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Genetic aspects of DDT resistance in the house fly, Musca domestica L. (Diptera).

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INTRODUCTION

The introduction of DDT into the insecticidal field of the United States in 1943 was followed by such widespread use of the material that by May of the following year pilot plant production was initiated in both the United States and Great Britain. In order to meet continually increasing demands for this material for the control of an expanding list of insects affecting the manpower of a nation at war, production facilities of both nations were increased and taxed until the end of World War II. So epoch-making was the insecticidal effectiveness of the material that in 1948 Paul Müller was awarded the Nobel prize in physiology and medicine for having revealed the nature of its insecticidal properties.

Many reasons may be advanced to account for the nearly universal success of the compound. However perhaps foremost of the reasons for its wide adoption (Frear, 1948) was its high toxicity to such a wide range of insects, including particularly those affecting man. A second factor fostering its success was its wide margin of safety to man and warm blooded animals. With the war progressively inflicting its effects through reduced supplies of natural insecticides, a condition incurred by curtailed

shipping facilities, DDT supplemented depleting supplies of natural insecticides at an opportune time.

Among the several insects successfully controlled by DDT was the house fly, Musca domestica L. (Muscidae, Diptera), perhaps the most common and abundant insect throughout the world, ranging from the subpolar regions to the tropics. Apart from the larva being the causative agent in occasional intestinal myiases, this insect probably is not the direct cause of other pathogenic disturbances in man. With its hairy body and legs to which many types of pathogenic organisms may adhere as it frequents and feeds upon animal filth and fecal waste, decayed and diseased animal and vegetable as well as other sources of pathogenic contamination, it serves however as an ideal mechanical carrier of many disease inducing organisms. By endozooic and epizooic contact transmission it disseminates and deposits in foods, liquids, exposed sores, open wounds, moistened surfaces as about the mouth, nose, and eyes of man, etc., pathogenic bacteria, protozoa and helminthic ova or larvae, chiefly those causing enteric diseases (Belding, 1942).

Some of the pathogenic bacteria associated with the house fly include the causative agents of Typhoid fever, Eberthella typhosa, Salmonella paratyphi, and S.

schottmülleri; of bacillary dysentery, Shigella (Bacillus) dysenteriae, and S. paradysenteriae; of Cholera, Spirillum cholerae (Vibrio comma); and of Anthrax, Bacillus anthracis.

Cysts of human intestinal protozoa have been reported (Matheson, 1950) taken up by the house fly and passed in its feces in a viable condition. Among these have been Endamoeba histolytica (Schaudin, 1903) Hickson, 1909*, the cause of amoebic dysentery; Endamoeba coli (Grassi, 1879) Hickson, 1909, a nonpathogenic commensal of the large intestine of man; Giardia lamblia Stiles, 1915 (syn. Giardia intestinalis Lambl, 1859) a controversially pathogenic inhabitant of the small intestine; and Chilomastix mesnili (Wenyon, 1910) Alexeieff, 1912, a nonpathogenic protozoan whose exact habitat in the human body has not been determined but of which the trophozoite probably lives in the large intestine or cecum.

The house fly assumes an important role (Matheson, 1950) as an intermediate host in the life cycle of helminths. According to Nicoll (Matheson, 1950) it has been found capable of ingesting and excreting in a viable condition ova of the pork tapeworm, Taenia solium Linnaeus, 1758; of

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* nomenclature after Belding, 1942

the dwarf tapeworm, Hymenolepis nana (Siebold, 1852) Blanchard, 1891; of the meromyrian nematode causing enterobiasis or pinworm infection (Belding, 1942), Enterobius vermicularius (Linnaeus, 1758) Leach, 1853 (syn. Oxyuris vermicularius (Linnaeus, 1758) Lamarch, 1816; and the whipworm, Trichuris trichiura (Linnaeus, 1771) Stiles, 1901.

Epizooically the fly may transmit from fecal deposits any eggs which adhere to its body setae or to its feet. In this respect the sticky substance exuded by the tenent hairs of the pulvillus which enables the fly to walk on ceilings, glass and other smooth surfaces, serves to gather up all sorts of bacteria, spores, cysts, and filth and to distribute them.

A considerable number of important human diseases are caused (Chandler, 1949) by filtrable viruses. These small causative agents are probably highly degenerative forms which have become dependent upon the host cells for enzymes and other materials which free-living organisms provide for themselves. In many ways the simplest viruses are comparable to genes in size and properties and are subject to mutations. Poliomyelitis is among the simplest viruses. Although considerable uncertainty exists as to the role that arthropods play in the transmission of this disease,

its epidemiology nevertheless strongly suggests their implication. The house fly is known to harbor this virus, acquiring it from human feces.

As a potential menace therefore to the health and welfare of mankind, control of the house fly has evolved into a comparatively incessant problem. The outstanding success of DDT initiated a period of fly control unsurpassed in all previous attempts. For approximately four to five years this compound appeared to be the answer to all fly control problems, effecting what was often referred to as a "panacea of control."

In 1947 however isolated reports appeared which described the failure of DDT as a control measure for this insect. These reports gradually became more and more numerous until 1948 and 1949 when a critical evaluation of the house fly control complex indicated that a resistance factor or factors had apparently developed within this insect which was capable of aborting the insecticidal effectiveness of DDT. Subsequent reports depicting various phases of this resistance concept generally agree that DDT resistant flies, in addition to DDT resistance, may simultaneously show an increased tolerance to other toxicants. However, the degree of tolerance to the conditioning material, in this instance DDT, is reportedly

greater than that to any other toxicant, related or otherwise.

Resistance to DDT in the house fly is currently rather widely reported, the majority of relevant data being derived from areas which have been subjected to repeated applications of the toxicant. This increasing failure of DDT to control the house fly has of necessity curtailed use of the chemical and has brought about adoption of other toxicants as control measures. Thus flies which have built up a resistance to DDT are deprived of exposure to the conditioning compound. Determination of the stability and duration of resistance under such circumstances becomes an objective of this study.

Available data depicting the various aspects of DDT resistance are confined to the adult life stage. Although an evaluation of tolerance levels in the immature life stages may be considered of a preliminary nature, a more complete understanding of resistance nevertheless hinges thereon.

House fly resistance thus far has developed largely in areas well isolated from each other. Interspersed between these areas are flies of the normal or susceptible level of tolerance to DDT. The question of the effect on tolerance induced by cross breeding with susceptible or

other resistant strains seems paramount to the future status of the resistance problem. In conjunction therewith a determination of the degree to which this ability to withstand increased dosages of DDT may be transmitted to successive progeny becomes a further objective of this study. An understanding of some of the genetic aspects concerned is relevant.

REVIEW OF LITERATURE

The sporadic development of insects which for some reason, physiological or morphological, seemingly abort the toxicity of certain chemical compounds and thus assume the role of a resistant strain is not new to the field of entomology. Whether or not the term "resistant" is appropriate for these insects must be determined in the light of future research. Perhaps a more appropriate term would be "tolerant." As employed in this study the term tolerant shall be construed to imply those strains of insects which can withstand relatively greater concentrations of a toxic agent than can the normal or non-tolerant strain.

In 1914 Melander found that in certain areas the San Jose scale, Aspidiotus perniciosus Comstock, had exhibited a decided resistance to the action of lime sulfur whereas in other areas the normal action of this toxicant was observed. Repeated experiments conducted eleven years later showed a marked increase in the tolerance of the scales despite a ten-fold increase in the concentration of the insecticide.

The extensive work of Quayle (1916, 1922, 1938, 1942, 1943) describing increased resistance to insecticides by certain scale insects, including the California red scale,

Aonidiella aurantii Mask., black scale, Saissetia oleae Bern., and the citricola scale, Coccus pseudomagnoliarum Kuw., has contributed greatly to the background information of insect resistance to insecticides. Quayle found that scales were not immune to hydrocyanic acid fumigation but in many instances the dosage required for effective control was such as to border on phytotoxicity except in rare cases of extremely favorable environment. He concluded (1922) that the greatest resistance was shown by scales on trees that had been fumigated regularly, once, or even twice a year. This work was later supported by observations of Woglum (1925).

Hough (1928, 1929) reported that larvae of the Colorado strain of the codling moth, Carpocapsa pomonella Linne, exhibited a higher rate of penetration of the sides of arsenical sprayed apples than did the Virginia strain. The F_1 generation of a cross between these two strains was less resistant to arsenicals (Hough, 1934) than the resistant parental line (Colorado) and capable of a higher rate of fruit entrance than the non-resistant parental line (Virginia). Haseman and Burk (1929), after feeding specific doses of arsenic to larvae of both the Colorado and Missouri strains of the codling moth and finding no differences in the lethal doses required, concluded that

both strains were equally susceptible. Haseman and Meffert (1933) furthered this work using the Colorado, Missouri, and Virginia strains. The resistance of all three strains was equivalent to sodium arsenite and lead arsenate when injected into the hemocoele or through the mouth into the digestive tract. Although these workers agreed with Haseman and Burk that resistance to arsenic had not developed in the Colorado strain they did indicate that the Virginia strain was easier to control than the Colorado strain under field conditions with arsenical toxicants.

Gough (1939) subjected the confused flour beetle, Tribolium confusum Duv., to fumigation with hydrocyanic acid gas, adjusting the dosage (concentration and exposure time) so that a relatively small number only of beetles survived. From the progeny of the survivors of a series of such exposures he was able to develop a strain of beetles which displayed a greater resistance to the hydrocyanic acid gas than did the normal. This resistance was shown to be hereditary with the greatest degree of resistance being found in the pupae, followed in order by the adults, larvae, and eggs. No morphological differences were detected.

