C. HARRISON

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, LONDON

GENETICAL ASPECTS OF DDT-RESISTANCE IN THE HOUSEFLY

Riassunto. — Risulta dai lavori pubblicati su questo problema che le esperienze d'incroci fra insetti resistenti ed insetti non resistenti ad un insetticida hanno apportato dati estremamente confusi: ciò non ha niente di sorprendente poichè quando si effetua una selezione per un particolare carattere avviene spesso che il soggetto scelto presenti nello stesso tempo altri caratteri. Può darsi che la resistenza constatata in un ceppo dato, sia dovuta a diversi fattori.

Gli esperimenti descritti in questo studio sono stati realizzati con un ceppo italiano di mosche comuni che resistono alla distruzione per mezzo del DDT ed anche alla paralisi prodotta da questo insetticida, proprietà che non presentano tutti i ceppi resistenti. L'incrocio di questo ceppo italiano con mosche non resistenti ha dimostrato che i due caratteri di resistenza alla paralisi e di resistenza alla distruzione si trasmettevano separatamente.

Résumé. — Il ressort des travaux publiés sur cette question que les expériences de croisement d'insectes résistant et d'insectes ne résistant pas à l'insecticide ont donné des résultats extrêmement confus. Cela n'a rien de surprenant, car lorsqu'on opère une sélection pour un caractère particulier, il arrive souvent que le sujet choisi présente en même temps d'autres caractères. Il se peut que la résistance constatée dans une souche donnée soit due à plusieurs facteurs.

Les expériences décrites dans cette étude ont été réalisées sur une souche italienne de mouches communes qui résistent à la destruction par le DDT, et aussi à la paralysie produite par cet insecticide, propriété que ne présentent pas toutes les souches résistantes. Le croisement de cette souche italienne avec des mouches non résistantes a prouvé que les deux caractères de résistance à la paralysie et de résistance à la destruction se transmettaient séparément.

Summary. — A review of the literature shows that the results of experiments in which non-resistant and insecticide resistant insects have been crossed are extremely confusing. This is not surprising since selection for one character often involves selection for other characters at the same time. There may be several factors contributing to resistance in one insect strain.

The experiments described in this paper deal with an Italian strain of houseflies resistant to kill by DDT, and also to the paralytic effect of this insecticide, a property which is not present in every resistant strain. When flies of this Italian strain were crossed with non-resistant flies there was evidence that the two properties of resistance to knock-down and resistance to kill were inherited separately.

Zusammenfassung. — Es wird durch die veröffentlichten Arbeiten über diese Frage hervorgehoben, dass die Erfahrungen über die Kreuzung zwischen Insekten, die gegen Insekticiden resistent und nicht-resistent sind, einige sehr unklar Ergebnisse gegeben haben.

Dass sollte aber uns nicht wundern, weil, wenn man eine Auswahl für eine bestimmte Eigenschaft ausführt, geschieht es häufig dass, das ausgewählte Subjekt ganz andere Eigenschaften entwickelt.

Es kann sein dass die festgestellte Resistenz in einer gegebenen Abstammung von verschiedenen Faktoren bedingt ist.

Die in diesem Studium beschriebenen Erfahrungen sind mit einer Abstammung von gewöhnlichen italienischen Fliegen ausgeführt worden; sie widerstehen der Vernichtung durch DDT und auch der Lähmung, die durch diesen Insekticide hervorgeruft wird. Diese Eigenschaften kommen nicht bei allen resistenten Abstammungen vor.

Die Kreuzung dieser italienischen Abstammung mit den nicht-resistenten Fliegen hat bewiesen dass, beide Resistenz-Eigenschaften zur Lähmung und zur Vernichtung sich getrennt übermitteln.

Much of the work on the resistance of insects to insecticides has been concerned solely with the problem of control. This has led to the development of new insecticides, but it has proved a less fertile field of investigation than was originally hoped, if only because insects have become resistant to many of these new insecticides also. Such observations have, however, helped to demonstrate the complexity of the problem, and point to the need for an approach that is concerned with

physiology, biochemistry and genetics and which might offer fundamental knowledge on the origins of resistance.

Research on the genetical aspect of resistance is not necessarily of academic interest alone, but may have immediate practical value in enabling predictions to be made on the emergence of resistant races, and on the maintenance or loss of resistance.

Smith (1941) suggests that populations of some insect species consist of single homozygous race, but that most populations contain concealed « potential » races. These potential races may become evident if the environmental or other conditions are favourable. Such a condition has arisen in many insect species within the last few years, but most notably in the housefly *Musca domestica* L. Strains of houseflies resistant to DDT have been obtained by selection both under field conditions and in the laboratory. When the selective pressure is removed the subsequent generations of flies may retain their resistance or, as has been reported in many instances, the resistance may fall. The rate at which it declines, however, varies considerably from strain to strain. The ability to withstand an abnormally high dose of DDT is clearly inherited in some way, but there is considerable confusion as to the precise mode of inheritance.

