THE INHERITANCE OF DDT RESISTANCE IN HOUSE FLIES

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CHAPTER I

INTRODUCTION AND LITERATURE

For centuries men have known that resistance to the effects of certain poisons could be built up in the human body by the habitual introduction of sub-lethal doses. Soon a dose that would be fatal to a non-resistant individual can be taken by the tolerant person without apparent ill effect. A common example of such a tolerance or resistance is that of smokers to nicotine. If a non-smoker smokes a cigar, the chances are that he will be deathly sick. A man who smokes regularly seems unaffected. Some people also have acquired a tolerance for such poisons as alcohol, opium, arsenic, and others. Such a tolerance in humans is transient, disappearing in a short time after the use of the poison is discontinued. There is no evidence that this type of tolerance lasts beyond a single generation.

Resistance of insects to insecticides may be built up in the same way. However, resistance in insects is known to last for many generations. How this resistance is transmitted, the number of genes involved, if any, and where these genes are located, has not been determined.

The purpose of this experiment is to determine the mode of inheritance of resistance and the factors influencing resistance to toxic materials.

Since house flies are common, and since their resistance to DDT was of comparatively recent origin, these insects were selected for this work. Resistant strains have appeared in widely separated localities. In 1950 resistance was noted in the flies in the dairy district around Corvallis, Oregon. Other strains have appeared in almost every locality in the world where DDT has been used as a means of control. Resistance is not confined to DDT in flies, but appears to be a general resistance to many insecticides. However, this study was confined to their resistance to DDT.

Resistance in insects to the effects of insecticides was noted only a few years after the insecticides were first used. Hough (16, p.256) reported a strain of codling moths that was resistant to arsenic in 1929. An arsenic resistant tick was found by Omer-Cooper (29, p.451) in 1945. Quayle (31, p.497) noted a specific resistance for phenothiazine in the screw worm. McGregor (19, p.62) found that citrus thrips now thrive on tartar emetic, a poison that once controlled them.

Haseman and Leonard (15, p.8) concluded that arsenic resistance in codling moths was not transient as resistance

was in humans, but that it was inherited. Gough (13, p.571) noted that resistance to HCN in the confused flour beetle was inherited.

There has been a great deal of work done on the California red scale. Lindgren (20, p.224) concluded that the resistance of this insect to HCN was brought about by a so-called protective stupefaction. Resistant scales apparently became stupefied sooner than susceptible ones and thus did not absorb as much of the poison. Hardman and Craig (14, p.187) proposed that the closing of the spiracle of resistant red scales kept them from getting a lethal dose. Lindgren and Sinclair (21, p.314) recovered more HCN from the bodies of fumigated non-resistant scales than from those of resistant ones. In 1940, Dickson (9, p.522) determined that the resistance factor in this insect depends on a single gene or group of closely linked genes located on the X chromosome.

Resistance in house flies to DDT was first discovered by Weisman (41, p.504) in Sweden and by Sacca (34, p.128) in Italy in 1947. Both men thought there were enough morphological differences between the resistant and non-resistant strains to warrant the conclusion that they were different races or even different species. Weisman found that the lethal dose for his resistant strain was 2.5 gamma of DDT, whereas for the susceptible strain 0.025

gamma was lethal.

In 1948, Lindquist and Wilson (23, p.276) developed a strain of DDT resistant flies in the laboratory at Orlando, Florida. Common flies were sprayed with DDT in such concentrations that about 90% were killed. This was repeated for 14 generations. Only 29% of the flies in the 14th generation were killed by a dosage that killed 68% of the regular stock. Wilson and Gahan (42, p.277) determined that this strain of resistant flies was also resistant to chlordane, pyrethrins plus piperonyl cyclonene, chlorinated camphene, rotenone, and thanite. By selective breeding, therefore, a race of flies having a general resistance had been developed.

One colony of this resistant strain was bred in the laboratory for 12 generations without exposure to DDT by King (18, p.497). He found that the resistance in the 12th generation was no greater than that of normal flies. This indicates an acquired resistance and not a genetic one. Other workers have had different results from this one as will be indicated later.

King and Gahan (19) collected resistant wild strains of flies from Texas, Georgia, North Carolina, California, and Florida. The resistance of these strains was apparently general, but the differential for other insecticides was not so great as for DDT. Resistance in these strains

was greater than in the susceptible laboratory strain, but was much less than that of the resistant strain of Wilson and Gahan.

Barber, Starnes and Starnes (3, p.143) postulated that small amounts of the active principle of the insecticide might be transmitted from the treated flies through the eggs and larvae to the pupae. This conclusion has not been proved. Barber and Schmitt (4, p.7) concluded that the resistance in their strain of flies was specific for DDT. This conclusion has also been questioned.

