regression lines of probit mortality on log dose of the parental strains and the hybrids showed an overlap between the heterozygote and each homozygote (Figures 17,

19, 21). i.e. susceptible homozygote (SS), heterozygote (RS) and resistant homozygote (RR) could not be 100% discriminated by any dose of DDT in either adults and first instar larvae. Davidson and Zahar (1973) and WHO (1976) proposed that the treatment of 4% DDT for one hour would kill all susceptible homozygote individuals. However, the results obtained from tests of the 16c10 strain which had been single family selected for homozygous susceptibility, showed that this dose did not kill all susceptible homozygotes. A two hour treatment killed them all but it also killed some heterozygotes and homozygote resistant individuals. Tog, Ian and Pala were found to show imcompletely dominant DDT resistance in the adult stage. This was also the case with the first instar larval test, except in the Ian strain where nearly completely dominant resistance was found (see Tables 11 and 12 and Figures 16 and 17). Discrimination of the three genotypes was certainly no better at the larval stage than with adults. This was contrary to expectations because it appears that the adult test method may be seriously affected by the knockdown effect which causes the mosquitoes knocked down most quickly to "escape" some of the specified time of exposure to the test paper and possibly to recover later after the mosquitoes have been removed from the exposure tube.

Three types of dieldrin resistance were found from these studies, namely semi-dominant in the case of the Pala strain, almost fully dominant in the case of the Tog strain and incompletely dominant in the Ian strain. The first two are well known in An. gambiae s.s. and in other anophelines (Davidson and Jackson, 1961a, 1961b; Davidson and Hamon, 1962; Davidson, 1964a), but the incompletely dominant type seems to have been found for the first time.

With respect to the linkage studies of the DDT and dieldrin resistance genes, because of the contradiction of the results of the tests, with larval treatment by DDT and by dieldrin, no conclusion can be drawn. Use of an additional marker gene in such a study may provide further information on whether there really is linkage between the two resistance genes. In this connection the gene colourless (Curtis and Chalkley, in prep.) is being used as a third marker in the An. gambiae species group to investigate this point (Curtis, personal communication).

Concerning the studies of susceptibility to infection, the evidence from the experiments on duration of infection showed that An. gambiae s.s. and An. stephensi tend to lose their P. y. nigeriensis infection as they age. Both species investigated are in fact not natural vectors of this parasite, therefore it may be that these results are not typical of better adapted pairs of intermediate hosts and parasites, for example An. balabacensis and P. falciparum or An. dureni and P. y. nigeriensis. On the other hand, van der Kaay and Boorsma (1977) stated that even though An. atroparvus is not a natural vector of P. b. berghei, it can harbour infective sporozoites of this parasite for up to 57 days after the infecting blood meal, which is about 26 days longer than was found for An. gambiae s.s. and An. stephensi with respect to P. y. nigeriensis in the present studies (Tables 24 and 25). It remains unknown whether or not the human malaria parasites can persist in their natural anopheline vectors throughout the whole of the life of the vector. This is a point of some importance and deserves to be studied further.

A study of survival of An. gambiae s.s. infected with P. y. nigeriensis showed that the parasite had an effect during its development on mosquito survival (Figure 27), such a phenomenon should also be studied with natural parasite and vector systems. In the case of filariae there is evidence for damaging effects of the pathogens on Aedes aegypti (Kershaw et.al., 1953; Townson, 1971).

According to the experiments on possible effects of sublethal doses of insecticide on vectorial capacity, the results obtained were different from those of Gaaboub and Busvine (1975, 1976) with Ae. aegypti and Brugia pahangi. In my studies on a totally different parasite-vector system there was no evidence of an effect of sublethal dose of DDT or malathion or Dimilin on the vectorial capacity of refractory or susceptible strains of An. gambiae s.s. or on a highly susceptible strain of An. stephensi. The results produced here showed no statistically significant evidence of any effects of the insecticides, but do not prove that there are absolutely no effects. However, sufficient replicates were studied and it can be concluded with confidence that the percentage change in vectorial capacity due to sublethal doses of these insecticides, if it exists at all, is too small to be of any practical importance.

DDT selection pressure of An. gambiae s.s. was shown to confer some degree of cross resistance to permethrin. DDT resistant Ae. aegypti strains from various parts of the Far East and Central and South America, where pyrethroids have never been used in the past, showed resistance to pyrethroids (Chadwick et.al., 1977; Prasittisuk and Busvine, 1977). This

was interpreted as being due to cross-resistance conferred by the DDT resistance genes. In the present work with A. gambiae s.s. direct proof of such cross resistance has been obtained. This work showed quite moderate resistance factors (2.4 x to 4.8 x) to permethrin resulting from cross-tolerance and from deliberate selection with this compound. The resistance factors were about three orders of magnitude less than those obtained by Priester and Georghiou (1978) following permethrin larval selection of C. p. fatigans (= C. p. quinquefasciatus). It remains to be seen in anophelines whether and how soon significant levels of resistance to permethrin and other pyrethroids will be built up in wild populations. In order to predict whether cross-resistance resulting from DDT selection will pre-adapt populations to evolve high levels of pyrethroid resistance, it is necessary to study in anophelines the mechanisms involved in pyrethroid resistance and those conferring cross-resistance with DDT.

SUMMARY

1. In the present study, laboratory cage experiments were carried out with the Tog strain of An. gambiae s.s. to demonstrate the concept of the "dilution" method of reducing DDT resistance by introducing males of the R70 strain of the same species carrying susceptibility genes. Although the resistance levels in a control cage were found to decline in the absence of releases, a much faster reduction of DDT resistance levels in the experimental cage resulted from the introduction of R70 males in a ratio of four R70 males to one cage population male. Further investigations showed that under DDT selection pressure applied to females of the cage population, the same 4:1 ratio of R70 males released could hold the susceptibility levels of the cage population constant and when the release ratio was reduced to 2:1, the cage population under selection pressure still showed no sign of any change.

2. The R70 strain allows a genetic sexing system, based on the translocation of a semi-dominant dieldrin resistance gene to the Y chromosome, to be used for separating the sexes in the larval stage. First instar larvae are exposed to dieldrin, the female larvae are susceptible and are killed, and essentially only males are reared. This system has been shown to be very reliable and stable. In addition to heterozygosity for dieldrin resistance, the R70 males were also found to be partially resistant to DDT, but no DDT resistance is transmitted to their female progeny. This would give an advantage to the release method if applied when a DDT spraying programme was in progress as there would be less risk of the released males being killed. Further-

more, the release of such translocated males introduces semisterility into the target population, which provides an additional genetic control method.

3. Single family selection was considered to be the most promising method for obtaining homozygosity for susceptibility and resistance. Two selected strains of An. gambiae s.s. were found by both adult and first instar larval testing to show incompletely dominant DDT resistance. Another strain was found to show nearly dominant DDT resistance in the larval test but incomplete dominance in the adult test. It was not possible to obtain DDT doses for 100% discrimination of the homozygotes from the heterozygotes in any of the three strains. Further DDT inheritance studies in the Tog strain, which was used in the population cage experiment, gave some evidence that DDT resistance is controlled by a single major gene.

4. All three strains also showed resistance to dieldrin and three types of this resistance were observed, namely, semi-dominant in the Pala strain, almost fully dominant in the Tog strain and incompletely dominant in the Ian strain. The first two types are well known in An. gambiae s.s. but the last type of resistance has not been previously reported in this mosquito species.

5. An attempt to investigate a possible linkage relationship between DDT and dieldrin resistance genes in the three strains was not conclusive but provided evidence of similarity of the resistance alleles.

6. The results obtained from infection studies gave evidence that An. gambiae s.s. and An. stephensi tend to lose their P. y. nigeriensis infection if they live very long. Moreover, the parasite appeared to damage An. gambiae s.s. hosts in the first few days after infection. It remains unknown whether these phenomena occur in the human parasite-vector system in nature.