The first genetical experiments with insecticide resistant strains were carried out by Hough (1928) on the codling moth *Carpocapsa pomonella* L. He crossed two strains which differed in their ability to enter apples sprayed with lead arsenate and found the progeny to be intermediate in resistance between the two parental strains. Dickson (1941) made mass crosses between non-resistant strains of the red scale insect *Aonidiella aurantii* Mask. and strains resistant to hydrogen cyanide. The results suggest sex-linked inheritance and the presence of major gene on the X chromosome affecting resistance. More recently reciprocal crossing experiments betwen DDT-resistant and non-resistant strains of the German cockroach *Blatella germanica* L. show that the inheritance mechanism for resistance is complex (Cochran et al., 1952). Both chromosomal and extra-chromosomal factors appear to be involved, the former probably including sex-linked and autosomal components. Many more crossing experiments have been carried out on strains of houseflies resistant to DDT.

La Face (1952) states that flies resistant to DDT when crossed with susceptible flies gave susceptible progeny but there was no evidence of simple Mendelian inheritance in successive generations. Supposedly resistance is controlled by many factors which combine and interact in some way, although no specific data are given in support of this

statement. Bruce and Decker (1950) and (1951) found that resistance is carried by both male and female flies. The F1, F2 and F15 generations of crosses between resistant and normal flies had a degree of resistance between that of the parents. The resistance in this case was determined by applying DDT in acetone to the back of the thorax and noting the kill after 24 hours. These results were based on mass crossing experiments. Again March (1952) could find no evidence of simple Mendelian inheritance with his strains of flies.

Keiding (1953) working in Denmark carried out single crosses between DDT resistant and normal flies and tested the resistance of the progeny by exposing the flies continuously to a DDT deposit and noting the time taken for knock-down to occur. The results of preliminary experiments showed a segregation in the F2 generation (3 susceptible + intermediate to 1 resistant) and in the backcross of an F1 fly to a resistant fly (1 susceptible + intermediate to 1 resistant) indicating the action of a single gene determining the major differences in resistance to DDT and with susceptibility prevailing but not fully dominant over resistance.

Apart from the chromosome number, virtually nothing is known of the basic genetics of the housefly. Crow (1952) therefore chose to work with Drosophila. Crow has found that there is at least one gene concerned with resistance on each chromosome and it seems likely that resistance is inherited by multiple factors. By using Drosophila stocks in which each major chromosome was marked with a known dominant mutation he produced flies with various combinations of resistant and susceptible chromosomes. In subsequent tests Crow proved that there was generally some dominance of genes for resistance, but there was a negative interaction; when one resistant chromosome was present a second had less effect than it would have done had the first not been there. Sex-linked and cytoplasmic factors appeared to play no part.

Tsukamoto and Ogaki (1953) working in Japan also used *Drosophila melanogaster* for genetical experiments on resistance. As a measure of resistance they counted the number of flies emerging from DDT treated media. In reciprocal crossing experiments between the Fukuoka DDT resistant strain and the Canton susceptible strains they found that DDT resistance was dominant over non-resistance and there was no linkage with sex chromosomes. Crossing experiments indicated one or a few genes of DDT resistance in the Fukuoka strain linked with the second chromosome, and located near the vestigial gene.

The results of these crossing experiments with Drosophila are of considerable interest, but it is conceivable that the resistance induced in

Drosophila is of a different nature from that occurring in DDT-resistant houseflies. Considerable difficulty has for instance been encountered in the selection of DDT-resistant strains of Drosophila in the laboratory, yet this is not the case with the housefly.

It is evident from the various papers quoted that there is a great diversity of opinion about the inheritance of resistance and yet so far as the housefly is concerned there is a greater agreement than might appear from a cursory glance at the literature. In no case is there any indication that cytoplasmic factors are responsible for resistance. Experiments in which paralysis has been used as the criterion of resistance point to a single factor inheritance. When kill at a particular dosage is used as the criterion of resistance, multiple factors appear to be responsible. This is not necessarily an outcome of the testing technique but most probably an expression of the different types of resistance exhibited by different strains. No all DDT-resistant strains of houseflies have the same properties; for example Busvine (1951) demonstrated that a Sardinian strain was readily affected by DDT, yet a large percentage of those flies affected recovered. Another strain, from Italy, was resistant to the paralytic effect as well as to kill by DDT.

It is well known that selection for one character entails an unavoidable selection for other characters, e.g. Mather (1949) when selecting Drosophila for a higher bristle number found that the selected lines also had a different number of spermathecae in the females, changes in mating behaviour, body pigmentation and eye form. Similarly selection of houseflies with DDT may be responsible for the selection of other characters some of which may themselves contribute towards resistance. Weismann (1949) found that a DDT-resistant strain from Sweden had tarsi with a thicker cuticle and also a greater degree of pigmentation. He believed that these factors were responsible for resistance. Later experiments involving the injection of insecticide into resistant flies proved this hypothesis unlikely, but such morphological differences may have been selected for at the same time as resistance and could contribute to resistance.