Boyce and Persing (1939) reported a strain of citrus thrips, Scirtothrips citri Moult., in the lemon groves of

the San Fernando Valley in California which were resistant to tartar emetic, a control measure which two years previously had been satisfactory. Under laboratory conditions this resistant strain was observed to be tolerant to four times the normally recommended dosage. McGregor (1944) was able to display this difference in resistance between resistant and non-resistant individuals after seven generations.

The work of Knipling (1942) showed that the response of the larvae of the screw-worm fly, Callitroga americana Cushing and Patton (syn. Cochliomyia americana Knipling, 1942), to phenothiazene (thiodiphenylamine) was variable. Successive generations of newly hatched larvae were reared on an artificial breeding medium to which sublethal amounts of thiodiphenylamine had been added. After eleven such generations the number of survivors among the resistant screw-worm larvae was approximately eighteen times greater than the number of survivors among the non-resistant strain. Knipling concluded in part that the increased tolerance of the special strain appeared to be specific for thiodiphenylamine since in tests with toxic concentrations of diphenylamine and diphenylene oxide there was no difference in the numbers of survivors for the two strains in the fifth generation. Thus a strain with an apparently acquired

resistance to thiodiphenylamine ($C_{12}H_9NS$) was developed whereas the same strain showed no resistance to diphenylamine ($(C_6H_5)_2NH$) nor to diphenylene oxide ($(C_6H_4)_2O$).

Mosna reported (1947) that the mosquito, Culex pipiens autogenicus Latina, taken from the marshes near Rome, Italy, could withstand the effects of DDT approximately ten times longer than the normal strain.

McGovran found (1939) that when house flies were exposed to a knock-down dose of pyrethrins and were subsequently exposed to a supposedly lethal dose, there was an apparent temporary resistance displayed.

Perhaps the first report of any variation from the accustomed panacea of house fly control with DDT was that of Missiroli. In 1947 he reported that the house fly in Naples, Italy had showed an increase in tolerance to DDT to such an extent that a comparatively inferior degree of control had been obtained. About the same time Wiesmann reported (1947) that control of the house fly with DDT in 1946 in the area north of Stockholm, Sweden was relatively poor compared to the degree of control obtained in previous years. The lethal dose for this resistant strain was observed to be from one hundred to two hundred times greater than that for the normal strain, unexposed to DDT. The

basis of comparison was the absorption of lethal dosages through the tarsi. Morphological differences reported by Wiesmann include the presence of a greater amount of pigmentation in the extremities, a stiffer tarsal hair tuft, larger tarsal joints and thicker pulvilli and articular membranes of the joints. In view of these distinct physiological and morphological differences Wiesmann concluded that there was sufficient basis for considering the two strains to be two separate races. Sacca (1947) concluded that resistant house flies in certain parts of Italy were a different strain than the normal or non-resistant house fly. He proposed the name Musca domestica var. tiberina.

In his work with Drosophila species Kalmus (1942) proceeded on the premise that the darkening of a dipteron's cuticle is induced by polyphenols (Frankel and Rudall, 1940) which simultaneously result in a hardening and dehydration of the cuticle. He assumed that the darker and harder cuticle would be less permeable to water than the lighter one. He showed that the structural changes in the cuticle which accompany this darkening decrease the permeability not only for water-soluble substances but also for mineral oils and for oil-soluble substances. It was not certain whether the differences in reaction of the

mutants to the chemicals were due to a common cause or to a sequence of unknown factors resulting in a differential general resistance. Kalmus proposed that since light and dark mutants occur in many insects greater protection against toxic substances afforded by a dark cuticle might become a selective advantage under various conditions. He further proposed that chemical selection could conceivably favor survival of the darker members of an insect population that had been subjected to insecticidal treatment, and thus foster the development of a tolerant race.

The work of Lindquist and Wilson (1948) in developing a strain of resistant house flies may be compared to the work of Knipling cited above. By spraying large populations of laboratory house flies with a dosage of DDT adjusted so as to produce an approximate 90 per cent mortality, survivors were obtained which exhibited, initially at least, a higher degree of tolerance to DDT. After fourteen successive generations of such survivors had been sprayed with DDT a marked difference in susceptibility was observed. The resistant strain was approximately two and one-third times more tolerant to DDT than the unexposed strain. Further tests with these progeny (Wilson and Gahan, 1948) showed the resistant strain to be more resistant than

the normal strain to chlordane, pyrethrins plus piperonyl cyclonene, chlorinated camphene, rotenone and Thanite.* As in the work of Knipling (1942) a resistant strain had been effected by artificial selection.

In consequence of the accidental contamination of their laboratory with benzene hexachloride, Blickle et al (1948) found that the house fly culture had been reduced to but a few survivors. From these individuals a culture has been developed in the same contaminated laboratory, successive generations being continually exposed to benzene hexachloride. When adult house flies which had not been exposed to benzene hexachloride were allowed to emerge in this same laboratory less than five per cent survived for more than twenty-four hours after emergence. The resistant flies showed approximately twice the degree of tolerance to DDT exhibited by the normal strain. The tolerance of this culture for other insecticides was greater than that of the unexposed culture but considerably less than its tolerance for the conditioning material. Blickle concluded in part that a degree of specific resistance to

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* A product of Hercules Powder Company, Wilmington 99, Delaware, described as secondary terpene alcohol thiocynl acetate. It is a 100 per cent active toxicant containing 82 per cent isobornyl thiocyanoacetate and 18 per cent other active terpenes.

the insecticide used to increase the resistance was indicated. An increase in tolerance to DDT, pyrethrins and Lethane 384 Special (aliphatic thiocyanate) was observed. This tolerance however was considerably below that exhibited for the benzene hexachloride.

At a resort hotel at Ellenville, New York, Barber and Schmitt (1948, 1949a, 1949b) encountered a colony of house flies which had developed a resistance to DDT and an increased tolerance for methoxychlor and TDE (2, 2 bis p-chlorophenyl) 1, 1 dichloroethane). Since, according to Barber and Schmitt, these flies showed no resistance at all to the residual effects of toxaphene, chlordane, parathion, the gamma isomer of benzenehexachloride or tetraethyl pyrophosphate they apparently had acquired a specific resistance to DDT. Babers (1949) however points out that an analysis of these data indicates that a mortality of 95.6 per cent in the laboratory strain as compared with a mortality of 60 per cent in the resistant strain would indicate some degree of resistance to chlordane within the resistant strain. Similarly a mortality of 96.7 per cent in the normal strain as compared with 77.8 per cent in the resistant strain would indicate some degree of tolerance for parathion in the resistant colony.

When flies of the 10th and 11th generations of the Ellenville strain were tested (Barber and Schmitt, 1948, 1949a, 1949b) against technical DDT, its pp' isomer and against methoxychlor they had apparently retained their tolerance for these materials. However these flies again, as in the first three generations, displayed no particular tolerance for other chemicals such as toxaphene, chlordane, parathion, gamma isomer of benzenehexachloride and tetraethyl pyrophosphate, despite their ability to withstand repeated knockdown by DDT.

March and Metcalf (1949) found that DDT residual sprays were relatively ineffective for fly control in various parts of California. Samples of these populations were collected and their progeny subjected to comparative tolerance tests with flies which had never been exposed to DDT. A range of tolerance from five hundred to twenty-five times that of the unexposed strain was observed. The Bellflower strain, one of the cultures used in this study, exhibited a tolerance for methoxychlor fourteen times greater than that of the unexposed strain and a tolerance for TDE two hundred times greater than the unexposed strain. March and Metcalf concluded that the failure of residual applications of DDT to control the house fly was due to the development of resistance to this toxicant within this insect.

Hansens et al (1948) and Hansens and Godin (1949) continued the work of Barber and Schmitt (1948, 1949a, 1949b) with the Ellenville line of flies. They found this strain of flies to be about fifteen times more resistant to DDT wettable powders than other strains considered and apparently little more resistant to methoxychlor than the normal NAIDM culture.

In 1949 King and Gahan reported that samples of flies collected from seven localities in five states all showed greater tolerance for residual deposits of DDT than laboratory colonies being reared in Florida which had never been exposed to DDT. A resistant colony which had been developed experimentally however showed more resistance than either of these samples. Residual applications of DDT applied at the rate of 0.2 gm. per square foot of surface, although previously an effective means of control, was now practically non-repellent to this experimental strain. Increased applications of 0.8 to 4.0 gms. per square foot showed some repellency. Heavy deposits of wettable chlordane and methoxychlor were non-repellent. In some cases they were even attractive. Indications were that the increased resistance to DDT was greater than to methoxychlor, chlordane, and benzenehexachloride.

A rapid development of insecticide-tolerant house flies was effected by Bruce (1950) through insecticidal treatment in both larval and adult stages, tolerance being greatly intensified through treatment of the larval rearing medium. The initial acquisition of tolerance for DDT and methoxychlor was slow but after it had been once established it became rapidly intensified and even increased to a point of equilibrium with the toxicant. After nine generations the NAIDM strain exhibited high tolerance for methoxychlor whereas it required eighteen generations to attain a similar tolerance for DDT. When progeny of the DDT resistant strain were reared in a methoxychlor-treated environment a strain was developed in five generations which exhibited higher tolerance for both DDT and for methoxychlor than did either the DDT resistant strain or the methoxychlor resistant strain.