Bettini and Barachini (5, p.91) combined Octa-Klor with DDT and controlled flies that were resistant to DDT alone. Blickle, Capelle and Morse (6, p.149) developed a benzene hexachloride resistant strain which was also resistant to DDT, pyrethrins, and Lethane 384 Special, but not as much so as to benzene hexachloride.

In 1949 Ferguson and Kearns (10, p.817) injected 100 micrograms of DDT into large milkweed bugs. They found that within 90 minutes 80 to 100 percent of this DDT had been broken down into non-toxic products. The extracts of these bugs were non-toxic to non-resistant houseflies. Sternberg, Kearns and Bruce in 1950 (40,p. 219) state that DDT is readily absorbed by both susceptible and resistant strains of flies. There is little

difference in the initial rate of absorption. The rate is greatest when the concentration of DDT outside the body is greatest and decreases as the concentration of DDT decreases. Small amounts of DDT are metabolized to form an unknown compound. Flies that are resistant to DDT rapidly metabolize DDT that has entered the body.

Ferguson and Kearns also state that DDT susceptible flies are not able to metabolize DDT to form DDE and DDA. In view of the work of Lindquist, et al. (22) this statement will have to be questioned. These latter workers found with the aid of radioactive DDT that DDT is also broken down by susceptible flies in about the same percentages as by resistant ones. They also found that only 13 to 20 percent of the DDT applied is absorbed in 24 hours. Less DDT was recovered from washes and extracts of flies that died from the treatment than from flies that were still living after treatment. The flies were washed with acetone to remove DDT that was on the exterior. By using other materials to wash the flies it was determined that no DDT was removed from the interior of the flies by the acetone. Thirty-one to 71 percent of the DDT that entered the flies through the cuticle was broken down to non-toxic products in both resistant and susceptible strains.

Perry and Hoskins (30, p.600) state that the addition of Piperonyl cyclonene to DDT inhibits the breaking down of DDT within the flies' bodies, and causes a larger kill.

A strain of resistant flies has developed in the dairy district of Southern California and is known as the Bellflower strain. March and Metcalf (25, p.95-96) state that this strain's resistance to DDT is 333 times that of the susceptible strain. They have concluded that the resistance in this strain is not due to variations in weight and vigor of the flies, nor to the failure of DDT to penetrate the cuticle. This enormous resistance of the Bellflower strain to DDT was maintained for over 15 generations bred in the laboratory, completely free from contact with DDT.

Bruce and Decker (7, p.122), working in Illinois, developed a DDT resistant strain in the laboratory. They were unable to produce resistance by treating adults. They treated both adults and larvae. Resistance increased slowly for about 12 generations of treatment, and then increased rapidly. This was noted in the development of the Orlando strain by Lindquist and Wilson. When the Illinois strain became highly resistant the larvae would tolerate over 200 times as much DDT as would susceptible larvae. Several other strains were developed that were

resistant to DDT and other toxicants in varying degrees. All strains studied retained their respective levels of tolerance to DDT when placed in a DDT free environment. In 30 generations no significant loss of DDT tolerance was seen. These results are in contrast with those of King, working with the Orlando strain. They are similar to observations made on the Bellflower strain.

Bruce and Decker made reciprocal crosses between their resistant flies and susceptible ones. They found that the F_1 generation was intermediate in resistance to DDT. They inbred the F_1 and their offspring for 15 generations and found that the range of resistance was greater in the F_1 and subsequent generations than in either parent strain. The range for the F_2 through F_{15} remained approximately the same. They concluded that the resistance factors are carried by both males and females. Males have been found by them and other workers to be easier to kill than females. This indicates that at least part of the resistance factors may be carried on the X chromosome. Their data also show that the resistance of the descendants of the resistant female X susceptible male is greater than that of the descendants of the reciprocal cross. This indicates that there may be cytoplasmic as well as genetic inheritance of DDT resistance.

The fact that the resistance of the Orlando strain disappeared after 12 generations without contact with DDT indicates that resistance in this strain is different from that in the Bellflower and Illinois strains. It may be that there are two or more kinds of resistance to DDT and that they are inherited in different ways. There may also be two or more kinds of resistance in the same strain.

CHAPTER II

MATERIALS AND METHODS

A colony of each of two resistant strains of flies, the Bellflower and the Orlando, and a colony of susceptible ones, were obtained from the U.S. Bureau of Entomology and Plant Quarantine at Corvallis. One line of Orlando was maintained without exposure to DDT or other insecticide for nine generations. The flies of the ninth generation were tested to see if their initial resistance had been maintained. One colony of Bellflower flies was maintained for six generations in a DDT free environment and the flies of the sixth generation were tested.

Only the Bellflower and the susceptible strains were used in the genetic study. Previous work had indicated that the resistance in the Bellflower strain was genetic. Reciprocal crosses were made between this strain and the susceptible strain. The resistance of the F_1, F_2, and back-crosses was determined.