The examination of an Italian DDT-resistant strain of flies which possesses both the property of resistance to paralysis and to kill suggests that in this strain there are at least two mechanisms contributing to resistance. Flies of this strain were capable of converting DDT to DDE but the speed at which DDT was degraded was so slow that it could not account for the immediate resistance to paralysis demonstrated (Winteringham et al., 1951). There appeared to be one mechanism

responsible for dehydrochlorination and one mechanism associated with resistance to knock-down. Although dehydrochlorination was clearly an important part of resistance, flies of this strain were also resistant to compounds which could not be detoxified in this way. BUSVINE (1953) found the Italian resistant strain to be resistant to 1, 1-dianisyl-neopentane (a compound with a somewhat similar structure to DDT but which cannot be dehydrochlorinated) while another DDT-resistant strain (Sardinian) was not resistant to this compound. Similarly WINTERINGHAM et al. (1952) found this strain to be resistant to 1 : 1bis (4-chlorophenyl)2-nitropropane, another compound which cannot be dehydrochlorinated. BUSVINE (1951) was also unable to find a correlation between the resistance of flies of the Italian strain to a series of DDT analogues and the ease of dehydrochlorination of these analogues *in vitro*, yet such a correlation did exist in a DDT-resistant strain which did not show resistance to paralysis.

The following genetical experiments again demonstrate that there are at least two mechanisms at work governing resistance to DDT in this Italian strain.

ORIGIN AND PROPERTIES OF FLY STRAINS USED IN CROSSING EXPERIMENTS.

The two strains used for most of these experiments were obtained in February 1948 from Italy, through the kindness of Professor MISSEROLI of the Istituto Superiore di Sanità, Rome.

1) *The Rome strain* was collected from an area where there had been no spraying with DDT. Flies of this strain were non-resistant and were maintained in the laboratory as a mass culture without coming into contact with any insecticide.

Rome flies exposed in glass cylinders treated with DDT in acetone to give a deposit of 10 mg/sq. ft were all knocked down within one hour (see graph 1). The population was homogeneous and the variation was no greater than might be expected in any population due to slight differences in breeding and environment. When DDT in P31 oil at a series of different concentrations was applied to the back of the thorax from a microsyringe and the number of flies dead after 24 hours counted, the population was normal and non-resistant.

A second non-resistant strain of English origin was used for a few experiments. This strain had properties similar to those of the Rome strain.

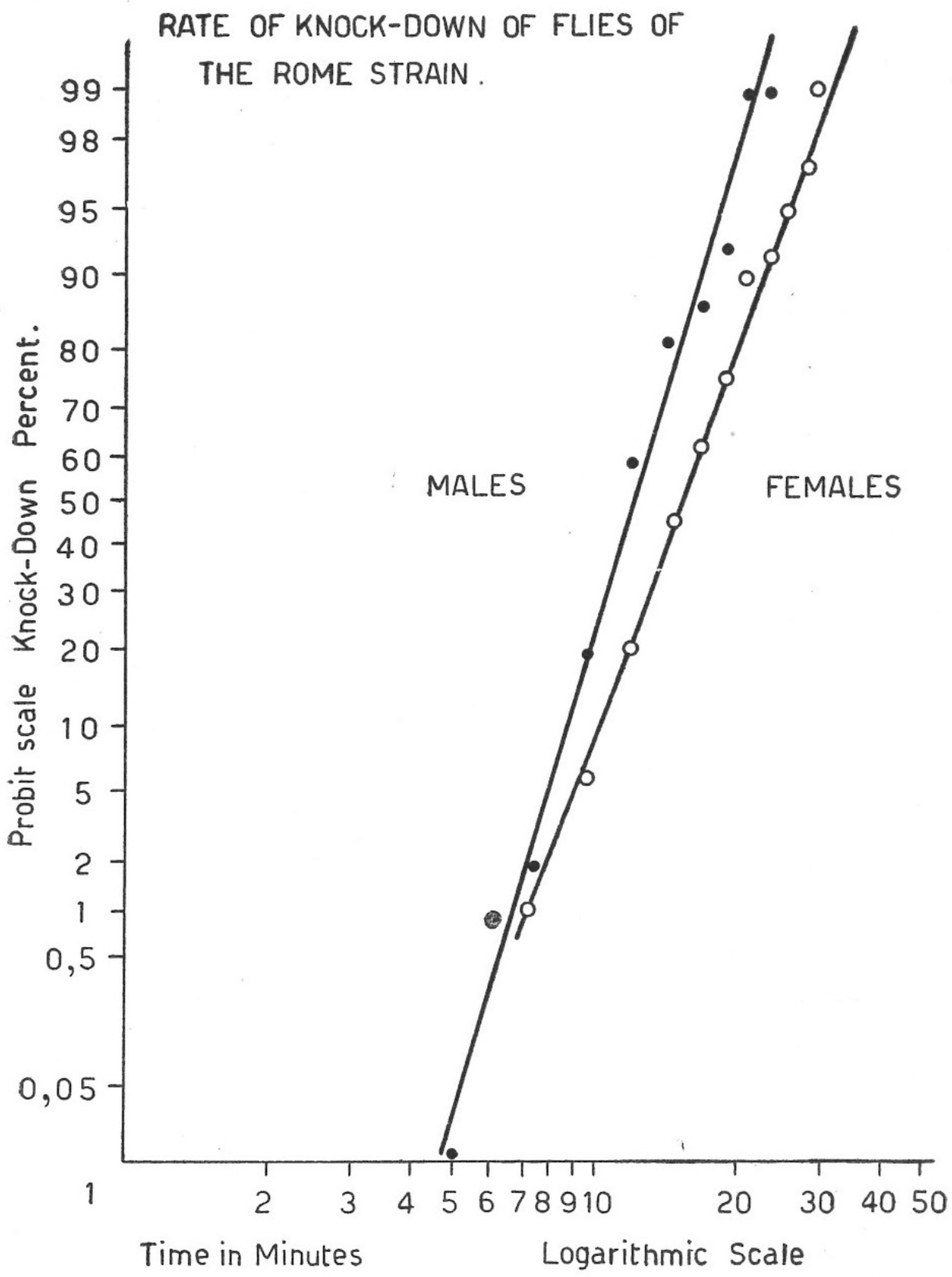

RATE OF KNOCK-DOWN OF FLIES OF THE ROME STRAIN.
MALES
FEMALES
Probit scale Knock-Down Percent.
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0,5
0,05
1
2 3 4 5 6 7 8 9 10 20 30 40 50
Time in Minutes
Logarithmic Scale

Fig. 1.