7. A series of experiments was performed to investigate the possible effects of sublethal doses of DDT, malathion and Dimilin on susceptibility to infection. No evidence was obtained for any effect of these insecticides on the susceptibility to P. y. nigeriensis of refractory and susceptible strains of An. gambiae s.s. or of a highly susceptible strain of An. stephensi. Similarly, no effect was found of these three insecticides on the rate of development of P. y. nigeriensis in An. gambiae s.s. and An. stephensi.

8. DDT selection pressure on the Tog strain of An. gambiae s.s. did not affect its susceptibility to P. y. nigeriensis infection, but did confer a small degree of cross-resistance to permethrin. On the other hand, permethrin selection pressure did not select for DDT resistance. The level of permethrin resistance resulting from deliberate selection with this compound and from cross-resistance with DDT was relatively low.

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Appendix 1. Mortalities after 4% DDT testing of female adults of the cage population, Cage Tog X, with the slopes of regression lines and the LT50 values.

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
6*	X1	4.0 (50)	4.0 (50)	42.1 (19)	54.1 (37)	2.4	6.8
	X2	2.6 (76)	5.0 (50)	22.2 (100)	52.0 (72)	2.3	8.4
	X3	3.2 (61)	9.4 (32)	40.9 (66)	50.0 (60)	2.2	6.9
	X4	0 (41)	3.3 (90)	14.6 (96)	45.3 (75)	5.1	7.0
	X5	6.4 (63)	12.0 (50)	16.0 (50)	50.0 (50)	1.6	10.9
	X6	0 (43)	12.0 (50)	-	48.8 (86)	5.0	6.6
7	X7	6.7 (60)	6.0 (100)	36.7 (60)	61.0 (54)	2.2	7.8
	X8	3.9 (51)	17.2 (58)	30.0 (90)	53.3 (60)	2.0	7.1
	X9	1.5 (65)	10.3 (68)	21.5 (93)	44.3 (88)	2.2	9.0
	X10	-	-	-	-	-	-
	X11	-	-	-	-	-	-
	X12	3.2 (31)	-	17.9 (28)	46.2 (26)	1.9	10.2
8	X13	6.9 (29)	6.7 (60)	18.0 (50)	53.4 (28)	1.8	9.8
	X14	0 (52)	4.8 (42)	20.0 (50)	47.4 (76)	5.2	6.6
	X15	3.2 (63)	6.4 (110)	-	48.0 (25)	2.1	9.0
	X16	0 (92)	2.3 (90)	14.6 (48)	45.3 (75)	5.1	7.0
	X17	2.9 (35)	10.0 (60)	8.3 (60)	51.7 (60)	1.9	11.2
	X18	1.7 (60)	12.5 (16)	26.7 (30)	53.3 (30)	2.4	7.3
9	X19	-	-	-	-	-	-
	X20	-	18.8 (64)	25.0 (40)	38.5 (65)	1.8	7.1
	X21	5.8 (87)	8.8 (60)	45.2 (31)	60.3 (73)	2.3	5.8
	X22	0 (30)	7.4 (27)	17.5 (65)	26.7 (15)	4.8	6.6
	X23	9.7 (72)	17.0 (47)	36.7 (30)	40.7 (54)	1.3	10.3
	X24	-	-	-	-	-	-

Appendix 1 (continued)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
10	X25	1.2 (161)	22.6 (84)	50.0 (60)	88.3 (60)	3.4	3.7
	X26	7.6 (53)	13.2 (38)	42.4 (118)	76.3 (76)	2.5	4.6
	X27	8.1 (37)	22.7 (22)	56.7 (30)	71.1 (45)	2.3	4.1
	X28	9.7 (31)	45.0 (20)	50.0 (36)	76.9 (52)	2.1	3.4
	X29	20.0 (10)	33.3 (30)	80.4 (46)	81.0 (42)	2.1	2.5
	X30	25.0 (20)	52.4 (21)	50.0 (24)	90.0 (30)	1.9	2.3
11	X31	21.1 (19)	66.0 (50)	81.4 (86)	86.7 (60)	2.1	1.8
	X32	50.0 (40)	73.3 (30)	83.3 (42)	83.0 (53)	1.1	0.72
	X33	44.4 (36)	65.0 (40)	74.3 (35)	96.0 (25)	2.0	1.3
	X34	56.5 (23)	70.2 (41)	83.6 (55)	-	1.4	0.78
	X35	63.5 (52)	52.2 (23)	-	94.0 (18)	1.6	0.94
	X36	67.7 (60)	74.3 (35)	95.6 (45)	46.7 (30)	1.7	0.60
12	X37	-	-	-	-	-	-
	X38	55.4 (65)	73.3 (60)	85.0 (60)	96.7 (60)	1.8	0.91
	X39	59.7 (62)	78.3 (60)	89.3 (75)	95.0 (120)	1.5	0.66
	X40	52.1 (91)	66.7 (36)	83.3 (30)	95.0 (60)	1.8	1.03
	X41	-	-	-	-	-	-
	X42	-	-	-	-	-	-
13	X43	42.9 (35)	63.4 (82)	85.0 (60)	95.0 (60)	2.1	1.27
	X44	34.0 (53)	66.7 (18)	90.5 (21)	93.3 (60)	2.2	1.35
	X45	68.0 (50)	96.4 (56)	95.0 (60)	97.6 (42)	1.5	0.28
	X46	-	-	-	-	-	-
	X47	61.3 (62)	93.3 (60)	86.8 (38)	100.0 (42)	3.3	0.89
	X48	-	-	-	-	-	-
14	X49	61.8 (34)	70.5 (44)	90.7 (43)	100.0 (71)	3.7	1.12
	X50	39.9 (198)	70.0 (60)	90.0 (33)	100.0 (30)	4.2	1.38
	X51	63.3 (60)	69.4 (111)	90.2 (51)	100.0 (45)	3.6	1.12

Appendix 1 (continued)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
10	X25	1.2 (161)	22.6 (84)	50.0 (60)	88.3 (60)	3.4	3.7
	X26	7.6 (53)	13.2 (38)	42.4 (118)	76.3 (76)	2.5	4.6
	X27	8.1 (37)	22.7 (22)	56.7 (30)	71.1 (45)	2.3	4.1
	X28	9.7 (31)	45.0 (20)	50.0 (36)	76.9 (52)	2.1	3.4
	X29	20.0 (10)	33.3 (30)	80.4 (46)	81.0 (42)	2.1	2.5
	X30	25.0 (20)	52.4 (21)	50.0 (24)	90.0 (30)	1.9	2.3
11	X31	21.1 (19)	66.0 (50)	81.4 (86)	86.7 (60)	2.1	1.8
	X32	50.0 (40)	73.3 (30)	83.3 (42)	83.0 (53)	1.1	0.72
	X33	44.4 (36)	65.0 (40)	74.3 (35)	96.0 (25)	2.0	1.3
	X34	56.5 (23)	70.2 (41)	83.6 (55)	-	1.4	0.78
	X35	63.5 (52)	52.2 (23)	-	94.0 (18)	1.6	0.94
	X36	67.7 (60)	74.3 (35)	95.6 (45)	46.7 (30)	1.7	0.60
12	X37	-	-	-	-	-	-
	X38	55.4 (65)	73.3 (60)	85.0 (60)	96.7 (60)	1.8	0.91
	X39	59.7 (62)	78.3 (60)	89.3 (75)	95.0 (120)	1.5	0.66
	X40	52.1 (91)	66.7 (36)	83.3 (30)	95.0 (60)	1.8	1.03
	X41	-	-	-	-	-	-
	X42	-	-	-	-	-	-
13	X43	42.9 (35)	63.4 (82)	85.0 (60)	95.0 (60)	2.1	1.27
	X44	34.0 (53)	66.7 (18)	90.5 (21)	93.3 (60)	2.2	1.35
	X45	68.0 (50)	96.4 (56)	95.0 (60)	97.6 (42)	1.5	0.28
	X46	-	-	-	-	-	-
	X47	61.3 (62)	93.3 (60)	86.8 (38)	100.0 (42)	3.3	0.89
	X48	-	-	-	-	-	-
14	X49	61.8 (34)	70.5 (44)	90.7 (43)	100.0 (71)	3.7	1.12
	X50	39.9 (198)	70.0 (60)	90.0 (33)	100.0 (30)	4.2	1.38
	X51	63.3 (60)	69.4 (111)	90.2 (51)	100.0 (45)	3.6	1.12

Appendix 1. (continued)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	X52	50.9 (222)	82.7 (52)	93.3 (60)	98.6 (69)	2.3	0.9
	X53	69.8 (53)	73.2 (41)	100.0 (60)	100.0 (24)	5.3	0.99
	X54	67.8 (245)	-	-	100.0 (30)	3.4	0.65

* Generation number following mass selection from Table 2 (and see Figure 25)

Appendix 2. Mortalities after 4% DDT testing of female adults of the control cage population, Cage Tog C, with the slopes of regression lines and the LT50 values.