After thirty-four generations of inbreeding the DDT resistant strains have maintained their tolerance. The progeny of crosses and of reciprocal crosses between the DDT resistant strain and the normal or unexposed strain displayed tolerance to a lesser degree than the tolerant parental line but to a greater degree than the non-tolerant parental line. Bruce concludes that both the male and female carry the resistance factors. Concerning the genetic

aspects of this study Bruce states, "In summation of the genetical studies it may be said that DDT resistance is caused by multiple gene characters regulating physiological processes." The expression "multiple gene characters" is questioned. Multiple or polymeric genes may regulate physiological processes. However since the term "characters" construes that which is visible externally, perhaps a misconception of genetics is herein represented.

Commenting on the work of Bruce, Decker (1950) states, "Where interbreeding with susceptible flies is permitted the degree of tolerance is progressively reduced with each generation of cross breeding."

MATERIALS

TEST ORGANISM.

The house fly was used as the test organism in all tests. Cultures were developed from pupae secured from various areas of the United States wherein DDT resistance in the house fly had been reported either as developing under field conditions after repeated applications of DDT or as the result under laboratory conditions of the artificial selection of insecticide-tolerant individuals.

In 1948 Barber and Schmitt encountered house flies at Ellenville, New York, which were able to withstand dosages of DDT which originally had effected a satisfactory degree of control. A colony established from these flies displayed a resistance differential to DDT when compared, under laboratory conditions, with the ordinary laboratory strain. From pupae of these Ellenville house flies a culture was established during 1949. This culture is hereinafter referred to as the New York strain.

In 1948 Blickle et al reported on house flies which had developed a resistance to benzene hexachloride as the result of accidental contamination of their laboratory. The progeny of those house flies surviving the benzene hexachloride contamination were retained in this same laboratory over a period of three years. During this time

the house flies were subjected to continual exposure to benzene hexachloride. In addition to resistance to benzene hexachloride these flies apparently are tolerant to DDT in the ratio of two to one when compared with the normal laboratory (in this instance flies which had never been exposed to benzene hexachloride or to DDT) strain. Early in 1949 a culture was established from pupae of this strain. It is hereinafter referred to as the New Hampshire strain.

Early in 1949 March and Metcalf reported that DDT residual spraying was apparently not effective for fly control in southern California. Laboratory tests showed that various house fly populations collected from Bellflower, San Jose, Ontario and Riverside, California exhibited varying degrees of tolerance for DDT. In each instance the tolerance was in excess of that exhibited by house flies which had never been exposed to DDT. Of these the Bellflower strain exhibited the greatest degree of tolerance, showing approximately five hundred times that of the laboratory strain. During the summer of 1949 a culture was developed from pupae of the Bellflower strain. It is hereinafter referred to as the California strain.

In 1950 Bruce reported on the development of a strain of insecticide-tolerant house flies which had been developed

under laboratory conditions. Treatment of the larval medium with DDT had enhanced the tolerance of these flies for DDT. Larval selection contributed greatly to the development of this tolerance. In a letter dated February 23, 1950 Bruce has stated that Multi-strain I exhibits over 1,000 times normal resistance to DDT when compared to the NAIDM strain. A culture hereinafter referred to as the Illinois strain has been established from larvae of this Multi-strain I.

A stock culture of laboratory flies which, as far as is known, was devoid of exposure to and likewise devoid of resistance to DDT, was maintained for purposes of comparison with the above described resistant strains.

Rearing. All flies were reared in accordance with conditions prescribed by the National Association of Insecticide and Disinfectant Manufacturers for the Peet-Grady Method (Anon., 1947). Adult flies which emerged from puparia were placed in number sixteen mesh wire screen cages measuring approximately 8 1/2 x 11 x 13 inches, reinforced with wooden supports. Approximately 200 flies were maintained in each such cage as stock cultures.

To each cage of flies a number four Lily paraffin-impregnated ice cream sundae dish of approximately five

ounce capacity and containing approximately four ounces of evaporated whole milk diluted with three parts of water was added as food. One part of 37 per cent formalin was added to approximately every 1500 parts of diluted milk to serve as a bactericide and anti-coagulant for the milk. One-half of a crumpled paper towel was then added to the diluted milk-formalin preparation to provide more surface area for feeding. Under normal conditions of temperature and relative humidity such a feeding was sufficient to last the flies for two days, requiring a change of food but every other day. This stock culture was an inbred population to which sufficient numbers of each new generation were added to maintain two hundred.

Eggs. Eggs were deposited on or after the third day following emergence of the adult. Eggs of practically any age could be obtained from the stock culture by changing the food dish at the proper time. They were deposited on the crumpled paper toweling.

During the early part of this study eggs were washed from the paper toweling with distilled water (Breakey and Miller, 1935) into small centrifuge tubes, gently agitated and allowed to settle. Those eggs and other extraneous matter such as milk coagulates and fly excreta which

remained floating were decanted. Ten cc. of water was added to the remaining eggs to remove them from the tube. These were then poured into a small cone-shaped pit in the surface of the rearing medium and covered with from one to two cc. of the rearing medium.

Later it was found more expedient to remove a group of eggs of desired number from the paper toweling with a pair of forceps and to deposit them directly within the surface of the rearing medium.

Rearing medium. The rearing medium was prepared at least one hour prior to placing the eggs therein. It consisted of approximately one and one-third cups (100 grs.) of crimped oats placed in an open-top pint glass jar to which was added an approximately equal volume (316 cc.) of evaporated whole milk which had been diluted with three parts of water. The jar was then covered. After soaking for one hour the oats were considerably swollen and softened. At this time the surface of the oats was above the unabsorbed diluted milk level. The eggs therefore could be placed just beneath the rearing medium surface and remain at a safe distance above the liquid.

The jar containing the rearing medium was then placed in a battery jar and surrounded to a height of about three

inches with dry sand which had been sifted through a 12 mesh wire screen strainer. The battery jar was covered with a cloth secured in place with rubber bands. This cloth covering reduced the loss of moisture from the rearing medium but more important, it greatly reduced the incidence of stray flies ovipositing in the rearing medium and thus contaminating the strain. The battery jar and contents were then placed in a constant temperature and relative humidity chamber where the temperature was maintained constant at 27° C., plus or minus 1° C., and the relative humidity at 65 per cent. During the latter part of this study the vavrious cultures were maintained under room conditions where the temperature remained relatively constant at 25° C., plus or minus 2° C., and the relative humidity remained at 55 per cent, plus or minus 5 per cent.

Larvae. The eggs hatched in from 24 to 48 hours. The larvae matured in from 4 to 6 days. With the approach of maturity (Spear, 1950) the larvae became restless, as evidenced by incessant crawling about the rearing medium surface. Following this behavior the larvae would crawl out of the rearing medium jar and drop into the surrounding sand where pupation was effected.

Pupae. After the larvae had crawled into the sand to pupate, the rearing medium jar was removed and the pupae left undisturbed for about three days. When pupae of any specific age were desired the sand was removed and replaced with fresh sand. The pupae were removed by sifting the sand through the 12 mesh wire screen strainer.

Each group of pupae was sorted for uniformity of size and age, other pupae being discarded. Of these, approximately 75 were measured into 6 ounce glass jars and then covered with a cloth secured in place by rubber bands. These jars were then segregated as to culture and age and, in the case of earlier work, placed in the constant temperature and relative humidity chamber or, as in later work, maintained under room conditions.

Adults. The majority of the adults emerged from the puparia in about five days. At this time a sufficient number of adults were added to the stock cultures to maintain the desired number. Flies that were to be subjected to insecticidal exposures were placed in tarlatan cages, hereinafter described, provided with diluted whole evaporated milk and formalin as above described, and left undisturbed for from three to five days. All flies used

for insecticidal exposures were permitted to age in this manner for at least three days and not more than five days. A second feeding was provided if needed.

EQUIPMENT.

American Aerovap. Application of insecticidal treatments to adult flies was in the form of an aerosol, effected with a vaporizer known commercially as the American Aerovap.* The original model of this device was designed for the production of a continuous phase bactericide in the air raid shelters of England during World War II. Model C was used in this study.

The Aerovap is fundamentally composed of three cylindrical, open-top receptacles which fit one inside the other. The outer receptacle, constructed of bakelite, is attached by machine screws to an extension arm to permit attachment of the unit to a wall surface. The base of the outer receptacle is equipped with female electrical contacts which receive the male contact elements of and serve to mount the intermediate cast aluminum receptacle. A groove in the bottom of the intermediate container, aligned with an opening in the outer receptacle, permits the insertion

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* A product of American Aerovap, Inc., 110 West 40th Street, New York 18, N. Y.

of a thermometer for observation of well temperatures. When the thermometer is removed it is replaced by a threaded cap which secures the intermediate receptacle in place. The intermediate receptacle is encompassed by the heating element, supporting and adjusted by a thermostatic control, adapted for external adjustment. The inner receptacle is an aluminum insecticide cup measuring approximately 8 cm. inside diameter and 5 1/2 cm. deep with a holding capacity of about 150 grams of DDT. It fits snugly into the intermediate receptacle.

To obviate any undue variation in the heating of the DDT, the same Aerovap, adjusted to 130° C., was used throughout.

Exposure chamber. The exposure chamber was a cube-shaped enclosure with inside measurements of 8 x 8 feet square and 7 feet 9 3/4 inches high. It was constructed of 2 x 4 inch wooden studding and 1/4 inch plywood. The studding was on the outside as framing and plywood on the inside thus forming a smooth inner surface, painted white. The chamber provided an exposure volume of approximately 500 cubic feet. Access to the chamber was by means of a door in the center of the front wall. The Aerovap unit was mounted in the center of the rear wall so that the

surface of the insecticide cup was 42 inches from the ceiling and 56 inches from the floor. This location was found (Spear, 1950) to be the most generally effective for treatment of house flies with the Aerovap.