2) *The Torre in Pietra strain* was taken from a district where DDT spraying had been intensive. When first obtained, it was not highly resistant, the average resistance being only four times greater than that of the normal strain (HARRISON, 1952). After culturing in the laboratory as a mass culture for six months the resistance to DDT had dropped. Selection of flies of this strain by exposure to residual deposits of DDT, however, increased the resistance enormously (HARRISON, 1952). This selected stock was used for experimental purposes and was designated the X strain. The resistance was maintained at a high level by selecting the most resistant individuals in the mass culture at intervals and breeding from them. This strain was resistant to DDT and slightly resistant to pyrethrins, but not to other insecticides. BUSVINE (1951) pointed out that flies of this strain were resistant not only to kill but also to the paralytic effect of the insecticide.

The rate of knockdown is shown in graph 2. The population appears to be heterogeneous, part being knocked down within 100 minutes and the rest of the population being affected at a much slower rate. Tests in which samples of flies were treated with DDT in P31 oil at different concentrations and the percentage kill noted after 24 hours showed that the population was more heterogeneous than the non-resistant strain. It was impossible to obtain 100 per cent. kill with this technique since P31 is saturated at 4 per cent.

STANDARDIZATION OF FLIES FOR CROSSING EXPERIMENTS.

The fly strains were maintained under standard conditions as mass cultures (HARRISON, 1950). Pupae of the two fly-strains were placed in separate tubes, males and females being separated on emergence so that there was no chance of mating having taken place. Pairs for mating were placed in six inch cube cages and the cotton wool pad soaked with milk and water in each cage was examined daily for eggs. Since breeding small numbers of flies is difficult, the eggs were allowed to hatch in the moist pad which was transferred to a small quantity of larval medium after two days. In this way the small amount of medium was prevented from dryng out before the larvae were established. The pupae were separated from the medium by hand.

Flies of the P1 and F1 generations in crossing experiments were allowed to mate (F1 sister - brother matings) and eggs obtained before the adults were tested for resistance.

THE INHERITANCE OF RESISTANCE TO PARALYSIS IN THE X STRAIN OF HOUSEFLIES.

Flies for pairing were chosen from the Rome and X populations at random and cultures from the eggs started. The resistance to paralysis of the parental flies was then tested by noting the time for knock-down when the flies were exposed in DDT treated beakers. It is obviously necessary in genetical experiments to have some criterion for distinguishing susceptible and resistant flies. Since all flies of the non-resistant strain were knocked down well within one hour this time point was used as the criterion of susceptibility.

The following crosses were made between Rome and X flies:

Nos. 1-4 X strain female (resistant) by Rome strain male (non-resistant)

Nos. 5-8 Rome strain female by X strain male.

The knock-down times of the parental flies are given in Table 1.

TABLE 1.

The knock-down times of parental flies used in crossing experiments.

Cross number	*Knock-down time in minutes*			
	Resistant X Susceptible		*Susceptible X Resistant*	
	female	*male*	*female*	*male*
1	between 180-300	9		
2	over 1,260	14		
3	240	16		
4	300	22		
5			15	dead
6			17	150
7			28	dead
8			38	over 360

The first filial generation (F1).

The knock-down time curve for the F1 generation follows that of the non-resistant Rome strain, but is not coincident with it, see graph 3. There is no fly in this generation with a resistance to DDT which resembles that of the resistant parent. The possibility of the deviation from the completely non-resistant strain in the F1 generation being due to hybrid vigour was tested by crossing two non-resistant strains and comparing the susceptibility of the progeny with that of