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
8*	C15	-	-	-	-	-	-
	C16	3.3 (30)	7.6 (66)	-	46.0	2.0	9.4
	C17	-	-	-	-	-	-
	C18	3.6 (28)	13.3 (30)	16.7 (60)	55.0 (60)	2.0	8.5
9	C19	10.0 (30)	4.0 (25)	30.0 (30)	55.6 (27)	1.8	8.3
	C20	3.7 (82)	0 (30)	13.3 (60)	50.6 (85)	3.1	12.3
	C21	-	-	-	-	-	-
	C22	4.6 (108)	12.1 (83)	20.0 (40)	60.0 (35)	2.0	7.5
	C23	3.9 (51)	17.2 (29)	30.0 (30)	53.3 (30)	2.0	7.5
	C24	3.3 (30)	5.6 (18)	-	-	-	-
10	C25	2.9 (68)	5.1 (78)	15.6 (90)	58.3 (60)	2.3	8.3
	C26	3.5 (57)	11.7 (60)	32.6 (43)	57.66(30)	2.3	6.5
	C27	9.7 (31)	10.0 (60)	48.0 (25)	58.7 (63)	1.9	5.8
	C28	-	10.7 (28)	14.3 (49)	38.5 (26)	1.6	14.2
	C29	6.8 (103)	16.7 (60)	30.5 (59)	58.3 (60)	1.9	6.7
	C30	6.3 (32)	24.0 (96)	45.6 (57)	68.3 (60)	2.2	4.6
11	C31	6.1 (33)	15.1 (73)	50.0 (30)	66.7 (60)	2.3	4.8
	C32	3.3 (60)	23.3 (30)	16.7 (12)	66.0 (47)	2.2	6.5
	C33	14.3 (19)	32.3 (31)	47.6 (21)	76.7 (30)	1.9	3.7
	C34	11.3 (62)	22.6 (31)	-	-	1.5	6.2
	C35	-	-	-	-	-	-
	C36	8.3 (72)	21.7 (60)	69.0 (29)	66.7 (30)	2.2	3.9
12	C37	-	-	-	-	-	-
	C38	10.9 (55)	23.3 (60)	36.7 (60)	75.0 (60)	2.0	4.5

Appendix 2. (continued)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression line	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	C39	8.3 (24)	14.3 (28)	39.1 (105)	66.7 (60)	2.1	5.4
	C40	6.6 (76)	20.0 (90)	36.8 (68)	70.8 (120)	2.2	4.9
	C41	11.7 (60)	23.3 (30)	45.0 (60)	71.7 (60)	2.0	4.4
	C42	-	-	-	-	-	-
13	C43	4.5 (67)	25.0 (40)	46.7 (60)	76.6 (64)	2.6	4.1
	C44	11.4 (88)	13.6 (66)	37.4 (131)	73.7 (38)	2.1	4.9
	C45	17.0 (106)	36.7 (60)	66.7 (60)	74.1 (27)	1.9	3.0
	C46	8.3 (24)	41.2 (17)	-	76.2 (42)	2.2	3.4
	C47	10.0 (70)	43.2 (74)	63.3 (60)	86.2 (65)	2.5	2.8
	C48	13.0 (69)	42.9 (28)	65.4 (52)	70.0 (30)	1.8	3.2
14	C49	21.7 (60)	36.7 (60)	65.0 (60)	80.6 (36)	1.9	2.7
	C50	-	-	50.3 (24)	71.4 (21)	1.9	4.0
	C51	31.7 (60)	35.0 (103)	53.7 (95)	88.3 (94)	1.8	2.5
	C52	22.2 (27)	26.7 (60)	61.7 (60)	91.5 (117)	2.4	2.7
	C53	25.6 (82)	46.7 (45)	80.0 (60)	81.8 (44)	2.9	2.8
	C54	5.2 (58)	13.3 (30)	43.3 (30)	86.7 (30)	3.0	4.0
15	C55	33.3 (72)	50.0 (90)	71.4 (49)	90.2 (82)	1.9	1.8
	C56	28.9 (45)	40.0 (35)	79.1 (67)	78.6 (28)	1.7	2.2
	C57	29.6 (44)	30.8 (26)	66.7 (30)	90.0 (60)	2.1	2.4
	C58	30.8 (13)	-	56.7 (30)	83.3 (30)	1.6	1.9
	C59	26.2 (42)	60.0 (60)	53.3 (60)	-	1.2	2.4
	C60	35.5 (31)	28.3 (46)	60.9 (23)	83.8 (37)	1.6	2.5
16	C61	31.4 (35)	54.6 (22)	60.9 (23)	90.0 (30)	1.8	1.9
	C62	-	33.3 (36)	84.2 (38)	96.7 (30)	1.9	1.2
	C63	30.6 (36)	43.3 (30)	62.9 (35)	88.9 (27)	1.9	2.2
	C64	46.7 (60)	-	-	92.3 (26)	1.7	1.1
	C65	26.1 (23)	50.0 (30)	84.8 (46)	96.0 (25)	2.7	1.8

Appendix 2. (continued)

Gener-ation	Egg batch No.	% Mortality (No. tested)				Slopes of regres-sion lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	C66	19.6 (46)	64.2 (67)	73.0 (63)	93.3 (30)	2.4	1.9
17	C67	20.0 (15)	26.7 (30)	86.7 (30)	96.2 (53)	3.2	2.2
	C68	-	-	-	-	-	-
	C69	54.6 (22)	77.8 (54)	100.0 (36)	-	6.0	1.1
	C70	57.6 (59)	85.7 (19)	89.5 (19)	93.3 (30)	1.4	0.52
	C71	47.4 (17)	76.7 (30)	100.0 (10)	93.3 (30)	9.9	1.7
	C72	26.3 (19)	50.0 (42)	84.2 (19)	200.0 (27)	4.7	1.7
18	C73	14.7 (34)	66.1 (56)	90.4 (52)	77.3 (22)	2.1	1.9
	C74	17.8 (45)	47.6 (63)	86.7 (60)	93.3 (30)	2.8	2.0
	C75	42.0 (100)	78.3 (60)	95.2 (21)	96.7 (30)	2.3	1.0
	C76	38.0 (50)	83.3 (30)	94.9 (78)	98.3 (60)	2.9	1.3
	C77	-	-	-	-	-	-
	C78	68.4 (57)	78.3 (60)	81.7 (60)	96.7 (30)	1.4	0.54

* Generation number following mass selection (see Table 2) and 2 generations following establishment of cage Tog X (and see Figure 25)

Appendix 2. (continued)