The flies were allowed to lay their eggs in NAIDM fly media, furnished by the Federal Bureau. The eggs hatched into larvae in a few hours. The larvae fed on the media for several days and then pupated. The pupae were separated from the media by hand and transferred to

holding jars or cages where the flies emerged in three or four days. After emerging, the flies were fed condensed milk and supplied with water before they were treated.

The flies were knocked out with CO_2 by means of a seltzer bottle and Sparklet bombs. They were kept immobile in a petri dish on ice until they could be treated. Care was taken that the flies were not injured by prolonged exposure to cold.

In order to check the resistance of any individual it was necessary to expose it to DDT. There are several methods of doing this. The topical method was used in this work. A measured amount of DDT was applied to the thorax of each fly. The insecticide was measured by means of a micrometer and a ¼cc. tuberculin syringe. The syringe was equipped with a .27 gauge needle bent to 90 degrees and with the point filed off so that the opening came at the end. Five thousandths on the micrometer depressed the plunger on the syringe enough to give a suitable dosage. This dosage was used in all tests. The amount of DDT in the dose was regulated by the percentage solution used. The DDT in all tests was in acetone solution. The acetone evaporated quickly, leaving the DDT on the thorax of the fly.

All tests were made when the flies were not more

than two days old. After that time the females became larger than the males. Wide mouth pint jars with Kerr tops were used to hold the flies after treatment. Twenty-five flies were placed in each jar to facilitate counting. The solid cap was replaced with a circular piece of screen wire.

Twenty four hours after treatment with DDT the flies were checked and the live ones counted. In any procedure such as this it must be taken into consideration that some resistant flies are going to die from environmental causes with or without treatment. Obviously, flies that have the genes for resistance but which are weakened by other conditions are likely to be in the number that die. In testing with a high dosage, therefore, the number of flies that live is too small to be taken as the only ones that are resistant. Conversely, when a low dosage is used, the number of flies that die is too large to be taken as all susceptible. Account has been taken of these facts in interpreting the results of this experiment.

At different times, although the same stock of flies is treated with the same dosage of insecticide and other conditions are kept as near constant as possible, different results are obtained. Taking this fact into consideration, all tests for comparative purposes were conducted on the same day and as near the same time as possible.

Individual and mass matings were made. In the individual matings, P_1 flies were poisoned after the eggs were laid. If a supposedly resistant fly died from the treatment or if a supposedly susceptible one lived after treatment, the eggs from that mating were discarded. Eggs were used only from matings where the resistant parent lived after treatment with a suitable dosage of DDT and the susceptible parent died from a much smaller dose. In the mass mating method several males of one strain were placed with several females of the other. All eggs of these matings were used. Reciprocal crosses were made. Comparisons were made between the resistance exhibited by the F_1 and the F_2 of the reciprocal crosses and between that of the F_1 and the F_2 of the individual and mass matings. The F_2 in all cases came from inbreeding the F_1 by the mass method. All F_2 were counted and treated in order to obtain a ratio as nearly correct as possible.

As soon as it became apparent that the results of the individual matings did not differ materially from those of the mass matings, the individual matings were discontinued. Results of these matings up to that time were combined with the mass mating results.

CHAPTER III

RESULTS

Loss of Resistance and its Reappearance in Orlando Flies

Resistant flies of the Orlando strain were used in the experiment only to determine if their resistance would be maintained when DDT was removed entirely from their environment. A test on flies of this strain at the beginning of the experiment showed that almost half of them were able to tolerate 10 micrograms of DDT (Table 1). Flies of this strain were not tested again until they had been in a DDT free environment for nine generations. The results of the tests on the flies of the ninth generation (Table 1) demonstrate that ninth generation flies were not able to tolerate one-tenth the amount of the insecticide as was tolerated by the original stock.

The survivors of the test on the ninth generation were inbred and their progeny tested (Table 1). There was a rapid resurgence of resistance following the return of DDT to the environment, even though a very small amount of the insecticide was used. Selection, of course, was involved here, but it is unlikely that selection alone could account for such a great increase in resistance in a single generation. No statistical analysis could be made because of the differences in dosages of DDT that were used.

Table 1

The Loss of Resistance in the Orlando Strain of Resistant House Flies from the Beginning of the Experiment to the Ninth Generation in a DDT Free Environment, and its Recovery when DDT was Returned to the Environment

Generation*	Flies tested		Dosage of DDT	Survival rate	
	Male	Female		Male	Female
			Micrograms	Percent	Percent
First	100	100	10	37	48
Ninth	25	25	10	0	0
	25	25	5	0	0
	171	142	1	6	27
F_1	24	29	5	21	48

*First generation flies came from stock that were treated every generation. Ninth generation flies were descendants of first generation flies. No DDT was given in any generation between the first and ninth. The F_1 are the offspring of the 33 flies surviving treatment in the test on flies of the ninth generation.