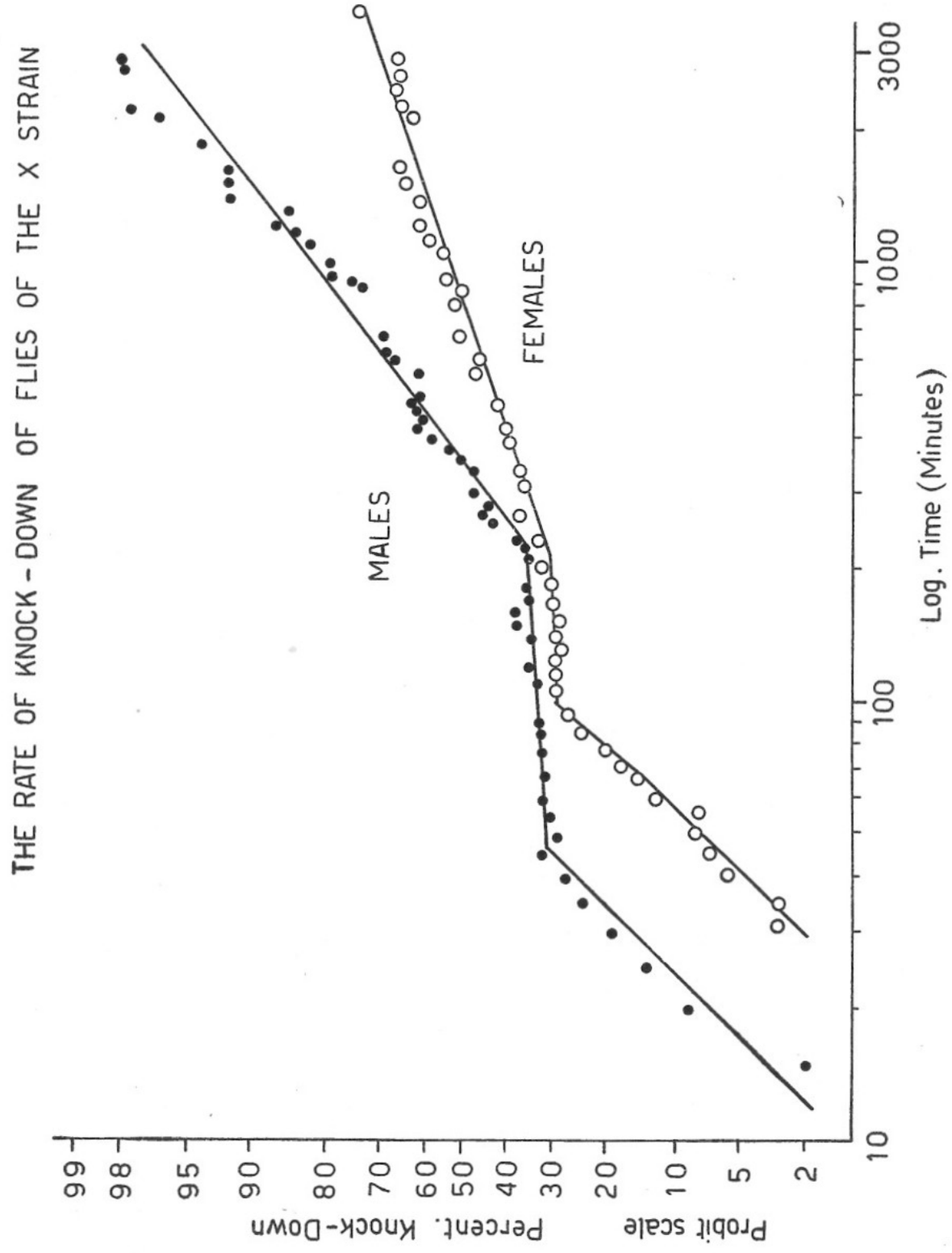

THE RATE OF KNOCK-DOWN OF FLIES OF THE X STRAIN
MALES
FEMALES
Percent. Knock-Down
Probit scale
99
98
95
90
80
70
60
50
40
30
20
10
5
2
10
100
1000
3000
Log. Time (Minutes)

Fig. 2.

the two parent strains. There was, however, no increase in resistance in the progeny of this cross.

The second filial generation (F2).

The knock-down time curve for this generation has the same characteristic shape in each case. There is a rapid knock-down of flies followed by a sudden change in the slope of the curve suggesting that the population is heterogeneous (graph 3). This change in rate is seen clearly in the frequency distribution diagram, graph 4. The proportion of non-resistant to resistant flies in this F2 generation appeared to approach 75 per cent to 25 per cent in many cases. The figures were therefore examined statistically in each cross to see whether the deviation from this expected 3 : 1 ratio was significant. The figures are given in Table 2.

TABLE 2.

Proportion of Non-Resistant to Resistant Flies in the F_2 Generation.

Cross	*No. of non-res. flies*	*No. of res. flies*	*Total No. flies*	*Per cent. non res.*	*Deviation from 3 : 1 ratio S. E. expected ratio*
1	37	10	47	79	0.64
2	46	16	62	74	0.18
3	66	31	97	68	1.61
4	275	93	368	74.7	0.13
5	35	19	54	65	1.70
6	208	85	293	71	1.58
7	205	89	294	70	1.97
8	40	23	63	64	2.04

There is no evidence in these results of resistance ranging between that of the two parents, either in the F1 or F2 generations. Segregation in the F2 generation also appears to be quite clear cut. These facts point to a simple Mendelian inheritance with a single gene governing resistance to knock-down and with non-resistance dominant over resistance although this dominance may not be complete. If this theory of resistance to knock-down is correct back crosses between F1 flies and the resistant parental strain should give progeny 50 per cent of which are susceptible to DDT and 50 per cent DDT-resistant.