The chamber was ventilated both before and after any individual exposure by a motor-driven exhaust fan which drew fresh air to the exposure chamber from outside the building and then exhausted the same through an outlet port. The inlet and outlet ports, located at the lower left rear and upper right front corners, respectively, of the exposure chamber were closed by sliding metal doors during exposure periods. These were opened during ventilation of the chamber.

Cages. The cages used for exposure of the flies to insecticidal applications were constructed (Spear, 1950) of tarlatan cloth machine-sewn into a rectangular bag of approximately 6 1/2 x 6 1/2 x 14 inches. On the end that would correspond to the open end of the bag a piece of corrugated cardboard, 6 1/2 x 6 1/2 inches, was stapled. This cardboard end was cut so as to allow an opening in the form of a door in the inner portion of the cardboard. Through this door flies and food were easily placed in the cage for the aging period. At the completion of the aging

period and prior to subjection of the flies to insecticidal exposure, the food dish and jar containing the empty pupal cases were removed. The flies were thus rendered available for insecticidal exposure without undue disturbance or injury.

TOXICANTS.

The chemical compounds employed as toxicants (Fig. 1) included DDT and some of its closely related analogs. The DDT, 2, 2 bis (p-chlorophenyl) 1, 1, 1 trichloroethane, was of aerosol grade, containing not less than 98 per cent of the p,p' isomer, with a melting point of not less than 103° C.

The di-fluoro analog, 2, 2 bis (p-fluorophenyl) 1, 1, 1 trichloroethane, conveniently referred to as DFDT, was used as an insecticide in Germany during World War II under the name Gix. It has been reported (Metcalf, 1948a) to be equivalent in toxicity to DDT but to be more rapid in effecting mortality. Because of greater volatility its residual action is far less than that of DDT. High cost of manufacture has been an inhibiting factor in its wider use. The material used was of technical grade.

The TDE analog, 2, 2 bis (p-chlorophenyl) 1, 1 dichloroethane, was synthesized by German workers during

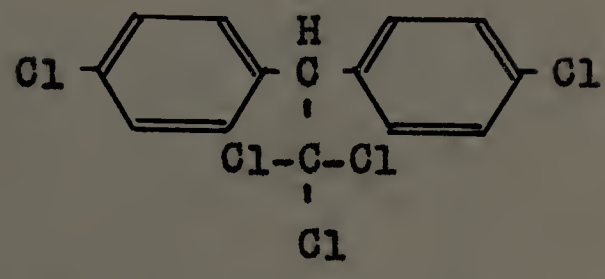
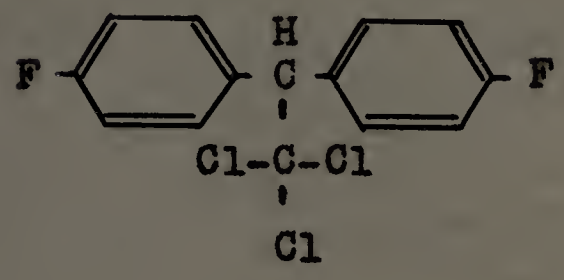
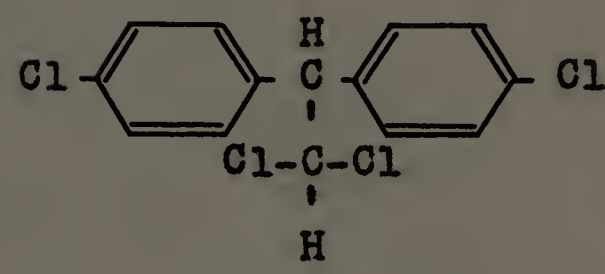
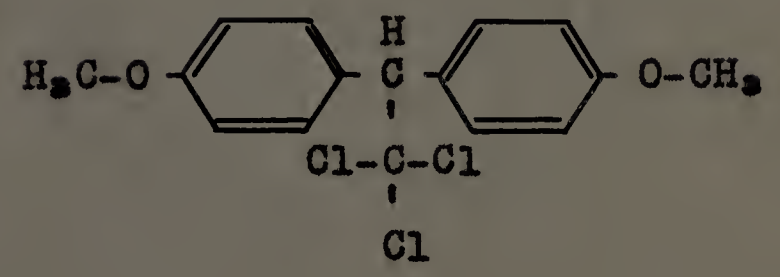
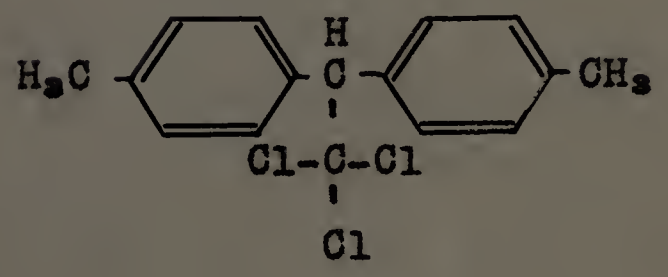
World War II and designated as Me 1700. This compound had been referred to as the DDD analog of DDT until recently but is now properly designated as the TDE analog (from the generic name tetrachlorodiphenylethane). According to German reports (Frear, 1948) it is approximately equal to DDT in toxicity. According to Ebeling (1950) it compares favorably with DDT against many pests. Metcalf (1948b) reported DDT and DFDT to be more toxic to insects than TDE. The aerosol grade was used.

The di-methoxy analog, 2, 2 bis (p-methoxyphenyl) 1, 1, 1 trichloro ethane, (also designated as 2, 2 di-p-anisyl- 1, 1, 1 trichloroethane), is more commonly known as methoxychlor. When used as a contact insecticide against house flies (Pril et al, 1945) methoxychlor gave a greater knockdown than the p,p' DDT but effected much slower mortality. These findings were supported by Bishopp (1946). Technical grade methoxychlor consisting of 88 per cent methoxychlor and 12 per cent related compounds was used.

The di-tolyl analog, 2, 2 bis (p-tolylphenyl) 1, 1, 1 trichloroethane, was found somewhat toxic to the larvae of the codling moth, Carpocapsa pomonella L., by Siegler and

Gertler (1944) but inferior to p,p' DDT. These workers found that substitution of a methyl group or of a methoxy group in the p,p' position in the diaryl trichloroethane ring resulted in a compound highly toxic to codling moth. A technical grade of the material was used.

FIGURE I

Compound tested	Structural formula
DDT 2, 2 bis (p-chlorophenyl) 1, 1, 1 trichloroethane	
DFDT analog 2, 2 bis (p-fluorophenyl) 1, 1, 1 trichloroethane	
TDE analog 2, 2 bis (p-chlorophenyl) 1, 1 dichloroethane	
Di-methoxy analog 2, 2 bis (p-methoxyphenyl) 1, 1, 1 trichloroethane	
Di-tolyl analog 2, 2 bis (p-tolylphenyl) 1, 1, 1 trichloroethane	

AEROSOLS.

The term aerosol, as suggested by the physicist F. G. Donnan (Roark, 1942) denotes a system of particles of ultramicroscopic size dispersed in a gas. In a broad use of the term Roark refers to an aerosol as a suspension of fine solid or liquid particles in air or gas. Ebeling (1950) considers aerosol as a generic term for the dispersal in air or other gas of solids or liquids in colloidal particle size. According to Johnstone (1950) aerosols are suspensions of finely divided particles in the air in which the rate of fall is small compared to the Brownian movement or turbulent diffusion. All solid and liquid particulates in the range of 0.01 to 1.00 microns in diameter are included. In each of the foregoing the term aerosol is to be construed as including the entire mixture of air and liquid and solid particles suspended therein rather than the particles or parent substance thereof exclusive of the air.

The physical properties of aerosols represent an intermediate state of matter devoid of the molecular properties of gases and the macroscopic properties of liquid and solid continua (Johnstone, 1950).

The actual deposition of the aerosol particulate is a source of variance. Because of the extremely small

mass of individual aerosol particles, their momentum in an air stream, according to Johnstone (1950), is very small. The particles tend to follow the stream line of the gas and circumvent an obstacle, deposition of small particles taking place with very low efficiency. Deposition efficiency depends on such factors as the diameter of the particle, the velocity of the gas stream, the density of the particle, the viscosity of the gas, and the dimension of the obstacle.

When the concentration of an aerosol increases, the restricted visibility concurrent therewith is due to a scattering of light. When the particles are submicroscopic and smaller than the wave length of light (Johnstone, 1950) their presence is manifested by a bright spot in a beam of light. The amount of light that is scattered depends not only on the concentration or number of particles per unit volume but also on the particle size and refractive index of the material. The number of particles per unit volume of aerosol is large although the total concentration in comparison with gas contaminants is very low.

According to Johnstone (1950) aerosols exhibit the property of coagulation in which the aerosol particles are brought together and coalesce. The rate of such

coagulation is proportional to the square of the concentration and almost independent of the particle size. The effective half-life of an aerosol depends largely on the concentration.

In aerosols of liquid droplets the smallest tend to evaporate rapidly, the large drops tending to grow at the expense of the small ones. In the case of a hygroscopic aerosol an increase in the relative humidity increases the particle size of the aerosol and stabilizes the aerosol. Solid particles may serve as nuclei for hygroscopic aerosols.

Because of the impact of air molecules, aerosols are in continuous movement, even in a quiet atmosphere, because of Brownian movement or diffusion. Such particles settle at an extremely slow rate, an atmosphere devoid of any particles being unattainable.

Johnstone (1950) describes electrical properties as another characteristic of aerosols. The particulates exhibit the ability to take on an electrical charge, and once having been charged, to be attracted to a pole of the opposite charge.

EXPERIMENTAL PROCEDURE AND DATA

EGGS.