Loss of Resistance and its Reappearance in Bellflower Flies

More than fifty percent of the flies in the Bellflower strain tolerated ten micrograms of DDT at the beginning of this study. One colony of flies was kept without contact with DDT for six generations. The flies of the sixth generation were treated with ten micrograms of DDT. The results show that resistance to DDT was lower after six generations in an environment in which there was

no DDT (Table 2). Resistance increased when survivors of the sixth generation were used as parents (Table 2). The difference in resistance between the original Bellflower flies and the sixth generation flies was significant.

The Bellflower flies of the sixth generation that survived treatment were interbred and their progeny treated with 10 micrograms of DDT (Table 2). The resistance of the progeny was significantly greater than that of the sixth generation flies. The increase in resistance here is not so great as that shown by the progeny of the ninth generation Orlando flies that survived treatment (Table 1).

Table 2

The Loss of Resistance by the Bellflower Strain of Resistant Flies Bred in an Environment Free of DDT and the Recovery of Resistance when DDT was Reintroduced into the Environment

	Flies tested		Survival rate	
Generation*	Male	Female	Male	Female
			Percent	Percent
First	100	100	48	62
Sixth	97	216	4	9
F_1	102	101	18	14

*The first generation was the original stock whose ancestors had been treated every generation and the survivors used for breeding. The sixth generation was produced by inbreeding these original flies and keeping the stock free of DDT for six generations. The F_1 are the offspring of the flies surviving treatment in the test on sixth generation flies. All flies were tested by treating with 10 micrograms of DDT.

Test for Sex Linkage

Reciprocal crosses (resistant males X susceptible females and susceptible males X resistant females) were made between Bellflower (resistant) flies and common (susceptible) ones. The F_1 flies from both types of matings were treated with five and ten micrograms of DDT. There was no great difference in resistance of flies produced by the two types of matings (Table 3). Statistical analysis failed to show a real difference in resistance between the F_1 from the reciprocal crosses; consequently, there is no indication of sex linkage.

Table 3

Comparison of the Survival Rates of the F_1 from Reciprocal Crosses of Bellflower X Susceptible Flies

Mating	Flies treated		Dosage of DDT	Survival rate	
	Male	Female		Male	Female
			Micrograms	Percent	Percent
Resistant male X susceptible female	131	166	5	37	54
Susceptible male X resistant female	712	336	5	40	44
Resistant male X susceptible female	78	80	10	17	34
Susceptible male X resistant female	74	37	10	30	46

Differences in the Survival Percentages of the F_2 Flies

When the F_1 flies from each cross were inbred, the F_2 flies from one cross proved to be much more resistant than those from the reciprocal cross. The F_2 flies produced by inbreeding F_1 flies from resistant females X susceptible males were much more resistant than the F_2 flies produced by inbreeding F_1 flies from susceptible females X resistant males (Table 4). This difference was significant as shown by the X^2 test. These data were secured by using F_2 flies that came from untreated F_1 flies. When F_1 flies that survived treatment with 10 micrograms of DDT were used to produce F_2 flies, there was also a significant difference in survival percentages, depending upon the kind of F_1 flies that were used to produce the F_2 flies (Table 4). When the F_1 flies were produced by mating resistant females to susceptible males, the resulting F_2 generation flies possessed more resistance than when the F_2 generation flies were produced from F_1 flies that were developed by mating susceptible females to resistant males (Table 4).

Table 4

Comparison of the Survival Rates of F_2 Flies from Reciprocal Crosses of Bellflower X Susceptible Flies

Composition of F_1 used to produce F_2 flies*	Results of tests made on F_2 flies			
	Flies treated		Survival rate	
	Male	Female	Male	Female
			Percent	Percent
Resistant males X susceptible females F_1 untreated	200	1348	6	5
Susceptible males X resistant females F_1 untreated	303	668	24	23
Resistant males X susceptible females F_1 treated	55	105	11	31
Susceptible males X resistant females F_1 treated	224	169	59	67

*The parents of part of these flies were not treated. The parents of the others were given 10 micrograms of DDT and the survivors were used to produce the flies treated in this test. All flies treated in this test got 10 micrograms of DDT.

Resistance Differences in Backcrosses to Susceptible Flies

When the F_1 flies were backcrossed to the susceptible flies, there was a marked difference in the amount of DDT that could be tolerated, depending on how the F_1 were produced. The backcross flies produced by mating F_1 (resistant male X susceptible female) flies to susceptible ones

could tolerate only one-tenth the quantity of DDT as backcross flies produced by mating F_1 (susceptible male X resistant female) flies to susceptible ones (Table 5). A statistical analysis could not be made to determine if the differences were significant because the dosages of DDT were different. It was determined, however, that the sex of the F_1 parent of the backcross flies made no difference.