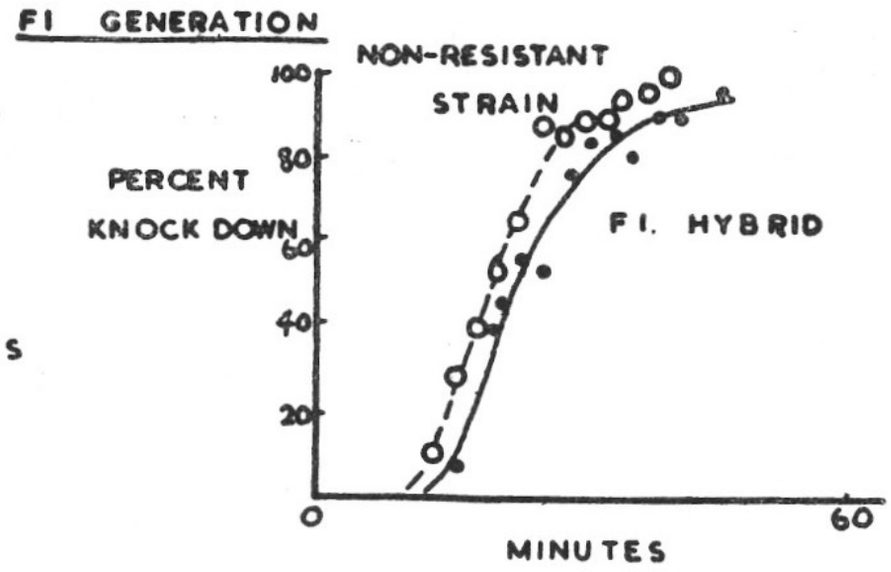
RATE OF KNOCK-DOWN OF FLIES OF CROSS 1. X♀ × R♂

P 1 GENERATION

TIME OF KNOCK DOWN

X ♀ 180 – 300 MINUTES

R ♂ 9 MINUTES



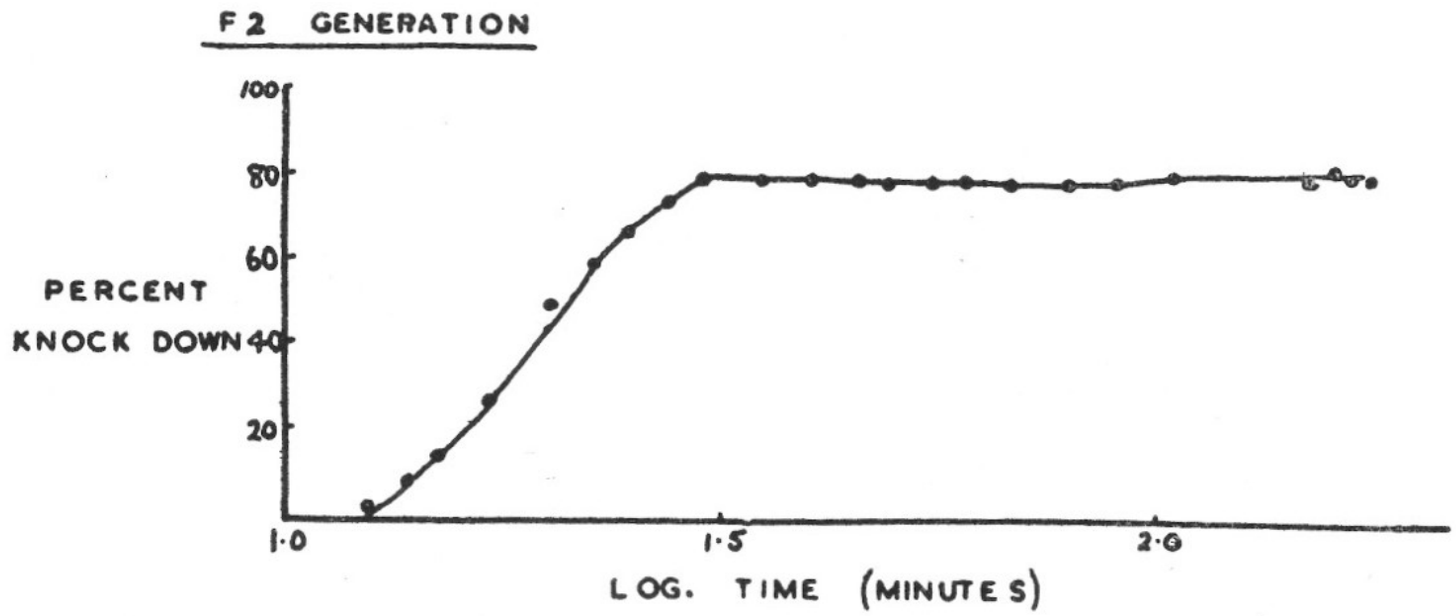


Fig. 3.

Backcrossing experiments.

The following backcrosses were carried out:

1. F1 hybrid female × resistant male (X strain)
   (F1 result of cross between Rome male and X female)
2. F1 hybrid female × resistant male (X strain)
   (F1 result of cross between X male and Rome female)
3. F1 hybrid male × non-resistant female (Rome strain)
   (F1 result of cross between X male and Rome female)

The knock-down times of the parental flies are given in Table 3.

TABLE 3.

Knock-down times of Parental Flies used in Backcrossing Experiments.

Backcross No.	*Knock-down times in minutes of parental flies when exposed in DDT treated beakers (10 mg sq. ft)*	
	Males	*Females*
1.	over 249 (X stock)	42 (F1 hybrid)
2.	over 270 (X stock)	46 (F1 hybrid)
3.	over 17 (Hybrid)	28 (Rome stock)

The progeny of the first two backcrosses showed a segregation into approximately one non-resistant to one resistant fly (see Table 4). The progeny of the third backcross were all non-resistant, a result which would be expected since non-resistance is dominant.