Gener-ation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	C66	19.6 (46)	64.2 (67)	73.0 (63)	93.3 (30)	2.4	1.9
17	C67	20.0 (15)	26.7 (30)	86.7 (30)	96.2 (53)	3.2	2.2
	C68	-	-	-	-	-	-
	C69	54.6 (22)	77.8 (54)	100.0 (36)	-	6.0	1.1
	C70	57.6 (59)	85.7 (19)	89.5 (19)	93.3 (30)	1.4	0.52
	C71	47.4 (17)	76.7 (30)	100.0 (10)	93.3 (30)	9.9	1.7
	C72	26.3 (19)	50.0 (42)	84.2 (19)	200.0 (27)	4.7	1.7
18	C73	14.7 (34)	66.1 (56)	90.4 (52)	77.3 (22)	2.1	1.9
	C74	17.8 (45)	47.6 (63)	86.7 (60)	93.3 (30)	2.8	2.0
	C75	42.0 (100)	78.3 (60)	95.2 (21)	96.7 (30)	2.3	1.0
	C76	38.0 (50)	83.3 (30)	94.9 (78)	98.3 (60)	2.9	1.3
	C77	-	-	-	-	-	-
	C78	68.4 (57)	78.3 (60)	81.7 (60)	96.7 (30)	1.4	0.54

* Generation number following mass selection (see Table 2) and 2 generations following establishment of cage Tog X (and see Figure 25)

Appendix 3. Mortalities after 4% DDT testing of female adults of the cage population, Cage A with the slopes of regression lines and the LT50 values (* Generation number following start of mass selection (see Table 2) and 9 generations after the establishment of the Tog X cage (and see Figure 25)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
15*	XA55	70.0 (30)	82.0 (61)	96.6 (58)	100.0 (33)	3.5	0.89
	XA56	-	-	-	-	-	-
	XA57	-	-	-	-	-	-
	XA58	57.8 (45)	76.7 (30)	93.3 (30)	93.3 (30)	1.6	0.66
	XA59	79.8 (99)	96.7 (60)	98.3 (60)	-	3.0	0.62
	XA60	-	-	-	-	-	-
16	XA61	-	-	-	-	-	-
	XA62	66.7 (30)	83.3 (30)	86.7 (45)	95.7 (23)	1.3	0.45
	XA63	56.3 (64)	90.0 (30)	89.5 (38)	100.0 (42)	2.7	0.97
	XA64	29.0 (62)	70.0 (60)	76.5 (17)	96.7 (30)	1.6	0.83
	XA65	-	-	-	-	-	-
	XA66	44.2 (77)	95.5 (66)	98.3 (60)	100.0 (30)	4.0	0.97
17	XA67	87.0 (23)	96.6 (59)	100.0 (30)	100.0 (38)	3.2	0.44
	XA68	67.1 (88)	90.7 (54)	96.4 (55)	100.0 (63)	3.4	0.83
	XA69	73.3 (45)	96.3 (27)	100.0 (60)	-	5.1	0.8
	XA70	77.5 (40)	97.0 (33)	100.0 (30)	100.0 (32)	3.6	0.56
	XA71	62.9 (35)	90.0 (30)	100.0 (18)	96.7 (30)	2.3	0.48
	XA72	56.7 (30)	70.0 (60)	100.0 (38)	100.0 (26)	4.6	1.0
18	XA73	61.2 (49)	88.0 (60)	100.0 (50)	100.0 (23)	5.7	0.99
	XA74	40.0 (65)	65.1 (86)	91.7 (60)	98.3 (60)	2.7	1.3
	XA75	67.7 (99)	81.7 (60)	96.7 (30)	100.0 (31)	3.6	0.92
	XA76	32.1 (56)	88.3 (60)	90.6 (106)	98.4 (61)	2.6	1.1
	XA77	82.6 (23)	87.5 (56)	100.0 (30)	100.0 (30)	3.6	0.62
	XA78	41.7 (36)	76.7 (30)	100.0 (30)	100.0 (60)	4.9	1.1

Appendix 4. Mortalities after 4% DDT testing of female adults of the cage population, Cage B with the slopes of regression lines and the LT50 values

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
15*	XB55	52.9 (34)	83.3 (60)	93.3 (30)	100.0 (35)	3.8	1.1
	XB56	77.1 (218)	-	-	96.3 (27)	4.5	0.68
	XB57	78.3 (60)	84.6 (26)	95.0 (40)	98.3 (60)	1.6	0.36
	XB58	51.1 (88)	62.5 (32)	-	-	-	-
	XB59	59.2 (125)	90.0 (60)	86.7 (60)	-	1.5	0.5
	XB60	41.5 (53)	75.0 (84)	73.3 (30)	96.7 (30)	2.0	1.2
16	XB61	40.0 (50)	-	-	100.0 (28)	4.4	1.1
	XB62	52.1 (121)	84.2 (19)	-	83.3 (30)	0.9	0.41
	XB63	38.2 (89)	80.0 (30)	83.3 (36)	88.9 (36)	1.6	1.0
	XB64	64.4 (132)	75.0 (36)	85.7 (28)	100.0 (15)	3.5	1.1
	XB65	56.3 (32)	62.5 (56)	72.2 (36)	90.0 (60)	1.2	0.93
	XB66	49.5 (107)	94.2 (69)	93.8 (32)	97.8 (45)	2.0	0.05
17	XB67	29.0 (93)	88.3 (60)	96.9 (32)	100.0 (34)	4.5	1.3
	XB68	36.5 (63)	52.8 (36)	87.5 (56)	96.4 (28)	2.5	1.5
	XB69	63.3 (31)	83.3 (30)	100.0 (22)	-	5.6	1.0
	XB70	49.6 (121)	-	90.0 (30)	-	2.2	1.0
	XB71	-	90.3 (31)	81.1 (37)	96.7 (30)	0.9	0.13
	XB72	34.7 (72)	58.7 (46)	94.7 (19)	-	3.3	1.4
18	XB73	-	-	-	-	-	-
	XB74	52.1 (71)	48.3 (60)	84.2 (76)	93.9 (32)	1.4	1.2
	XB75	48.5 (97)	74.0 (73)	96.7 (30)	96.7 (30)	2.3	0.95
	XB76	35.1 (94)	95.7 ([illegible])	100.0 (32)	96.7 (30)	2.9	0.71
	XB77	25.0 (32)	83.3 ([illegible])	100.0 (30)	100.0 (30)	7.3	1.14
	XB78	47.1 ([illegible])	90.0 (60)	91.7 (60)	93.3 (60)	1.6	0.[illegible]

Appendix 4. (continued)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression line	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
19	XB79	46.4 (168)	45.0 (60)	84.8 (46)	90.0 (60)	1.7	1.4
	XB80	14.1 (135)	28.3 (120)	77.9 (86)	100.0 (30)	5.2	2.1
	XB81	33.7 (107)	68.7 (67)	83.8 (65)	95.7 (23)	2.3	1.4
	XB82	44.2 (120)	66.2 (74)	83.3 (60)	96.7 (60)	2.2	1.3
	XB83	21.8 (87)	68.3 (60)	85.6 (90)	-	3.1	1.7
	XB84	23.1 (39)	70.0 (120)	-	96.7 (30)	2.8	1.6
20	XB85	48.3 (29)	73.3 (30)	88.9 (54)	92.3 (26)	1.7	0.95
	XB86	40.6 (106)	65.9 (41)	84.5 (58)	-	1.3	0.75
	XB87	38.6 (57)	67.2 (64)	89.2 (28)	-	2.6	1.3
	XB88	27.1 (133)	74.2 (93)	83.8 (24)	-	2.6	1.5
	XB89	-	-	-	-	-	-
	XB90	-	-	-	-	-	-
21	XB91	-	-	-	-	-	-
	XB92	41.6 (113)	-	-	-	-	-
	XB93	31.5 (73)	48.9 (45)	-	90.0 (30)	1.9	1.9
	XB94	34.8 (89)	42.9 (42)	75.5 (53)	93.3 (30)	2.2	1.8
	XB95	23.2 (48)	54.0 (50)	78.0 (59)	90.0 (24)	2.1	1.8
	XB96	28.8 (73)	64.3 (42)	-	-	3.1	1.5
22	XB97	39.1 (42)	63.3 (90)	86.6 (119)	95.8 (24)	2.3	1.4
	XB98	34.8 (86)	-	-	-	-	-
	XB99	33.3 (30)	68.3 (41)	73.9 (23)	91.1 (56)	1.8	1.5
	XB100	12.5 (56)	76.2 (63)	80.7 (31)	-	3.4	1.8
	XB101	-	-	-	-	-	-
	XB102	31.2 (77)	47.4 (38)	90.9 (33)	-	3.0	1.6
23	XB103	28.9 (104)	37.7 (60)	82.1 (56)	86.7 (30)	2.1	2.1
	XB104	-	-	-	-	-	-
	XB105	-	-	-	-	-	-