Various workers have intermittently reported upon the efficacy of different toxicants as ovicidal treatments for the control of insect pests. Certain of these data evaluate compounds in terms of effectiveness against eggs of the house fly. However the relatively recent development of DDT resistance in the house fly probably has not afforded sufficient time for appraisal of any degree of resistance in the egg stage. As far as known no data are available depicting tolerance or susceptibility to insecticides in eggs of resistant strains in comparison with the eggs of normal strains. A consideration of this aspect of the resistance problem evolves into the concern of the initial phase of this study whereby it becomes fundamental to this study to ascertain whether eggs of DDT resistant strains of house flies exhibit any greater tolerance to DDT than eggs of normal strains.

Many of the reports pertaining to DDT resistance have either clearly expressed or otherwise inferred that the presence of DDT resistance is often accompanied by a resistance to some of the analogs of this compound. Other

reports (Barber and Schmitt, 1948) indicate the development of a specific resistance for DDT. A third group of data (Blickle et al, 1948) describe resistance for the conditioning toxicant as well as a lesser resistance for unrelated toxicants. In view of the diversified nature of these data it appeared advisable to include certain of the analogs of DDT in these tests in order to determine whether differential resistance to DDT and not its analogs might be expressed in the egg stage. It is not the purpose of this work however to establish lethal dosage levels for the toxicants concerned but rather to determine the presence or absence of resistance in the egg stage as above described.

Procedure. In 1935 Breakey and Miller described a procedure for comparing the relative ovicidal properties of different contact insecticides. Eggs of the blowflies, Phormia regina Meig., and Lucilia sericata Meig., Angoumois grain moth, Sitotroga cerealla Olivier, Mediterranean flour moth, Ephestia kuehniella Zeller, and short-tailed mealy bug, Pseudococcus citri Risso, were treated with various toxic materials under equivalent conditions of environment. The procedure provides for the relative evaluation of the ovicidal effect of each

material used. The technique developed in this study is a modification of the foregoing procedure adapted to a more expedient evaluation of the toxicants under consideration.

At the beginning of each ovicidal test a 9 cm. filter paper was placed in each of a sufficient number of 9 cm. petri dishes to provide duplicate dishes for each strain of flies tested as well as for each chemical compound being considered. This sheet of filter paper aided materially in absorbing the excess toxic solution from the eggs and aided more rapid drying. A second sheet of filter paper was scored with parallel lines one-half centimeter apart and then scored into quadrants and placed over the first sheet in the petri dish.

Five cc. of a 10 per cent solution in acetone of DDT and of each of the analogs considered (Fig. 1) were placed in glass vials of 10 cc. capacity, duplicate vials being prepared for each material for each strain. A solution of 0.01 per cent rotenone in acetone (Jones and Davidson, 1931, and Davidson and Jones, 1931) was used as a basis of comparison. Controls consisted of acetone-treated, and untreated eggs processed in a similar manner.

All eggs used were less than twenty-four hours old. By removing the feeding dish from each of the culture

cages and replacing it with fresh food from twelve to twenty-four hours before the tests were initiated, eggs of the desired age were obtained. In order to secure a randomized sample of eggs from any particular strain, representative portions of all egg masses in the feeding dish were removed from the crumpled paper toweling with a pair of forceps and placed in approximately 10 cc. of distilled water in a centrifuge tube. The masses of eggs were separated by gentle agitation and then allowed to settle. Those eggs and other extraneous matter which remained floating were removed from the water and discarded. When the eggs had settled in the centrifuge tube they were easily removed by a medicine dropper of uniform bore, measuring 0.10 cm. inside diameter. The tip of the dropper was extended below the surface of the water until it just touched the settled eggs. By carefully releasing the constricted bulb a rather uniform volume of eggs was withdrawn from the water into the dropper. The dropper was then held in an upright position to permit the eggs to settle to its tip. By careful constriction a desired volume of eggs with a relatively insignificant amount of water was emitted to the solutions of toxicants in each of the 10 cc. vials.

The eggs were gently agitated in each vial to assure contact of the toxicant with the complete surface of the eggs and were then allowed to settle. Eggs were immersed in the toxic solutions in this manner for from fifteen to thirty seconds. Removal of the eggs was accomplished as above described for withdrawing the eggs from the distilled water. As a precaution against contamination different droppers were used for each of the toxicants. After the eggs had settled in the dropper they were extruded to each of the four quadrants of the scored filter paper in the petri dish. Petri dishes were left open for approximately one hour following deposition of the eggs to allow evaporation of the solvent. After complete drying had been accomplished one cc. of water was distributed over each of the treated egg masses to bring about a more even dispersal of the eggs over the filter paper, such dispersal greatly facilitating subsequent differentiation between hatched and unhatched eggs. Because of the relative insolubility of DDT and its analogs and of rotenone (1 part in 6,000,000) in water this procedure did not visibly affect the actual deposit of these toxicants upon the egg surface.

The moisture content of each petri dish was maintained at a level sufficient to obviate excessive dessication by placing a small piece of water-moistened absorbent cotton in the petri dish apart from the eggs. The period of incubation for the untreated controls was taken as the criterion by which the effectiveness of each of the treatments was assayed. When normal hatching had occurred in these controls, hatched and unhatched eggs in all dishes were counted. The effectiveness of the treatments was computed in terms of per cent hatched.

Tests in 1949. During the summer of 1949 eggs of the California, New Hampshire, and Laboratory strains were treated as above described. A culture of Rohm & Haas flies which had been reared in accordance with NAIDM specifications was included in the foregoing tests to serve as an adjunct to the Laboratory culture, and to assist in comparatively determining whether chemical resistance had become established in the Laboratory strain. This test was replicated four times using approximately 100 eggs from each strain for each treatment (Table I).

The percentage of eggs that hatched following treatment with DDT or with its analogs is not strikingly different one from the other. A possible exception to

this similarity however occurs in the eggs of the New Hampshire strain treated with the di-fluoro analog and with TDE. Since these variations were not repeated in tests conducted in 1950 they are not considered of importance.

Tests in 1950. During the summer of 1950 a second series of tests was conducted to further evaluate the ovicidal effect of these toxicants upon the previously tested strains as well as upon strains acquired since the 1949 tests. During this interim the Illinois and New York strains had been acquired. Data from exposure of adults of the Rohm & Haas strain and Laboratory strain to DDT indicated no particular difference between the two strains in tolerance to DDT. On the assumption then that the Laboratory strain was devoid of any chemical resistance, particularly to DDT, that would interfere with the execution of this study, the Rohm & Haas strain was omitted from further consideration in this problem.

Approximately 200 eggs per strain per treatment were used in these tests, four replicates being used. As in the 1949 tests, percentage of hatching was used as the criterion (Table II) by which treatments were evaluated.

No particular difference could be detected in percentage of hatching effected by the different controls and those eggs treated with DDT or with its analogs. Treatment with an acetone solution of rotenone induced a mortality in approximate agreement with results obtained by other workers (Jones and Davidson, 1931; Davidson and Jones, 1941; Richardson, 1943).

Table I

Per cent Hatching of House Fly Eggs

Treated with DDT and Some of its Analogs

Compound tested	Strain Rohm & Haas	Calif.	N. H.	Lab.
DDT	93.06	92.02	95.00	93.79
Di-fluoro	94.06	92.34	79.55	95.08
TDE	89.60	86.75	77.42	93.33
Di-methoxy	82.09	91.09	93.47	93.88
Di-tolyl	93.63	94.76	99.07	96.71
Rotenone (.01 per cent in acetone)	8.04	7.32	7.94	7.63
Acetone	92.08	93.79	91.58	91.96
Water	91.78	94.06	93.25	90.20
Untreated	94.58	93.45	91.03	91.65

Maximum variation 3.25 per cent

Table II

Per cent Hatching of House Fly Eggs

Treated with DDT and Some of its Analogs

Compound tested	Strain Ill.	Calif.	N. Y.	N. H.	Lab.
DDT	91.00	92.24	92.24	89.39	90.08
Di-fluoro	92.79	92.54	92.32	93.81	94.23
TDE	93.63	91.54	92.06	91.41	90.72
Di-methoxy	91.18	89.46	90.75	92.62	89.79
Di-tolyl	89.32	91.11	88.65	89.89	89.49
Rotenone (.01 per cent in acetone)	9.24	8.64	9.67	7.98	8.43
Acetone	92.75	91.19	91.04	92.47	91.09
Water	91.02	90.84	93.01	95.45	94.28
Untreated	91.92	93.43	90.74	91.30	92.67

Maximum variation 3.50 per cent

LARVAE.

The successful use of DDT for control of noxious insect pests inimical to man and plants was soon extended to control of the incessantly abundant house fly. First efforts were directed toward control of the adult and resulted in exceptional success now well known to all. Cognizant of the important part played in control of this pest by sound sanitation practices it was logical that the larvicidal effectiveness of the toxicant be determined as a possible replacement for existing measures or as an adjunct thereto. Many reports are available depicting both success and failure of DDT as a larvicide for the house fly.

Olson and Dahms (1945) obtained practically no larvicidal effectiveness from an emulsion of 5 parts of a 5 per cent DDT in kerosene to 10 parts of Triton 770 and 85 parts of water mixed with dewatered sewage sludge or applied to the surface thereof. A 10 per cent dust similarly applied although ineffective as a larvicide prevented the emergence of most of the adults from the puparia. The few adults which did emerge died within two hours. Shaw and Bourne (1946) found DDT non-effective as a larvicide when applied to maggot-infested litter.