TABLE 4

Segregation of Progeny of Backcrosses.

Backcross	*No. of non-res flies*	*No. res. flies*	*Total no. flies*	*Per cent non-res.*	*Deviation from 1 : 1 ratio S.E. of expected ratio*
1	23	23	46	53	0.43
2	26	27	53	49	0.15

THE FREQUENCY OF KNOCK-DOWN OF F 2 GENERATION FLIES

OF CROSS NUMBER 6.

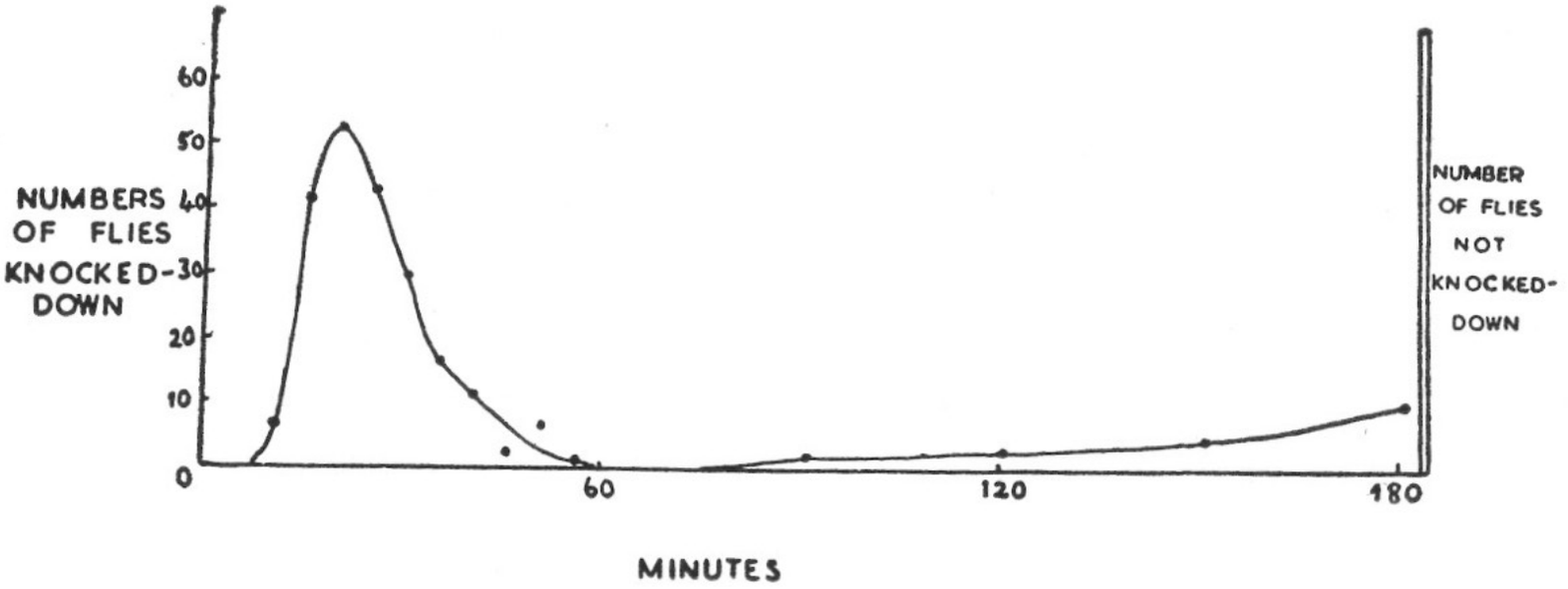


Fig. 4.

THE INHERITANCE OF RESISTANCE TO KILL BY AN APPLIED DOSE OF DDT IN THE X STRAIN OF FLIES.

Individual flies of the non-resistant strain and the X resistant strain were crossed and the progeny tested by applying DDT in the mineral oil P31 from a microsyringe to the back of the thorax at a series of different concentrations. The flies were transferred to clean cages and the number dead after 24 hours was noted. One grave disadvantage in these crossing experiments is that it is impossible to determine the resistance of the individual parental flies used. Moroever the number of flies obtained in the F1 generation is usually insufficient to carry out an adequate test at a large number of different concentrations. For this reason three crosses were made at the same time for each reciprocal pair, and tested the same day under precisely the same conditions. The results of each type of cross were then combined.

F1 generation.

Flies of this generation whether progeny of non-resistant female X resistant males or vice versa were homogeneous, the resistance being considerably higher than in the non-resistant strain and lower than in the resistant strain (see Table 5).

F2 generation.

The F2 generation was very heterogeneous, some flies being as susceptible as those of the non-resistant parental generation, some as resistant as the resistant strain and many having an intermediate resistance (see graph. 5).

In both the F1 and F2 generations the resistance was intermediate between that of the parental strains, but the spread of resistance was greater in the F2 generation than in the F1. This is indicative of multiple factor inheritance and agrees with results obtained by other workers when using this method of determining resistance.