Appendix 4. (continued)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 value (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	XB106	28.3 (60)	56.7 (90)	75.0 (32)	-	2.1	1.8
	XB107	-	-	-	-	-	-
	XB108	-	-	-	-	-	-

* Generation number following start of mass selection (see Table 2) and 9 generations after the establishment of the Tog X cage (and see Figure 25)

Appendix 5. Mortalities after 4% DDT testing of female adults of the cage population, Cage C with the slopes of the regression lines and the LT50 values

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs)
		1 hrs.	2 hrs.	4 hrs.	8 hrs.		
15*	XC55	43.3 (30)	76.7 (60)	93.3 (30)	97.5 (40)	2.4	1.1
	XC56	74.2 (209)	93.3 (33)	-	98.5 (66)	1.6	0.32
	XC57	87.7 (81)	88.2 (17)	88.9 (27)	100.0 (30)	2.6	0.55
	XC58	45.7 (35)	-	-	-	-	-
	XC59	81.3 (80)	-	-	-	-	-
	XC60	64.1 (89)	91.7 (35)	-	-	3.4	0.78
16	XC61	71.8 (39)	100.0 (19)	-	97.1 (34)	1.5	0.4
	XC62	71.8 (78)	-	-	100.0 (30)	3.5	0.68
	XC63	56.0 (75)	96.7 (30)	92.3 (26)	100.0 (30)	3.4	0.85
	XC64	75.7 (148)	86.0 (50)	92.3 (13)	100.0 (30)	3.1	0.79
	XC65	66.7 (66)	75.8 (62)	93.0 (57)	98.3 (60)	2.0	0.7
	XC66	69.8 (129)	90.2 (51)	100.0 (34)	100.0 (30)	5.3	0.9
17	XC67	59.3 (54)	81.5 (54)	93.3 (30)	100.0 (20)	3.7	1.1
	XC68	59.5 (111)	83.3 (30)	83.3 (30)	100.0 (56)	3.5	1.1
	XC69	56.3 (32)	89.7 (39)	100.0 (49)	-	5.9	1.0
	XC70	64.0 (136)	-	-	-	-	-
	XC71	78.0 (59)	85.2 (27)	94.6 (74)	96.7 (30)	1.3	0.25
	XC72	59.2 (130)	80.0 (30)	83.3 (36)	100.0 (19)	3.5	1.1
18	XC73	-	-	-	-	-	-
	XC74	-	-	-	-	-	-
	XC75	-	-	-	-	-	-
	XC76	80.6 (102)	69.2 (26)	100.0 (28)	96.7 (30)	3.7	1.3
	XC77	-	-	-	-	-	-
	XC78	66.7 (81)	80.0 (60)	96.7 (60)	98.3 (60)	2.0	0.64

Appendix 5. (continued)

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression line	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
19	XC79	47.9 (71)	83.3 (30)	87.0 (77)	96.7 (60)	1.9	0.89
	XC80	47.8 (92)	75.0 (60)	88.2 (17)	-	2.1	1.0
	XC81	45.0 (60)	71.4 (77)	78.2 (78)	90.2 (51)	1.5	1.1
	XC82	66.7 (150)	83.2 (125)	92.2 (90)	98.3 (60)	2.8	1.3
	XC83	-	-	78.8 (60)	-	-	-
	XC84	60.7 (56)	73.5 (98)	100.0 (46)	-	5.7	1.1
20	XC85	74.1 (54)	83.3 (54)	95.4 (21)	96.7 (30)	1.4	0.4
	XC86	72.5 (69)	82.2 (73)	100.0 (18)	96.6 (29)	2.2	0.43
	XC87	62.2 (135)	86.4 (81)	94.4 (36)	-	2.1	0.68
	XC88	56.3 (119)	80.0 (50)	100.0 (33)	-	5.9	1.1
	XC89	-	-	-	-	-	-
	XC90	-	-	-	-	-	-
21	XC91	-	-	-	-	-	-
	XC92	55.2 (58)	78.7 (47)	-	96.7 (30)	1.9	0.81
	XC93	66.7 (27)	73.7 (38)	93.3 (30)	-	1.8	0.66
	XC94	70.6 (51)	-	-	-	-	-
	XC95	-	-	-	-	-	-
	XC96	57.5 (40)	-	-	-	-	-
22	XC97	67.4 (86)	75.6 (41)	-	100.0 (30)	3.8	0.95
	XC98	64.2 (134)	95.5 (22)	-	-	4.4	0.83
	XC99	56.8 (37)	78.0 (50)	100.0 (24)	-	5.9	1.1
	XC100	74.3 (101)	80.0 (30	91.4 (35)	100.0 (21)	3.2	0.88
	XC101	74.5 (47)	-	-	-	-	-
	XC102	67.2 (64)	77.8 (54)	86.7 (30)	96.7 (30)	1.5	0.58
23	XC103	55.6 (36)	-	-	-	-	-
	XC104	-	-	-	-	-	-
	XC105	52.9 (87)	74.0 (43)	92.5 (40)	-	2.3	0.73

Appendix 5. (continued)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	XC106	52.0 (98)	74.4 (39)	87.1 (27)	97.7 (43)	2.1	0.98
	XC107	-	-	-	-	-	-
	XC108	-	-	-	-	-	-

* Generation number following start of mass selection (see Table 2) and 9 generations after the establishment of the Tog X cage (and see Figure 25)

Appendix 6. Mortalities after 4% DDT testing of male adults of the cage population, Cage Tog X with the slopes of regression lines and the LT50 values

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
6*	X1	10.0 (40)	12.0 (50)	33.3 (30)	45.8 (48)	1.5	8.6
	X2	5.6 (36)	3.3 (30)	33.3 (27)	62.0 (50)	2.4	6.7
	X3	4.0 (101)	6.0 (50)	33.3 (75)	66.0 (50)	2.5	6.0
	X4	3.3 (30)	8.0 (25)	35.4 (49)	55.9 (34)	2.4	6.6
	X5	6.5 (77)	10.0 (50)	20.6 (34)	44.0 (50)	1.5	11.8
	X6	5.4 (37)	10.0 (50)	18.1 (83)	57.1 (35)	1.9	8.5
7	X7	6.4 (78)	10.5 (86)	26.1 (92)	60.8 (75)	2.0	7.0
	X8	4.6 (65)	2.4 (85)	23.6 (55)	52.4 (63)	2.2	9.0
	X9	2.5 (122)	10.0 (110)	24.1 (79)	45.1 (90)	2.0	9.0
	X10	5.2 (58)	7.0 (71)	30.6 (111)	52.8 (125)	2.0	7.8
	X11	4.3 (47)	9.9 (71)	18.0 (50)	62.0 (50)	2.1	7.5
	X12	8.0 (25)	-	28.6 (28)	60.0 (30)	1.8	6.7
8	X13	0 (36)	12.0 (50)	-	48.8 (43)	5.0	6.6
	X14	2.6 (38)	5.0 (40)	22.0 (50)	52.0 (75)	2.3	8.4
	X15	3.6 (55)	10.0 (11)	-	42.0 (50)	1.8	10.4
	X16	2.9 (103)	3.3 (60)	41.7 (48)	58.7 (75)	2.7	6.4
	X17	3.6 (28)	3.3 (90)	21.7 (60)	58.3 (60)	2.1	8.5
	X18	6.7 (30)	8.9 (45)	23.3 (30)	60.0 (30)	2.0	7.5
9	X19	0 (50)	4 (25)	32.3 (31)	33.3 (42)	5.0	6.8
	X20	3.2 (31)	9.4 (64)	40.9 (66)	50.0 (60)	2.2	6.9
	X21	7.2 (125)	11.7 (60)	30.0 (20)	56.4 (55)	1.8	7.3
	X22	0 (60)	11.1 (36)	37.5 (24)	54.3 (35)	5.4	5.6
	X23	3.7 (27)	16.7 (54)	33.3 (33)	60.0 (85)	2.2	6.1
	X24	-	-	-	-	-	-