Table 5

Resistance of the Offspring from Backcrosses of the F_1 X Susceptible Flies

Composition of backcross flies	Results of tests made on backcross flies				
	Flies tested		Dosage of DDT*	Survival rate	
	Male	Female		Male	Female
				Percent	Percent
F_1 females (resistant males X susceptible females) X susceptible males	283	290	1	16	19
F_1 females (susceptible males X resistant females) X susceptible males	219	29	10	8	21
F_1 males (resistant males X susceptible females) X susceptible females	353	416	1	14	16
F_1 males (susceptible males X resistant females) X susceptible females	174	280	10	3	6

*Micrograms

Resistance Differences in Backcrosses to Resistant Flies

In backcrosses of the F_1 flies to the resistant parent stock, there was a significant difference in the survival rates of the backcross flies depending on how the F_1 flies were produced. When F_1 males from a resistant female X a susceptible male were bred back to resistant females, their offspring had a significantly higher survival percentage than the backcross offspring of F_1 males from a resistant male X susceptible females (Table 6). When F_1 females were bred back to resistant males the backcross flies had a resistance in accordance with the way the F_1 flies were produced. If the F_1 flies used in the backcross resulted from crossing resistant females X susceptible males, the resulting backcross flies were more resistant than when the F_1 flies used in making the backcross resulted from crossing susceptible females with resistant males (Table 6).

Resistant females mated to F_1 males produced backcross flies with less resistance than resistant males mated to F_1 females (Table 6). The backcross flies resulting from the crossing of F_1 females with resistant males emerged from the pupae two days later than the ones produced by mating F_1 males to resistant females. This difference in development time of two days may be the

cause of the difference in resistance. A test to see if a difference in the time taken for development has any effect on resistance was later made. The results of this test are presented in the next section and in Table 7.

Table 6

Resistance in Flies Produced by Backcrossing F_1 Flies to the Resistant Parent Stock

Composition of flies	Results of treating flies with DDT*			
	Number treated		Survival rate	
	Male	Female	Male	Female
			Percent	Percent
F_1 males (resistant males X susceptible females) X resistant females	218	236	6	12
F_1 males (susceptible males X resistant females) X resistant females	60	107	13	21
F_1 females (resistant males X susceptible females) X resistant males	82	167	17	23
F_1 females (susceptible males X resistant females) X resistant males	247	262	28	30

*Each fly was treated with 10 micrograms of DDT.

Effect of the Length of Development Time upon Resistance

First generation Bellflower flies, first generation Orlando flies, eighth generation Bellflower flies, and

eleventh generation Orlando flies were used to test the effect upon resistance of the length of time it takes a fly to develop from egg to adult. Whereas, in all the foregoing tests this development time ranged from 9 to 12 days, in this instance the time was increased to 19-23 days. Slowing down of the development process was accomplished by lowering the temperature in the fly room during the period when the flies were passing through the larval and pupal stages of development. There was a marked increase in resistance, resulting from increased time in development, in all four kinds of flies tested. The first generation flies of both the Bellflower and the Orlando strains showed a significant increase in resistance over the original flies of the same stocks (Table 7). The eighth generation Bellflower flies, which took 19-23 days to develop, were more resistant than sixth generation Bellflower flies which took 9-12 days to develop (Table 7). The Orlando flies of the eleventh generation, developed in 19-23 days, were more resistant than ninth generation flies developed in 9-12 days. There was almost as great a survival percentage of these eleventh generation flies when treated with 10 micrograms of DDT as there was in the sixth generation flies receiving 1 microgram of DDT (Tables 1 and 7).

The evidence presented in Table 7 is not entirely conclusive, since other flies of the same stocks raised in the regular way were not available for testing at the same time. The evidence strongly indicates, however, that resistance is directly proportional to development time.

Table 7

Comparison of the Resistance of Flies Developed in 19-23 Days with That of Flies of the Same or Comparable Stocks which were Developed in 9-12 Days*

Kinds of flies tested	Time	Number tested		Survival rate	
		Male	Female	Male	Female
	Days			Percent	Percent
Bellflower, first generation	19-23	143	152	52	70
Bellflower, first generation (Table 2)	9-12	100	100	48	62
Bellflower, eighth generation	19-23	245	192	9	13
Bellflower, sixth generation (Table 2)	9-12	97	216	4	9
Orlando, first generation	19-23	111	172	55	70
Orlando, first generation (Table 1)	9-12	100	100	37	48
Orlando, eleventh generation	19-23	241	238	4	16
Orlando, ninth generation (Table 1)	9-12	25	25	0	0

*All flies were treated with 10 micrograms of DDT.

CHAPTER IV

DISCUSSION

The loss of resistance in the flies of the Orlando strain (Table 1) may be a manifestation of the phenomenon known as Dauer-modification. A Dauer-modification is defined as "a cytoplasmic change induced by an environmental factor which decreases in penetrance and expressivity in succeeding generations in the absence of the inducing stimulus" (8, p.55). The cytoplasmic factor apparently reproduces itself rapidly enough to maintain resistance only in the presence of DDT. When DDT is removed, the resistance decreases until by the ninth generation very little is left.