Other workers have found DDT to be effective as a larvicide. McGovran et al (1944) reported that when 0.025 per cent and 0.013 per cent by weight of DDT was mixed with the feeding media it caused 88 per cent and 77 per cent mortality, respectively. Simmons and Wright (1944) sprayed manure piles infested with maggots with a 0.25 per cent emulsion. Each pile contained approximately 16 cubic feet of manure and was treated with eight to ten gallons of spray or at the rate of 0.6 gallons per cubic foot. No flies had emerged at the end of 18 days. Control was rated as excellent.

From the foregoing observations it is obvious that considerable controversy exists as to the actual value of DDT as an effective larvicide against the house fly. In view of this apparent lack of agreement the purpose of the present portion of this study becomes two-fold: (1) to determine the relative effectiveness of DDT and its analogs as larvicidal applications against both the resistant strains and the laboratory strain, and (2) to ascertain whether the presence of DDT resistance in the adult fly in any manner affects the tolerance to DDT of the larval stage.

Procedure. Residual tests were conducted by adding one cc. of a 10 per cent solution in acetone of technical DDT and of its analogs to a nine cm. filter paper suspended on three needle points (correspondence with R. L. Metcalf, dated 23 May 1950). The solution spread evenly over the entire surface by capillarity, leaving a known amount of deposit quite evenly distributed. When completely dry the filter paper was placed in a nine cm. petri dish (March and Metcalf, 1949). Food and moisture were provided by placing a small piece of absorbent cotton, moistened with diluted evaporated whole milk, in the center of the filter paper. Twenty-five two-day old larvae were placed in the petri dish and covered. Duplicate dishes were prepared for each strain, all dishes being kept in the constant temperature and relative humidity chamber.

Pupae were removed daily, as formed, from the treated filter paper and placed in clean petri dishes. The per cent ultimate survival was computed (Table III) on the basis of the number of adults which emerged from the puparia and lived 48 hours or longer thereafter. The test was replicated eight times.

A consideration of these data from the aspect of the relative effectiveness of DDT and the analogs shows the di-fluoro analog to be the most toxic, the average survival

of all strains treated therewith being 51 per cent. In contrast DDT indicated the least larvicidal action, the average survival of all strains thereto being 65.25 per cent. TDE, methoxychlor and the di-tolyl analog are all intermediate in effectiveness between the di-fluoro analog and DDT. The respective survival averages of all strains treated with these compounds were 59.30, 58.70, and 59.20 per cent. There is little variation in tolerance to the different materials expressed by the laboratory strain. Exposure to all compounds results in just over 50 per cent mortality or, as in the case of the di-tolyl analog, just 50 per cent mortality.

An appraisal of the above data (Table III) from the aspect of comparative tolerance displayed by larvae of the resistant and non-resistant strains indicates a survival gradient. The greatest degree of resistance is displayed by the Illinois strain, followed in decreasing magnitude respectively by the California, New York, New Hampshire, and Laboratory strains. The average tolerance to all materials exhibited by the Illinois strain is 34 per cent greater than that of the Laboratory strain, whereas the tolerance of the California and New York strains exceeds that of the Laboratory strain by 20 and 11 per cent, respectively. The average tolerance of the New Hampshire strain is but slightly in excess of that of the Laboratory strain.

Table III.

Per cent Survival of Larvae Exposed to Surfaces Treated with DDT and Some of its Analogs

Compound tested	Strain Ill.	Calif.	N. Y.	N. H.	Lab.
DDT	86.25	75.00	65.00	52.50	47.50
Di-fluoro	68.00	55.00	50.50	44.00	41.00
TDE	81.50	65.00	57.00	50.50	42.50
Di-methoxy	87.00	64.50	52.50	45.50	44.00
Di-tolyl	74.50	65.50	56.00	50.00	50.00
Acetone	95.00	93.50	92.50	94.00	93.00
Water	95.00	94.00	94.25	94.00	94.25

Maximum variation plus or minus 7.00 per cent

PUPAE.

The effectiveness of DDT or of its related analogs as a control measure for the pupal stage of the house fly is not of particular economic importance. Control measures designed for pupal control alone would not be feasible. However Olson and Dohms (1945) and McDuffie (1946) have reported that residual applications of DDT on house fly puparia have inhibited the successful emergence of the adults to such an extent that only a few actually emerged from the puparia and a comparatively small number lived more than a few hours after emergence.

Studies of the comparative tolerance of the pupal stage of each of the house fly strains to the toxicants considered have been included in order to ascertain whether or not a strain of house fly adults exhibiting resistance to one or more of these compounds would display this same resistance or correlated resistance in the pupal stage of their progeny.

Procedure. Three-day old pupae from each of the strains were selected for uniform and representative size. The average weights of these pupae, computed from samples of 200 pupae for each strain, were as follows:

Illinois	22.3	mg.	per	pupa
California	21.4	"	"	"
New York	20.7	"	"	"
New Hampshire	17.6	"	"	"
Laboratory	23.9	"	"	"

Ten cc. of a 10 per cent solution in acetone of DDT and of each of the materials considered was placed in a 25 ml. test tube, duplicate tubes being prepared for each material for each strain.

Twenty-five pupae for each treatment were placed in a cylindrical number 12 mesh wire container measuring approximately 1 cm. in diameter and 5 cm. in height. The uppermost end was hinged on a wire loop to permit easy and rapid opening or closing with the point of a dissecting needle. The container was attached to the end of a piece of nickel-chrome wire about 20 cm. long.

Pupae for a particular treatment were placed in the container, the cover closed, and immersed for 15 to 20 seconds in the appropriate test tube of toxicant. Upon removal from the toxicant the pupae were gently shaken in the basket to remove any excess toxic solution and then emptied on a 9 cm. filter paper in a 9 cm. petri dish. After the solvent had completely evaporated the filter paper

was moistened with 1 cc. of distilled water and covered. As adults emerged they were collected in tarlatan cloth cages, fed on diluted evaporated whole milk with which absorbent cotton had been moistened, and observed daily. Only those adults which survived for 48 hours or longer following emergence were considered to have successfully emerged. This test was replicated eight times at irregular intervals over a two months period, a total of 400 pupae from each strain being used. The effectiveness of the various compounds was computed (Table IV) in terms of average per cent emergence.

Marked toxicity by DDT and its analogs to the pupal stage of all strains is apparent. No appreciable difference is noted however in the response of the various resistant strains to any particular treatment. All resistant strains show a higher percentage of emergence and consequently a higher level of tolerance to these compounds than the Laboratory strain.

A comparison of the relative toxicity of the various compounds clearly places the di-fluoro analog apart from all others as the most toxic. All resistant strains are more susceptible to this material. The Laboratory strain shows a marked susceptibility to the compound far in excess of that to any other compound.

Per cent emergence by pupae of the New Hampshire strain is comparable to that of other strains irrespective of the treatment rendered. Since the New Hampshire strain is primarily a BHC resistant strain with a presumably low secondary level of resistance to DDT, the high degree of resistance to DDT and analogs thereof herein displayed is not clearly understood. It is reasonable to expect that the presence of DDT resistance in the Illinois, California, and New York strains might simultaneously foster a degree of tolerance to these analogs of DDT. Several investigators have reported that DDT resistance is accompanied by a lesser resistance to some of the analogs of this compound. Whether or not the factors governing the expression of resistance to BHC are in any manner associated with resistance to DDT is largely conjecture. However in the absence of any directly related data, it seems logical to assume that the presence of the factors for BHC resistance induces a resistance for other toxicants, in this instance DDT and the analogs considered.

There appears to be but little difference in the effectiveness of the di-tolyl, di-methoxy or DDT compounds, their relative toxicities being below that of the di-fluoro analog, decreasingly in the order named. TDE appears to be the least effective.

March and Metcalf (1949) reported that the enormous differences in the susceptibility of the various fly strains to residual applications of DDT were not due to variations in the weights and vigor of the flies. A comparison of the data above (Table IV) with the average weights of the pupae indicates that no striking correlation apparently exists between pupal weight and susceptibility of the emerging adult to residual applications of DDT and some of its analogs.

Table IV

Per cent Emergence and Survival for 48 Hours from House Fly Puparia Treated with DDT and Some of its Analogs

Compound tested	Strain Ill.	Calif.	N. Y.	N. H.	Lab.
DDT	64.25	65.00	63.75	65.25	51.50
Di-fluoro	51.50	51.00	50.75	53.00	20.00
TDE	68.50	66.00	67.00	67.00	52.00
Di-methoxy	60.50	62.25	62.00	64.00	54.50
Di-tolyl	61.50	59.00	58.00	60.00	42.75
Acetone	92.00	91.25	93.25	92.25	92.00
Untreated	97.00	97.25	96.50	96.75	95.75

Maximum variation plus or minus 5.50 per cent

ADULTS.

Depiction of DDT resistance in the house fly has, from all available data, been limited to the adult stage of the insect and more specifically to its ability to withstand dosages of the toxicant which previously had effected a satisfactory degree of control. When it is realized that DDT is perhaps the most nearly universal insecticide ever employed it is not surprising that the gradual development of resistance thereto by the house fly should be followed by reports of diversified nature and from widely scattered corners of the world.

A range of resistance to DDT varying from twice to over one thousand times that of the respective laboratory strains concerned has been reported. An expression of these multiples in terms which might be compared to the susceptibility of the Laboratory strain herein considered however becomes impracticable. The varying degrees of vigor and susceptibility inherent in the different Laboratory strains used as a basis of comparison as well as the wide variety of techniques employed by the various investigators have contributed in greater or lesser magnitude to the variations encountered.