Appendix 6. (continued)

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression line	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
10	X25	4.2 (95)	12.6 (135)	43.3 (30)	83.3 (60)	3.2	4.3
	X26	4.4 (45)	12.8 (29)	49.2 (124)	86.4 (88)	3.2	3.9
	X27	4.4 (46)	16.7 (18)	61.7 (60)	84.5 (71)	3.1	3.6
	X28	16.1 (31)	26.7 (30)	57.6 (33)	73.7 (38)	1.9	3.6
	X29	8.0 (25)	40.0 (30)	61.5 (78)	85.0 (40)	2.6	3.1
	X30	20.8 (53)	46.7 (30)	63.2 (38)	86.7 (30)	1.9	2.3
11	X31	10.4 (29)	-	74.0 (119)	85.0 (60)	2.3	2.2
	X32	21.7 (60)	50.0 (30)	59.2 (49)	79.6 (44)	1.7	2.6
	X33	52.6 (38)	48.4 (31)	80.0 (25)	90.0 (30)	1.5	1.2
	X34	46.7 (30)	76.0 (25)	75.5 (49)	-	1.3	0.91
	X35	36.8 (38)	41.7 (24)	-	92.9 (28)	2.1	1.8
	X36	65.0 (60)	53.3 (30)	85.0 (40)	86.7 (30)	1.0	0.67
12	X37	-	-	-	-	-	-
	X38	47.4 (78)	58.3 (60)	80.0 (60)	90.0 (60)	1.6	1.2
	X39	53.3 (60)	73.3 (60)	86.1 (115)	98.3 (60)	2.2	1.0
	X40	33.3 (78)	50.0 (40)	80.0 (30)	100.0 (60)	4.4	1.7
	X41	-	-	-	-	-	-
	X42	-	-	-	-	-	-
13	X43	41.5 (53)	54.6 (97)	80.0 (60)	95.0 (60)	2.1	1.5
	X44	20.3 (64)	54.6 (22)	86.4 (22)	88.3 (60)	2.4	1.9
	X45	40.8 (49)	81.7 (60)	86.7 (30)	96.3 (27)	2.1	1.1
	X46	-	-	-	-	-	-
	X47	44.8 (67)	85.0 (60)	84.4 (32)	97.1 (35)	2.0	1.0
	X48	-	-	-	-	-	-
14	X49	26.1 (46)	65.6 (32)	87.1 (62)	98.5 (68)	3.1	1.6
	X50	18.1 (72)	32.8 (58)	83.1 (83)	96.7 (30)	3.2	2.2
	X51	33.3 (60)	60.0 (150)	95.1 (41)	97.7 (44)	2.9	1.4

Appendix 6. (continued)

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	X52	33.1 (84)	79.5 (117)	91.7 (60)	98.7 (72)	2.8	1.3
	X53	63.2 (76)	86.2 (58)	96.7 (67)	96.4 (28)	1.7	0.5
	X54	58.0 (69)	82.8 (93)	90.0 (30)	100.0 (60)	3.6	1.1

* Generation number following mass selection from Table 2 (and see Figure 26)

Appendix 7. Mortalities after 4% DDT testing of male adults of the control cage population, Cage Pop C with the slopes of regression lines and the LT50 values

Generation	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
8*	C15	5.2 (77)	6.5 (62)	8.9 (45)	54.3 (70)	1.8	11.6
	C16	4.3 (70)	5.9 (51)	-	54.0 (50)	2.1	8.0
	C17	2.5 (80)	5.5 (109)	18.3 (60)	51.7 (60)	2.2	8.9
	C18	3.7 (54)	13.3 (30)	20.0 (60)	53.3 (60)	2.0	8.3
9	C19	6.7 (30)	6.0 (50)	36.7 (30)	61.0 (18)	2.2	6.5
	C20	5.2 (155)	6.7 (60)	28.3 (60)	66.0 (50)	2.3	6.4
	C21	6.7 (60)	15.0 (60)	-	66.2 (65)	2.2	5.3
	C22	8.6 (117)	12.4 (89)	21.4 (42)	57.1 (42)	1.7	8.3
	C23	11.1 (18)	14.7 (75)	36.7 (30)	70.0 (30)	2.0	5.2
	C24	6.7 (30)	16.7 (24)	-	-	-	-
10	C25	2.5 (80)	3.6 (139)	16.7 (60)	46.7 (30)	2.2	10.3
	C26	8.1 (62)	15.0 (60)	35.1 (37)	57.1 (55)	1.8	6.6
	C27	2.5 (40)	8.3 (60)	39.5 (38)	53.9 (52)	2.4	6.5
	C28	-	18.8 (37)	25.0 (40)	58.1 (31)	1.8	7.1
	C29	5.5 (109)	15.0 (120)	41.3 (63)	63.3 (60)	2.2	5.5
	C30	4.6 (65)	15.0 (80)	40.0 (60)	65.0 (60)	2.3	5.4
11	C31	8.6 (35)	16.2 (37)	46.7 (30)	71.7 (60)	2.2	4.6
	C32	6.0 (100)	26.7 (60)	47.2 (36)	58.5 (53)	2.0	5.2
	C33	21.2 (33)	28.3 (30)	60.0 (30)	86.7 (30)	2.2	3.0
	C34	3.3 (30)	31.3 (32)	-	-	4.5	2.6
	C35	-	-	-	-	-	-
	C36	10.5 (76)	25.0 (60)	58.3 (24)	81.8 (55)	2.5	3.4
	C37	-	-	-	-	-	-
	C38	9.3 (75)	25.4 (67)	55.0 (60)	70.0 (60)	2.1	4.1

Appendix 7. (continued)

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression line	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	C39	0 (21)	7.4 (27)	47.8 (90)	66.7 (60)	5.9	5.1
	C40	4.9 (123)	11.4 (88)	61.5 (89)	75.0 (120)	2.8	4.2
	C41	16.0 (75)	23.5 (51)	50.0 (60)	74.5 (51)	1.9	3.9
	C42	0 (26)	19.7 (61)	60.0 (30)	-	8.7	3.3
13	C43	2.9 (33)	15.8 (34)	51.7 (60)	75.0 (84)	2.9	4.4
	C44	7.2 (111)	11.9 (59)	49.2 (124)	83.4 (60)	2.8	4.0
	C45	18.9 (111)	38.3 (60)	70.0 (60)	76.9 (39)	1.9	2.8
	C46	3.7 (54)	30.8 (13)	-	70.0 (60)	2.4	4.4
	C47	21.2 (85)	41.7 (72)	71.1 (90)	77.4 (58)	1.8	2.6
	C48	21.5 (65)	45.8 (24)	59.2 (71)	80.0 (30)	1.7	2.7
14	C49	16.7 (42)	43.3 (60)	71.7 (60)	81.6 (49)	2.1	2.6
	C50	-	-	52.2 (23)	79.2 (24)	2.5	3.8
	C51	16.7 (60)	39.6 (101)	48.1 (52)	86.6 (97)	2.1	3.0
	C52	10.3 (58)	25.0 (60)	74.1 (112)	95.2 (124)	3.4	2.7
	C53	18.3 (60)	41.6 (77)	73.3 (60)	74.6 (55)	1.8	2.7
	C54	9.8 (82)	30.0 (30)	53.3 (30)	90.0 (30)	2.8	3.1
15	C55	16.3 (80)	47.4 (116)	58.0 (50)	83.3 (90)	2.0	2.7
	C56	10.0 (40)	30.0 (30)	65.4 (52)	90.0 (30)	2.9	2.9
	C57	26.2 (42)	40.0 (40)	56.7 (30)	93.3 (60)	2.3	2.3
	C58	15.4 (13)	-	72.4 (29)	80.0 (30)	2.2	2.7
	C59	26.0 (50)	60.0 (60)	76.2 (42)	-	2.3	1.8
	C60	34.6 (26)	33.3 (27)	53.3 (30)	92.7 (55)	2.0	2.3
16	C61	27.8 (36)	44.2 (52)	56.7 (30)	86.7 (30)	1.8	2.4
	C62	35.7 (14)	50.0 (24)	83.8 (37)	93.3 (30)	2.2	1.6
	C63	38.6 (57)	36.7 (30)	87.5 (40)	92.3 (26)	2.2	1.7
	C64	46.2 (39)	-	-	100.0 (24)	4.2	1.1
	C65	26.6 (49)	44.8 (30)	89.7 (48)	90.0 (30)	2.3	1.9