The Dauer-modification theory, however, falls short of completely explaining resistance in the Orlando strain. Logically, if this theory were the true explanation, all offspring of resistant parents would be expected to have equal resistance. This is not the case. Resistance seems to range from a very small degree to a very large one in flies of the same hatch with the same breeding.

The fact that resistance is greater when transmitted through the female and the fact that there is no segregation according to any recognizable genetic pattern of the resistance factor or factors, both point to the

conclusion that the resistance factor is carried in the cytoplasm. The fact that resistance lasts at full strength for one generation after being transmitted by the male points to the conclusion that there is interaction between the cytoplasm and one or more genes. If this is the case, the genes transmitted by the male are apparently conditioned by the male's own cytoplasm to produce resistance in the F_1 flies. Apparently the cytoplasmic factor has to be there for resistance to be maintained, but the effect of this factor on the genes lasts through the next generation. The reproduction of this cytoplasmic factor would also necessarily depend on a nuclear gene.

It has been shown that a small amount of the cytoplasmic substance may be transmitted in the sperm (8, p.20). This may explain why a few of the F_2 flies produced by inbreeding F_1 flies from resistant males X susceptible females were able to survive a treatment with 10 micrograms of DDT.

Sonneborn has shown (38, pp.336-340) that there are specific particles in the cytoplasm of the so-called "killer" strain of Paramecia that are responsible for the "killer" character. He has shown that these particles reproduce themselves when a certain gene is present

in the nucleus. This nuclear gene which Sonneborn has named "K" and the cytoplasmic particles which he has called "kappa" must both be present in the cell for the "killer" character to be perpetuated. Kappa does not necessarily reproduce itself at the same rate as the cell itself divides; nor does it necessarily divide equally between the two daughter cells at mitosis (36, p.20). Although the gene K is responsible for kappa reproduction, it cannot initiate production of kappa if there is none present in the cell (38, p.339). Sonneborn and his co-workers have actually seen these kappa particles. They have also been able to reduce or destroy kappa by raising the temperature of the medium in which the animal lives (39, p.320).

It seems reasonable to think that resistance to DDT in the Orlando strain of flies may be due to particles in the cytoplasm which act like Sonneborn's kappa. In this case, however, it appears that DDT itself may be necessary for the reproduction of the particles in addition to a nuclear gene similar to Sonneborn's, particularly if the weather is warm and development is fast.

This nuclear gene may account for reproduction of the kappa-like substance, but it apparently needs the stimulus of DDT in the environment to maintain reproduction

of the cytoplasmic substance at a high level, under the conditions of this experiment. It is certainly true that as soon as DDT was reintroduced into the environment of ninth generation flies there was a marked increase in resistance in the offspring of the flies surviving treatment (Table 1). One explanation for this increase in resistance may be preferential selection. However, it seems unreasonable to believe that selection alone could be responsible for such a great increase in resistance in one generation.

Time was limited when this experiment was set up. For that reason the temperature in the fly room was kept above 70° F. throughout the experiment except during the last test. Sometimes the temperature was much higher because of summer heat. A heater equipped with a thermostat set at 70° was in use all the time. No provision was made for keeping the temperature from climbing higher. As a result of these conditions, the development time of the flies from egg to adult was shortened to 9 to 12 days. Sometimes the development period was still shorter, particularly in Orlando flies.

The fact that the temperature was kept at a high level may account for the decline in resistance. As stated above, the cytoplasmic particles in Sonneborn's

Paramecia were reduced in number or destroyed by raising slightly the temperature of the environment in which the animal lived.

Another possible explanation for the decline in resistance of these flies may be that the cytoplasmic particles reproduce at a certain rate unless stimulated by DDT, and that, under the conditions of this experiment, this rate was too slow to keep up with the accelerated rate of fly reproduction. This would cause a reduction of resistance. It was noted during the early stages of the experiment that flies emerging from the pupae last appeared to be more resistant than those emerging first. It was not known whether this longer time in the development stages caused the production of the resistance particles to keep up with the reproduction of the flies, which in turn caused the flies to exhibit more resistance, or whether resistance itself caused a slower process of development. It was thought that if environmental conditions were altered so that the process of development was slowed down, resistance might be maintained.

A test was made to determine if this idea had any merit. The temperature in the fly room was lowered about 15° F. for this test. The test was made in October when the outside temperature would not raise the temperature

in the fly room. The period of development increased to 19 to 23 days. The results of this test showed that resistance was increased in all four kinds of flies tested. First generation flies were significantly more resistant than were first generation flies at the beginning of the experiment. These results are not entirely conclusive, however, because no flies of the same stock which had been reared in the usual way were available for testing at the same time. The evidence strongly indicates, however, that a longer development period or a lower temperature, or both, may cause an increase in resistance. Production of the resistance substance may be adversely affected by a high temperature except in the presence of DDT, or the reproduction rate of the substance may be too slow to keep pace with the rapid reproduction rate of the flies in hot weather.