Locations of resistant house fly populations have remained quite isolated from each other. Surrounding

populations of non-resistant flies, devoid of any known degree of resistance to DDT or other chemicals, may thus be presumed to intermingle with the resistant populations and to effect cross breeding. The effect on tolerance thus induced has been reported (Bruce, 1950) as attenuant. Decker (1950) has alleged that tolerance is progressively reduced with each generation of cross breeding but has not offered substantiating data.

Continued use of DDT could conceivably foster, within certain limits, an increase in the incidence of resistant populations. The concomitant decrease in frequency of non-resistant populations that might ensue would then increase the probability of cross breeding between resistant populations. Whether such inter-breeding should be termed cross breeding is questionable. Data are not available to indicate the effect on tolerance induced by such crosses.

As a partial consequence to the curtailed use of DDT brought about by the resistance problem, other chemicals have been gradually adopted as control measures. Instances have been reported wherein resistance to these newer chemicals has presumably developed. The existence of populations with resistance to chemicals other than DDT is to be recognized. Cross breeding between two such

populations, each with an expressed resistance to a different chemical, is to be anticipated. Again data are not available to describe the effects on tolerance of the progeny brought about by such crosses.

A consideration of the foregoing digest of some of the more generalized aspects of DDT resistance in the house fly resolves into consideration of the following as objectives of the present portion of this study:

A. To establish relative levels of tolerance for each of the strains considered;

B. To determine the duration and stability of resistance when resistant cultures are deprived of exposure to the conditioning toxicant;

C. To assay the effect on tolerance induced by successive generations of cross breeding between resistant and non-resistant strains and between two resistant strains;

D. To evaluate the level of tolerance evolving from crosses between two resistant strains, each with expressed resistance to a different compound.

In general two theories have been advanced (Metcalf, 1948b) to account for the toxicological efficiency of DDT. Both lines of reasoning have developed from the Overton-Meyer theory (Meyer and Hemmi, 1935; Ferguson, 1939) which

correlates lipoid solubility (oil/water distribution coefficient of physiologically active materials) and activity. Despite the common origin of these two theories they are, in nearly all respects, in direct contrast to each other.

Läuger, Martin and Müller (1944) contended that two characteristics combined in the DDT molecule are responsible for the high insecticidal efficiency observed. The trichloromethyl group provides lipid solubility while the bis-(p-chlorophenyl)-methylene group constitutes the toxic portion of the molecule. Contrariwise Martin and Wain (1944) have contended that the p-chlorophenyl component accounts for the lipid solubility and that the trichloromethyl arrangement is actually accountable for the toxicity of the compound. Martin (1946) considers that the toxicity is a direct result of three properties combined in the molecule which (1) provide a means of penetration and concentration at the locus of action, (2) afford sufficient stability to reach the action site, and (3) provide for the release of hydrogen chloride when the compound becomes absorbed at the action locus. Martin has indicated that the effectiveness of certain alkoxy analogs of DDT is not adequately explained on the basis of the dichlorophenyl group being present.

Other theories have been brought forth in explanation of the action of DDT. Gavaudan and Poussel attribute (Metcalf, 1948b) the toxicity to an indifferent narcotic acting on a lipoidic substrate. Hurst considers (Metcalf, 1948b) that DDT and its analogs may be taken up and stored in the phospholipids of cell membranes resulting in an indirect blocking of cytochrome oxidase and succinic dehydrogenase. Hurst further suggests that a transformation from the narcotic to the lethal stage is associated with an irreversible decrease in lipoprotein stability.

Sex ratio. In consequence of numerous reports concerning the relative susceptibility of male house flies to insecticides as opposed to the comparative vigor of females, randomized samples of adults from each strain were examined for sex. Of approximately 1000 flies so examined from each strain, approximately equal numbers of each sex were found. The sex ratio is therefore considered 1:1, with a maximum observed variation of plus or minus 2 per cent.

Procedure. Feeding cups and glass jars containing empty puparia were removed from the tarlatan cloth cages

previously selected for insecticidal exposure. Additional cages similarly prepared were used as controls. Any cage containing more than two dead flies was discarded in order to obviate as much as possible the probability of accidental contamination and of its effects becoming expressed within the test results. Only flies uniformly 3 to 5 days old were used in these exposure tests, flies of the same age being used throughout each individual test.

The exposure chamber was ventilated by forced draft for at least one hour, usually longer, prior to each test, the ventilation ports being closed during each exposure period. The floor of the exposure chamber was covered with clean newspapers upon which the cages of flies were placed with the cardboard end downward. The cages were arranged in a Latin square design, usually of 5 columns and 5 rows.

The heating unit of the Aerovap was turned on by a switch outside of the exposure chamber. Observations of well temperature, room temperature and relative humidity were made at thirty minute intervals during the exposure period. In all exposure tests the length of the exposure period was computed from the time the Aerovap was turned on. Thus a period of approximately twenty minutes elapsed during the beginning of each test before the unit

reached a temperature of 130° C. A variation of plus or minus 1.0° C. was observed in the well temperature of the Aerovap unit. Room temperature and relative humidity varied somewhat in accordance with outside conditions.

After the exposure period was completed the cages of flies were removed to an uncontaminated area and placed on clean newspapers. The flies were fed with a sugar and water solution absorbed on a crumpled paper towel. Mortality counts were made 24 hours after the beginning of the exposure period. Per cent mortality was computed according to the method described by Abbott (1925).

Mortality versus weight of DDT evolved. In order to obtain a relative level of tolerance to DDT for each of the strains which could be used as a basis of comparison with subsequent exposures, a series of ten tests, replicated five times, was conducted. Flies were exposed to DDT as a continuous phase aerosol evolved from the Aerovap unit for varying periods of time ranging from 30 minutes to five hours. Each test consisted of 25 cages of flies, 5 cages being used for each strain.

The average amount of DDT evolved during the different exposure periods increased at an approximate rate of 0.02 grams per 30 minutes of increased exposure. The average

amount of DDT dispersed in each of the exposure periods was plotted against the average per cent mortality produced in each strain (Fig. 2). Per cent mortality produced by 30 minutes exposure was negligible, there being less than 0.005 grams of toxicant evolved. This is understandable when it is realized that approximately 20 minutes are required to allow the heating unit of the Aerovap to produce a well temperature of 130° C. The remaining 10 minutes therefore is not a sufficient length of exposure time for the Aerovap to produce a concentration of aerosol adequate to effect an appreciable mortality.

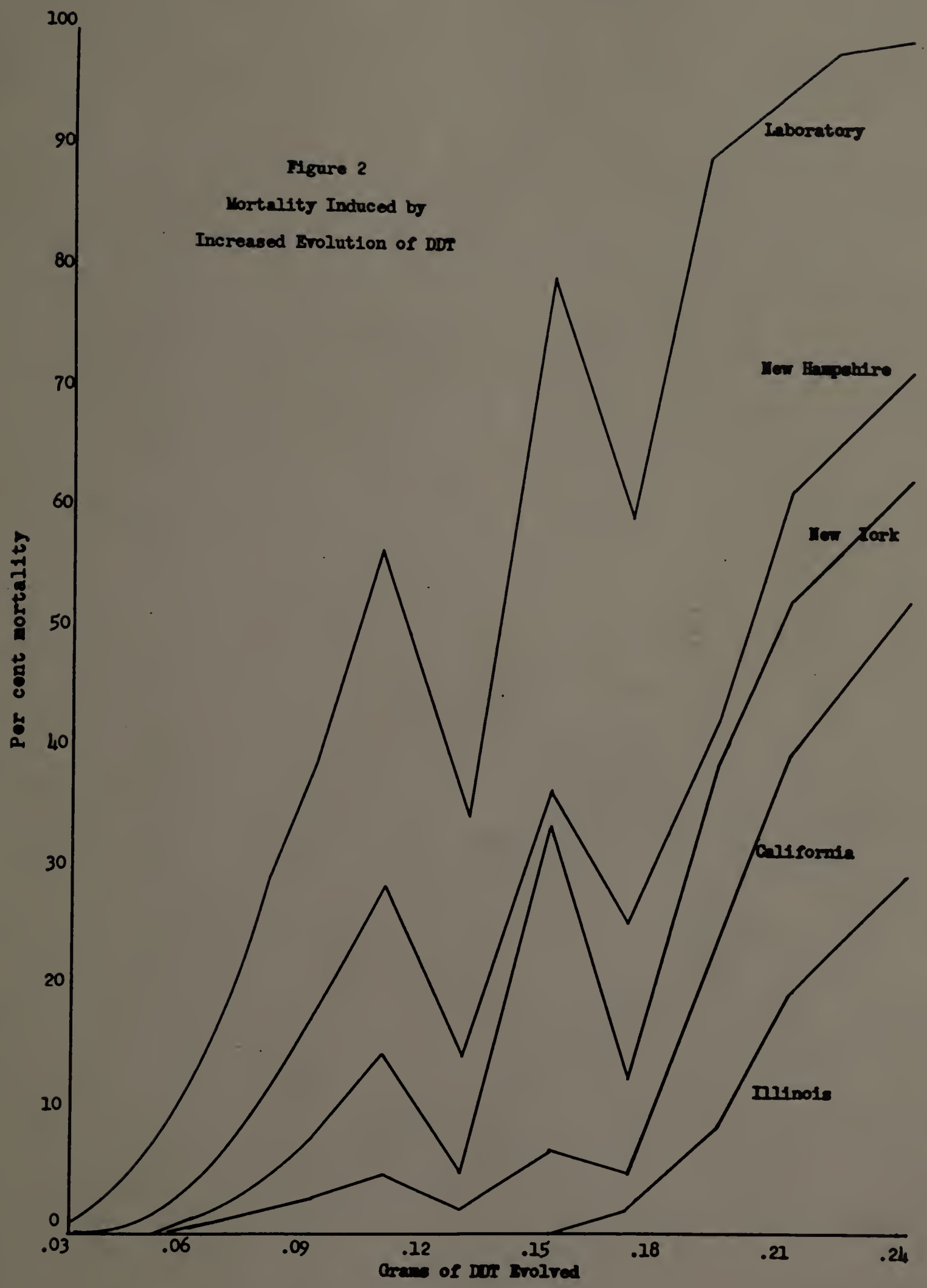
A consideration of these data clearly shows that an increase in the respective weights of DDT evolved during different exposure periods does not necessarily induce increased mortality. There is however a general tendency for the respective weights of DDT dispersed to coincide with an increase in mortality. The distribution of weights dispersed and per cent mortality produced approach the pattern of a normal mortality-concentration curve. There are however fluctuations within this mortality-dispersion distribution which render it unreliable for use as a standard of comparison for subsequent exposures.