Appendix 7. (continued)

Gener-	Egg batch No.	% Mortality (No. tested)				Slopes of regression line	LT50 values
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	C66	18.4 (49)	59.7 (72)	66.2 (68)	96.7 (30)	2.8	2.0
17	C67	22.7 (22)	66.7 (30)	80.0 (30)	95.1 (61)	2.5	1.7
	C68	-	-	-	-	-	-
	C69	36.0 (25)	62.5 (56)	100.0 (20)	-	4.7	1.4
	C70	60.7 (61)	68.8 (16)	77.3 (22)	90.0 (30)	1.1	0.7
	C71	55.6 (18)	60.0 (30)	76.9 (26)	96.7 (30)	1.9	1.1
	C72	23.3 (43)	46.1 (76)	66.7 (60)	97.1 (34)	2.8	2.1
18	C73	37.3 (51)	30.2 (63)	79.2 (24)	95.0 (40)	2.4	1.9
	C74	14.8 (81)	43.8 (73)	73.3 (60)	92.9 (28)	2.8	2.4
	C75	35.8 (120)	50.0 (60)	85.0 (20)	100.0 (30)	4.4	1.6
	C76	28.6 (56)	83.3 (30)	91.3 (46)	95.0 (60)	2.3	1.2
	C77	-	-	-	-	-	-
	C78	47.4 (57)	68.3 (60)	85.0 (60)	100.0 (30)	4.0	1.3

* Generation number following mass selection (see Table 2) and 2 generations after the establishment of the Tog X cage (and see Figure 26)

Appendix 8. Mortalities after 4% DDT testing of male adults of the cage population, Cage A with the slopes of regression lines and the LT50 values (* Generation number following start of mass selection (see Table 2) and 9 generations after the establishment of the Tog X cage (and see Figure 26)

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
15*	XA55	23.9 (46)	76.7 (60)	100.0 (72)	100.0 (40)	5.4	1.3
	XA56	38.8 (67)	87.5 (32)	86.0 (57)	99.0 (95)	2.6	1.1
	XA57	-	-	-	-	-	-
	XA58	45.2 (31)	66.7 (30)	86.7 (30)	96.7 (30)	2.2	1.2
	XA59	67.8 (118)	88.3 (60)	95.0 (60)	-	2.3	0.69
	XA60	-	-	-	-	-	-
16	XA61	85.2 (27)	82.0 (61)	98.2 (54)	100.0 (36)	3.1	0.65
	XA62	49.4 (77)	73.5 (34)	90.3 (31)	96.7 (30)	2.1	1.0
	XA63	36.2 (47)	73.3 (30)	89.5 (38)	100.0 (50)	4.3	1.4
	XA64	29.0 (62)	71.0 (62)	91.3 (23)	96.7 (30)	2.7	1.4
	XA65	84.2 (38)	80.7 (62)	87.0 (23)	100.0 (60)	2.8	0.71
	XA66	40.2 (82)	92.3 (52)	95.2 (62)	100.0 (30)	4.0	1.1
17	XA67	72.0 (25)	82.0 (50)	100.0 (30)	100.0 (40)	4.1	0.8
	XA68	72.1 (61)	83.3 (48)	96.3 (54)	98.3 (60)	1.8	0.5
	XA69	78.8 (33)	94.3 (35)	100.0 (60)	-	4.9	0.76
	XA70	89.0 (42)	92.3 (26)	95.2 (62)	100.0 (38)	4.0	1.1
	XA71	68.8 (32)	93.3 (30)	100.0 (22)	96.7 (30)	2.1	0.35
	XA72	46.4 (56)	70.0 (30)	100.0 (36)	100.0 (17)	4.9	1.1
18	XA73	59.1 (44)	85.7 (63)	95.5 (44)	100.0 (32)	3.7	0.99
	XA74	29.5 (61)	41.6 (101)	85.0 (60)	98.3 (60)	3.1	1.8
	XA75	49.7 (141)	83.3 (30)	94.3 (53)	100.0 (23)	3.9	1.2
	XA76	18.6 (59)	56.7 (60)	91.7 (36)	96.7 (61)	3.1	1.8
	XA77	42.9 (28)	76.2 (21)	90.0 (30)	100.0 (26)	4.1	1.3
	XA78	25.8 (31)	71.7 (60)	96.7 (30)	95.0 (60)	2.4	1.2

Appendix 9. Mortalities after 4% DDT testing of male adults of the cage population, Cage B with the slopes of regression lines and the LT50 values

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
15*	XB55	41.7 (24)	53.3 (60)	90.0 (30)	100.0 (27)	4.3	1.5
	XB56	53.7 (67)	90.2 (41)	95.0 (60)	100.0 (89)	3.7	1.0
	XB57	69.1 (90)	82.0 (50)	90.5 (42)	96.7 (60)	1.5	0.47
	XB58	14.7 (34)	80.7 (31)	88.9 (36)	93.3 (30)	2.7	1.6
	XB59	51.7 (89)	83.3 (60)	85.0 (60)	-	2.5	1.2
	XB60	58.9 (90)	72.3 (47)	86.7 (30)	100.0 (30)	3.7	1.2
16	XB61	40.0 (50)	-	-	92.7 (41)	1.9	1.4
	XB62	47.3 (91)	57.7 (26)	77.8 (18)	96.7 (30)	2.1	1.3
	XB63	16.4 (73)	73.3 (30)	90.3 (31)	96.3 (27)	3.0	1.7
	XB64	35.9 (64)	70.0 (50)	95.5 (22)	100.0 (27)	4.5	1.4
	XB65	30.6 (36)	60.7 (56)	85.4 (41)	90.0 (60)	2.0	1.6
	XB66	40.7 (91)	87.9 (58)	85.5 (37)	96.7 (30)	2.2	0.99
17	XB67	19.8 (81)	76.7 (60)	93.2 (59)	100.0 (34)	4.8	1.5
	XB68	23.3 (60)	62.5 (64)	98.3 (60)	96.9 (32)	3.2	1.5
	XB69	44.8 (29)	82.8 (29)	100.0 (12)	-	6.4	1.2
	XB70	50.0 (48)	-	76.7 (30)	-	1.2	1.0
	XB71	-	88.2 (34)	65.2 (23)	100.0 (30)	4.2	1.5
	XB72	56.3 (32)	56.7 (30)	90.0 (30)	-	1.9	1.0
18	XB73	-	-	-	-	-	-
	XB74	21.1 (19)	36.7 (60)	86.0 (57)	90.0 (33)	2.6	2.1
	XB75	37.2 (78)	47.7 (44)	93.3 (60)	96.7 (30)	2.7	1.5
	XB76	10.6 (66)	50.0 (26)	94.3 (35)	100.0 (30)	6.7	1.7
	XB77	19.1 (21)	60.0 (30)	86.7 (30)	96.7 (30)	3.0	1.8
	XB78	32.7 (101)	85.0 (60)	88.3 (60)	88.3 (60)	1.7	1.0

Appendix 9. (continued)