The Bellflower strain of flies may not have exactly the same sort of resistance as that exhibited by the Orlando strain. Their decline in resistance was much slower than that of the Orlando strain. It was noted during the experiment that the flies of this strain emerged from the pupae from 2 to 4 days later than the flies of the Orlando strain. The eggs were laid the same day and were incubated under the same conditions. This

fact suggests that the kind of resistance may be the same and that the theory of the speed of reproduction of flies being greater than the speed of reproduction of the resistance particles may be the correct answer. In this strain, also, the last flies to emerge appeared to be more resistant than the first.

March and Metcalf (25, pp.95-96) had no decline in resistance in their Bellflower strain in 30 generations. The flies used in this experiment may have been slightly different genetically. However, March and Metcalf treated their flies at a greater age (2 to 4 days). They also were probably not concerned with getting their generations as fast as was necessary in this work. Again the time element looms as a factor to be considered. In March and Metcalf's work the production of the resistance substance may have had time to keep up with the reproduction of the flies.

The offspring of the surviving Bellflower flies treated in the sixth generation showed a significant increase in survival percentage over the flies of the sixth generation. This could have resulted from selection alone. However, the resistance of the flies in the sixth generation was fairly high. It is not likely that selection alone would have accounted for all the increase.

It is suggested that the DDT itself acted as a stimulus to the production of the cytoplasmic substance just as it did in the Orlando strain and that the resistance in the two strains is the same. Other factors account for the difference in the time required to go from egg to adult in the two strains and this time difference in turn accounts for the difference in the rates of decline in resistance. The time for development in both strains was cut as short as possible in this experiment and this factor alone may have caused the decline in resistance in both strains of experimental flies. It is suggested that with a rate of development which is slow enough, there would be no decline in resistance in either strain. This in turn suggests that resistant flies in the wild, carried over the winter season when the environment is unfavorable and development is necessarily slow, would emerge in the spring with an enormous resistance to DDT, especially if there were small amounts of DDT in the environment.

CHAPTER V

CONCLUSIONS

1. The factor responsible for resistance to DDT in Bellflower and Orlando house flies is carried in the cytoplasm of their cells.

2. The factor responsible for DDT resistance is particulate. No alternate conclusion can be drawn from the data obtained in this experiment.

3. When DDT is introduced into the environment of DDT resistant house flies whose resistance has declined, the offspring of these treated flies have much more resistance than the parents. Part of this added resistance is accounted for by selection. The remainder of the increase in resistance may be due to a stimulating effect of DDT on the reproduction rate of the resistance particles or to the activation of new particles by the DDT.

4. It seems reasonable to assume that the reproduction of the cytoplasmic resistance particles is under the control of one or more nuclear genes.

5. Numerous observations and the results of one test indicate that the speed of development from egg to

adult influences resistance in DDT resistant house flies. Apparently, the longer the development period the greater the resistance.

6. There is evidence that the cytoplasmic resistance particles act indirectly by influencing nuclear genes to produce resistance, rather than acting directly to produce it themselves.

7. Resistance to DDT is at full strength for one generation after being transmitted by a resistant male. Apparently, the genes transmitted by the resistant male are conditioned by the male's own cytoplasm to cause resistance in the F_1 flies.

8. A few F_2 flies, all of whose resistance comes from one resistant grandfather, are fully resistant. This indicates that a few of the cytoplasmic resistance particles are transmitted in the sperm by the resistant male.

BIBLIOGRAPHY

1. Auerbach, C. and M. Moser. An analysis of the mutagenic action of formalin. Heredity 4(Part 2): 272-273. 1950.

2. Anonymous. Genetics and cancer: Symposium on the genetics of cancer. British journal of cancer 2(Part 2): 87-176. 1948.

3. Barber, George H., Ordway Starnes, and Eleanor B. Starnes. Resistance of house flies to insecticides. Soap and sanitary chemicals 24(11):120, 121, 143. 1948.

4. _____ and John B. Schmitt. House flies resistant to residual sprays. New Jersey Agricultural Experiment Station bulletin 742. 8p.

5. Bettini, S. and B. Barachini. Primi resultatidella lotta con L'octa Klor ed il Gamaesano contro le mosche domestiche resistente al DDT. Rivista di parassitologia 9(2):85-91. 1948.

6. Blickle, Robert L., Asher Capelle and W.J. Morse. Insecticide resistant house flies. Soap and sanitary chemicals 24(8):139, 141, 149. 1948.

7. Bruce, W.N., and G.C. Decker. Housefly tolerance for insecticides. Soap and sanitary chemicals 26(3):122. 1950.

8. Caspari, Ernst. Cytoplasmic inheritance. In Advances in genetics v.2. New York, Academic press, 1948. pp. 1-65.

9. Dickson, R.C. Inheritance of resistance to HCN fumigation in the California red scale. Hilgardia 13:515-522. 1941.

10. Ferguson, W.C. and C.W. Kearns. The metabolism of DDT in the large milkweed bug. Journal of economic entomology 42(5):810-817. 1949.