Both single factor and multiple factor inheritance of resistance has been demonstrated for different housefly strains using different testing techniques. The experiments described in this paper show that in the particular Italian DDT-resistant strain of houseflies used there are at least two mechanisms contributing to resistance which are inherited separately. Other evidence gives weight to the suggestion that one mechanism is concerned with the dehydrochlorination of DDT and the other is associated with resistance to paralysis. Resistance to paralysis,

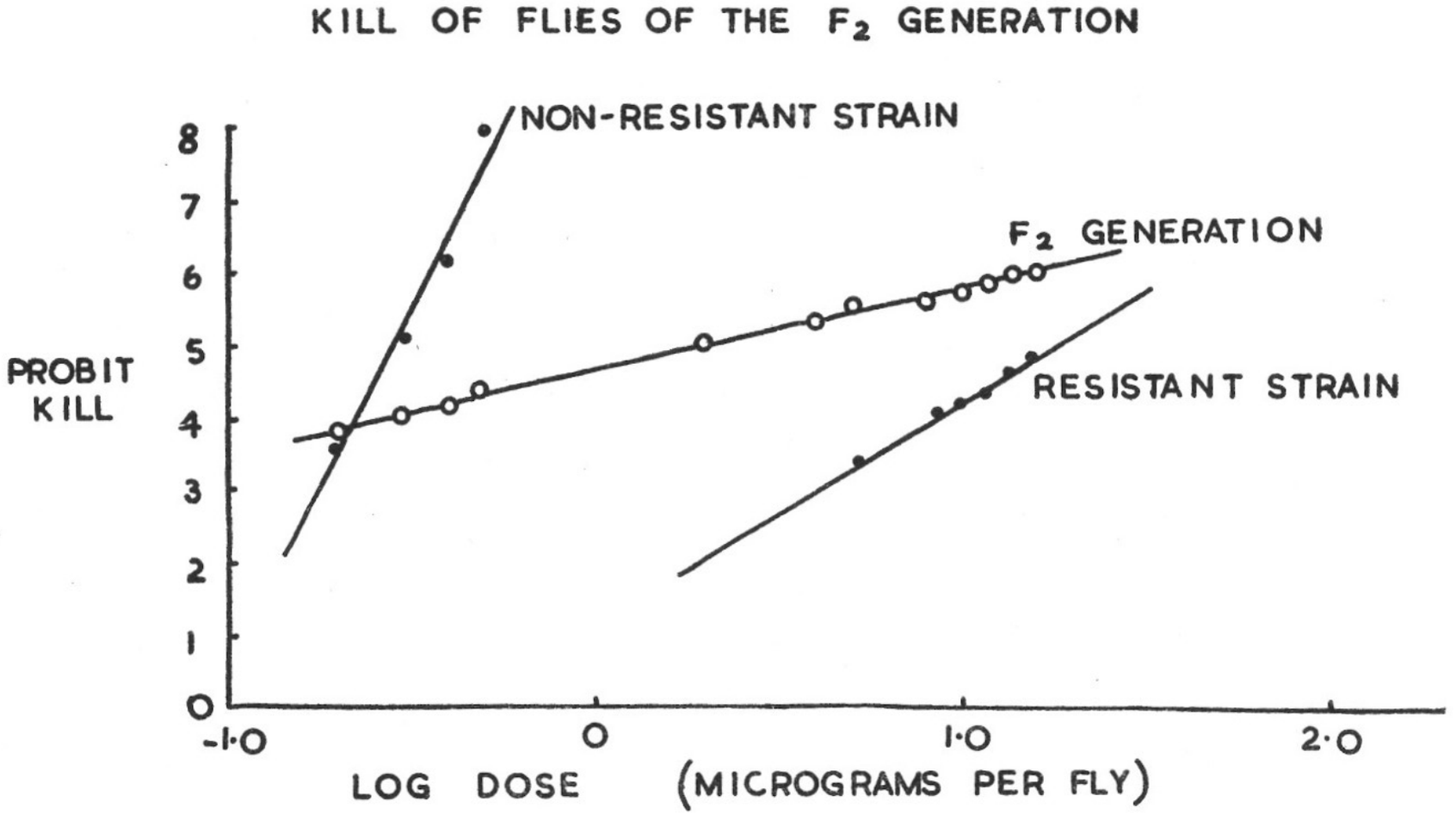

KILL OF FLIES OF THE F_2 GENERATION
NON-RESISTANT STRAIN
F_2 GENERATION
RESISTANT STRAIN
PROBIT KILL
8
7
6
5
4
3
2
1
0
-1·0
0
1·0
2·0
LOG DOSE (MICROGRAMS PER FLY)

Fig. 5.

TABLE 5.

Percentage kill of flies of P1, F1 *and* F2 *Generations of Crosses between Non-Resistant and DDT-resistant strains, when DDT is applied to the back of the Thorax of Individual Flies.*

Dose of DDT in P31 in micrograms fly	0.2	0.3	0.4	0.5	2	4	6	8	10	12	14	16
Generation												
P1	9	58	90	100			13	20	28	36	40	46
F1					0	11	62	93	99			
F2	11	18	21	28	50	64	72	74	78	80	83	84

since it does not occur in every resistant strain of houseflies, may be a chance feature of selection and may be of minor importance in the complete problem of resistance. Similarly other resistant strains may exhibit a number of peculiarities which may have been selected for at the same time as resistance and which may or may not contribute to resistance. There appears to be no evidence in crossing experiments with the housefly that cytoplasmic or sex-linked characters play any part in inheritance of resistance.

It is not known wheher a similar complex situation exists in resistant races of other insect species, but the diverse results obtained by different workers suggest that this may be so.

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