Generations	Egg batch No.	% Mortality (No. Tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
19	XB79	33.7 (104)	66.7 (60)	81.6 (49)	93.3 (60)	2.1	1.5
	XB80	15.5 (129)	27.5 (12)	61.3 (62)	93.3 (30)	2.8	2.7
	XB81	44.8 (87)	59.7 (57)	72.7 (66)	76.7 (43)	1.0	1.2
	XB82	52.9 (121)	66.7 (60)	85.6 (90)	98.4 (62)	2.3	1.1
	XB83	35.2 (91)	65.2 (46)	86.7 (90)	-	2.5	1.4
	XB84	39.7 (58)	58.2 (67)	-	93.3 (30)	2.0	1.4
20	XB85	55.2 (29)	88.5 (61)	91.9 (37)	100.0 (20)	3.6	1.0
	XB86	16.4 (146)	73.3 (66)	90.3 (31)	96.3 (27)	3.0	1.7
	XB87	44.4 (36)	71.9 (57)	86.4 (22)	-	2.1	1.1
	XB88	32.6 (181)	46.6 (58)	83.3 (36)	-	2.4	1.7
	XB89	-	-	-	-	-	-
	XB90	-	-	-	-	-	-
21	XB91	-	-	-	-	-	-
	XB92	51.7 (60)	-	-	-	-	-
	XB93	44.4 (36)	38.9 (36)	-	96.7 (30)	2.4	1.6
	XB94	35.7 (70)	78.1 (32)	78.3 (46)	96.7 (30)	2.2	1.3
	XB95	29.9 (97)	67.9 (56)	73.9 (88)	87.5 (24)	1.7	1.6
	XB96	34.2 (38)	66.7 (60)	-	-	2.8	1.4
22	XB97	35.7 (56)	66.7 (90)	84.8 (79)	100.0 (22)	4.3	1.5
	XB98	36.6 (41)	-	-	-	-	-
	XB99	57.1 (21)	75.5 (53)	79.2 (24)	96.7 (30)	1.7	0.86
	XB100	21.9 (64)	60.6 (33)	89.7 (66)	-	3.4	1.7
	XB101	-	-	-	-	-	-
	XB102	49.1 (53)	48.0 (50)	93.3 (30)	-	2.53	1.2
23	XB103	32.4 (71)	68.3 (60)	89.1 (46)	93.3 (30)	2.2	1.1
	XB104	-	-	-	-	-	-
	XB105	-	-	-	-	-	-

Appendix 9. (continued)

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	XB106	38.3 (60)	64.4 (90)	64.7 (34)	-	1.12	1.5
	XB107	-	-	-	-	-	-
	XB108	-	-	-	-	-	-

* Generation number following start of mass selection (see Table 2) and 9 generations after the establishment of the Tog X cage (and see Figure 26)

Appendix 10. Mortalities after 4% DDT testing of male adults of the cage population, Cage C with the slopes of the regression lines and the LT50 values

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
15*	XC55	90.0 (30)	60.0 (60)	87.5 (40)	96.6 (29)	0.8	0.13
	XC56	53.6 (56)	73.1 (41)	87.1 (62)	100.0 (97)	3.8	1.2
	XC57	78.9 (71)	82.6 (23)	89.7 (29)	100.0 (30)	3.0	0.78
	XC58	-	-	-	-	-	-
	XC59	63.0 (46)	86.7 (30)	88.3 (60)	-	1.4	0.46
	XC60	34.2 (41)	85.0 (20)	-	-	4.8	1.2
16	XC61	67.4 (43)	8.8 (22)	-	100.0 (39)	3.8	0.9
	XC62	58.3 (24)	-	-	100.0 (30)	1.8	0.77
	XC63	15.0 (20)	70.0 (30)	90.2 (51)	83.3 (30)	2.3	1.8
	XC64	25.5 (47)	58.3 (60)	95.7 (47)	96.7 (30)	3.0	1.6
	XC65	51.2 (86)	65.1 (63)	82.7 (52)	90.0 (60)	1.4	0.98
	XC66	59.3 (60)	89.7 (107)	100.0 (41)	97.8 (45)	2.6	0.57
17	XC67	45.6 (57)	86.8 (53)	93.3 (30)	100.0 (24)	3.9	1.1
	XC68	68.8 (32)	82.1 (67)	93.4 (61)	100.0 (63)	3.4	0.92
	XC69	29.9 (67)	96.8 (62)	100.0 (41)	-	7.1	1.2
	XC70	46.8 (77)	-	93.3 (38)	-	2.6	1.1
	XC71	40.8 (49)	87.5 (16)	82.1 (78)	100.0 (30)	3.9	1.2
	XC72	54.9 (51)	63.3 (30)	100.0 (40)	100.0 (19)	4.7	1.1
18	XC73	50.0 (36)	50.0 (30)	83.8 (37)	100.0 (30)	4.0	1.5
	XC74	14.3 (42)	63.3 (30)	80.0 (60)	100.0 (30)	4.9	1.8
	XC75	18.4 (49)	28.3 (60)	95.0 (40)	100.0 (30)	5.3	1.9
	XC76	20.0 (65)	50.0 (20)	86.7 (30)	90.0 (30)	2.5	2.0
	XC77	56.7 (60)	70.5 (61)	80.0 (60)	86.7 (30)	1.0	0.65
	XC78	55.6 (81)	66.7 (60)	91.7 (60)	95.0 (60)	1.8	0.88

Appendix 10. (continued)

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LC50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
19	XC79	16.9 (65)	53.3 (60)	89.8 (59)	93.3 (60)	2.8	1.9
	XC80	23.2 (108)	45.0 (60)	100.0 (20)	-	7.4	1.5
	XC81	78.9 (71)	82.6 (46)	89.7 (58)	100.0 (53)	3.0	0.78
	XC82	21.4 (117)	83.3 (96)	81.7 (60)	93.3 (60)	2.3	1.5
	XC83	-	-	87.0 (46)	-	-	-
	XC84	47.6 (42)	88.9 (45)	96.0 (50)	-	3.0	0.95
20	XC85	47.0 (34)	89.3 (56)	96.7 (30)	96.7 (30)	2.1	0.75
	XC86	45.8 (24)	67.9 (84)	87.5 (16)	96.7 (30)	2.2	1.2
	XC87	33.3 (87)	80.0 (35)	96.2 (26)	-	3.7	1.3
	XC88	37.6 (117)	80.0 (30)	81.8 (22)	-	2.0	1.2
	XC89	-	-	-	-	-	-
	XC90	-	-	-	-	-	-
21	XC91	-	-	-	-	-	-
	XC92	46.3 (54)	75.0 (28)	-	100.0 (30)	4.3	1.2
	XC93	55.6 (72)	71.8 (39)	93.3 (60)	-	2.3	0.94
	XC94	35.9 (39)	-	-	-	-	-
	XC95	-	-	-	-	-	-
	XC96	61.5 (26)	-	-	-	-	-
22	XC97	45.8 (59)	78.1 (32)	-	100.0 (30)	4.3	1.2
	XC98	67.5 (117)	76.5 (34)	-	-	0.9	0.81
	XC99	58.8 (34)	82.0 (50)	100.0 (26)	-	5.8	1.1
	XC100	43.3 (60)	76.9 (26)	93.2 (44)	100.0 (27)	4.1	1.3
	XC101	58.2 (79)	-	-	-	-	-
	XC102	44.0 (25)	66.7 (36)	93.3 (60)	96.7 (30)	2.3	1.2
23	XC103	35.1 (37)	-	-	-	-	-
	XC104	-	-	-	-	-	-
	XC105	33.3 (87)	80.0 (70)	96.2 (52)	-	3.7	1.3

Appendix 10. (continued)

Generations	Egg batch No.	% Mortality (No. tested)				Slopes of regression lines	LT50 values (hrs.)
		1 hr.	2 hrs.	4 hrs.	8 hrs.		
	XC106	59.2 (71)	80.9 (47)	96.7 (30)	98.0 (50)	2.1	0.74
	XC107	-	-	-	-	-	-
	XC108	-	-	-	-	-	-

* Generation number following start of mass selection (see Table 2) and 9 generations after the establishment of the Tog X cage (and see Figure 26)