11. Goldschmidt, Richard B. Physiological genetics. New York, McGraw - Hill, 1938. 375p.

12. Goldschmidt, Richard B. Fifty years of genetics. The American naturalist 84(818): 313-339. 1950.

13. Gough, H.C. Resistance of the flour beetle to HCN. Annals of applied biology 26:533-571. 1939.

14. Hardman, N.F. and Roderick Craig. A physiological basis for the differential resistance of the two races of red scale to HCN. Science 94:187. 1941.

15. Haseman, Leonard and R.L. Meffort. Are we developing a strain of codling moths resistant to arsenic? Missouri agricultural experiment station research bulletin 202. 1933. 11p.

16. Hough, W.S. Resistance of codling moth larvae to arsenical sprays. Journal of agricultural research 38:255-256. 1929.

17. Jones, D.F. The interrelation of plasmagenes and chromogenes in pollen production in maize. Genetics 35(5):507-511. 1950.

18. King, W.V. Some results of recent work on newer insecticides. American journal of tropical medicine 28:487-497. 1948.

19. King, W.V., and J.B. Gahan. Failure of DDT to control house flies. Journal of economic entomology (in press). 1950.

20. Lindgren, D.L. The stupefaction of red scale by HCN. Hilgardia 11:224. 1938.

21. _____ and Walton B. Sinclair. Relation of mortality to amounts of HCN recovered from fumigated resistant and non resistant citrus scale insects. Hilgardia 16:314. 1944.

22. Lindquist, Arthur W., et al. Penetration and metabolism studies of DDT on house flies with the aid of C^{14} labeled DDT. (Unpublished data).

23. Lindquist, Arthur W. and H.G. Wilson. Development of a strain of resistant house flies to DDT. Science 107:276. 1948.

24. Macdonald, J.M. Production of phenocopies in Drosophila by chemical substances. Heredity 2(Part 3): 408. 1948.

25. March, Ralph B. and R.L. Metcalf. Laboratory and field studies of DDT resistant house flies in southern California. The Bulletin, California Department of Agriculture, 39(2):93-101. 1949.

26. McGovran, E.R., W.N. Sullivan, and G.L. Phillips. Resistance to insecticides. The effect of knock-down on the resistance of house flies to pyrethrum sprays. Soap and sanitary chemicals 15(8): 88-90. 1939.

27. McGregor, E.A. Notes on the resistance of citrus thrips to tartar emetic. California citrograph 29(3):62. 1944.

28. Natman, P.S. Nuclear and cytoplasmic inheritance of resistance to infection by nodule bacteria in red clover. Heredity 3(Part 3):263-291. 1949.

29. Omer-Cooper, J. An arsenic resistant tick. Nature 156:450-451. 1945.

30. Perry, A.S. and W.M. Hoskins. Detoxification of DDT by resistant house flies and inhibition of this process by piperonyl cyclonene. Science 3(2892):600. 1950.

31. Quayle, H.J. The resistance increase in insects to insecticides. Journal of economic entomology 36(4):497. 1943.

32. Richards, A.C. and L.K. Cutkomp. Correlation between the possession of a chitinous cuticle and sensitivity to DDT. Biology bulletin 90(2): 97-108. 1946.

33. Riley, H.P. Genetics and cytogenetics. New York. Wiley, 1948. 596p.

34. Sacca, Giuseppe. Sull' esistenza di mosche domestiche resistenti al DDT. Rivista di parassitologia 8(2/3): 127-128. 1947.

35. Snyder, L.H. The principles of heredity. Boston, Heath, 1946. 450p.

36. Sonneborn, T.M. The cytoplasm in heredity. Heredity 4(Part 1): 11-36. 1950.

37. Sonneborn, T.M. The dependence of the physiological action of a gene on a primer and the relation of primer to gene. The American naturalist 79 (783): 318-339. 1947.

38. Sonneborn, T.M. Gene and cytoplasm. Proceedings national academy of science. 29: 338-340. 1943.

39. Sonneborn, T.M. Paramecium and euplotes. In Advances in genetics v. 1. New York, Academic press, 1948. pp. 264-355.

40. Sternberg, James, C.W. Kearns, and W.N. Bruce. Absorption and metabolism of DDT by resistant and susceptible house flies. Journal of economic entomology 43(2):218-219. 1950.

41. Wiesman, R. von. Untersuchungen uber das physiologische Verhalten von Musca domestica L. verschiedener provenienzen. Schweizerische Entomologische Gesellschaft Mitteilungen 20:484-504. 1947.

42. Wilson, H.G. and J.B. Gahan. Susceptibility of DDT resistant house flies to other insecticidal sprays. Science 107:276-277. 1948.