It is to be noted that the average per cent mortalities produced at the o.19 gram dispersal level were

Figure 2

Mortality Induced by

Increased Evolution of DDT



approximately 90 per cent and 8 per cent for the non-tolerant laboratory and the highly resistant Illinois strains, respectively. The California, New York, and New Hampshire strains displayed intermediate levels of relative tolerance, decreasing respectively in the order named. The evolution of 0.19 grams of DDT was effected in approximately 4 hours of exposure.

This lack of closer correlation between increased weights of DDT evolved and per cent mortality produced is probably ascribable to a complex of biological, physical and chemical factors rather than to either of these factors acting alone. The age of the flies, although constant throughout each individual exposure, is nevertheless a potential source of variation. The vigor of flies is known to vary from day to day, temperature and relative humidity not being the least of the contributing factors.

Insects are all poikilothermic, devoid of any precise mechanism for regulating body temperatures. Their body temperatures therefore follow closely the temperatures of the surrounding medium. Within limits, the higher the temperature the more rapid becomes the rate of metabolism. However the many physical and chemical reactions involved in metabolism are so complex that any expression of the

nature of the effect of temperature upon these biological processes would of necessity be limited and specific, not general.

DDT has been shown to increase the metabolism of insects and deplete reserves, probably by an overstimulation from the central nervous system. According to Welsh and Gordon (1947) the primary action of DDT in insects is probably increased afferent discharge, leading to hyperactivity and reflex incoordination. It is likely that the continuous activity of the nervous system in DDT-intoxicated insects causes metabolic exhaustion and death. This occurs very slowly and tremors often persist for 24 to 48 hours before the onset of paralysis. At low temperatures tremors may continue for weeks before death occurs. The low susceptibility of various species of insects to DDT may be partly due to greater stability of the peripheral nerve fibers in such insects or to differences in the tendency of the exoskeleton to allow DDT to accumulate. This latter action must precede that of the toxic nerve action.

In summation of the foregoing and in view of Johnstone's (1950) discussion of the properties of aerosols these fluctuations in the mortalities effected (Fig. 2)

are considered induced by environmental factors affecting either the vigor of the test organism or the efficiency of toxicant deposition or both.

Mortality versus length of exposure period. In view of the lack of correlation in the preceding tests between per cent mortality produced and weights of DDT evolved, further tests were carried out to compare the effect of length of the exposure period upon per cent mortality produced. Flies were subjected to ten different exposure periods ranging in length from 30 minutes to five hours. As in the preceding series, 25 cages for each exposure and 5 cages for each strain were used. The average per cent mortality produced in each strain was plotted against the length of the respective exposure periods (Fig. 3).

The mortality produced by the 30 minute and one hour exposure periods was too low to be of value in this work. The laboratory strain displayed an average mortality of about 17 per cent at the 30 minute exposure level and about 36 per cent at the one hour exposure level. None of the resistant strains displayed any mortality at the 30 minute exposure level. At the one hour exposure level the mortality of the resistant strains was less than 10 per cent. Because of this low mortality and the accompanying

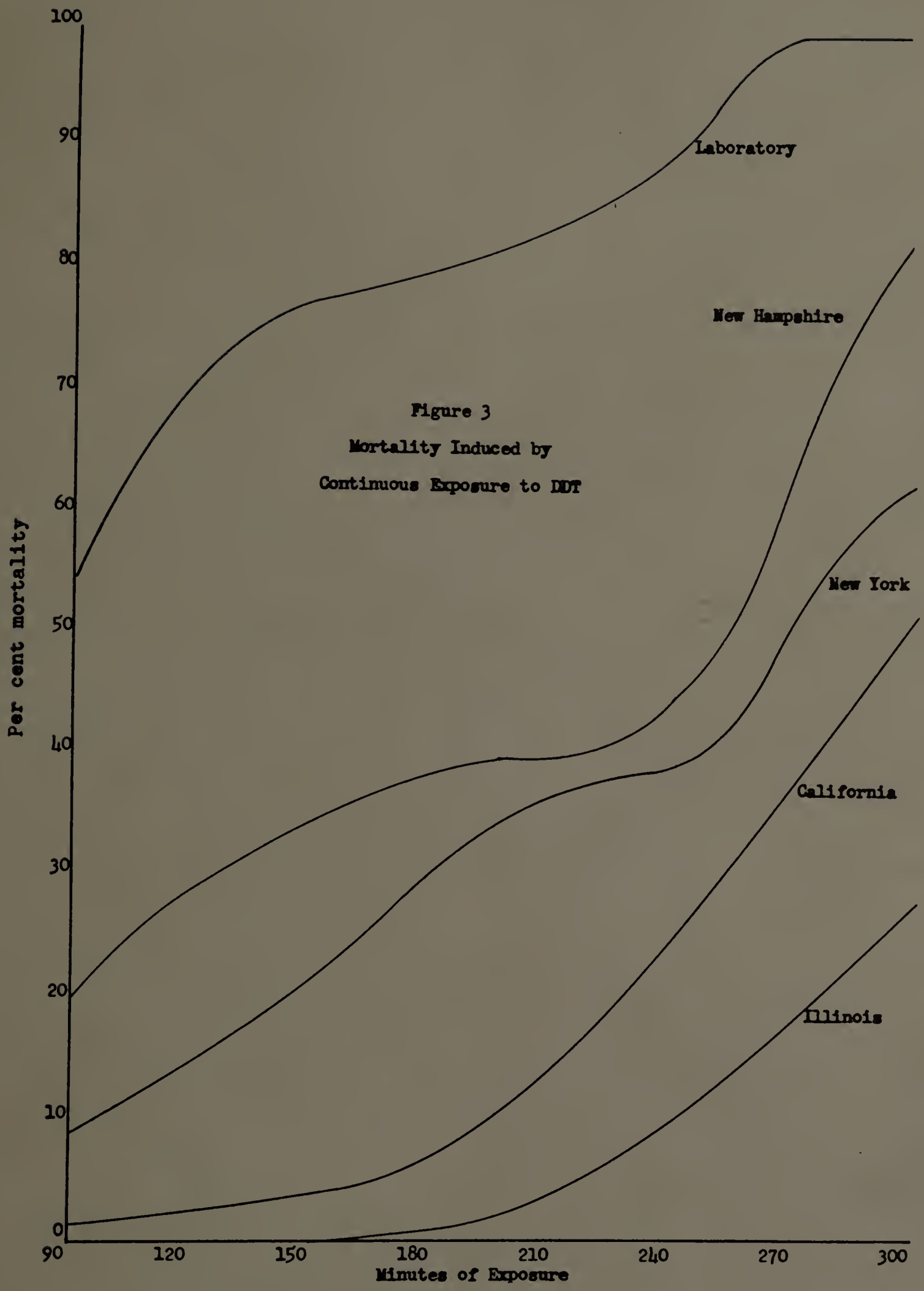


Figure 3

Mortality Induced by

Continuous Exposure to DDT

uncertainty of clear differentiation between the tolerance of the resistant strains, these data are omitted from Figure 3.

The data for the exposure periods ranging from 90 minutes to five hours indicate that within broad limits a correlation exists between the average per cent mortality produced and the length of the exposure period. These results are in agreement with the findings of Spear (1950).

The average mortality levels produced by the four hour exposure period places the highest mortality, that of the non-resistant laboratory strain, at about 90 per cent while that of the lowest mortality, that of the most resistant strain, falls at about the 10 per cent level. The intermediate levels of tolerance, representative of the remaining strains, are well distributed between the upper and the lower levels of mortality. The maximum observed variation in mortality for the four hour exposure period was plus or minus 4.50 per cent while that of the remaining exposure periods was plus or minus 7.50 per cent. The relative tolerance of each strain is in agreement with the results obtained in the preceding tests. The weights of DDT evolved during the four hour exposure periods varied from 0.158 grs. to 0.237 grs. with a mean average dispersal weight of 0.194 grs.

In the preceding section the reasons advanced to partially account for the lack of correlation between per cent mortality produced and weight of DDT evolved are equally applicable to this series of exposures. Despite these factors the length of exposure period as a means of establishing relative levels of tolerance appears better adapted to the scope of this problem than does the weight of compound dispersed.

Relative levels of resistance. In order to more adequately establish levels of tolerance to DDT for each of the resistant strains additional exposures of 4 hours duration were carried out for all strains. The procedure was identical to that followed in the preceding section. Fifteen exposures were effected.

The average mortality produced in each strain was generally aligned with that effected by equivalent exposure periods in the preceding section. While the relative mortality of the California and New York strains was somewhat lower than previously observed (Figs. 2 and 3) that of the remaining strains showed but little deviation. The variation was plus or minus 5 per cent. The following average mortalities were observed:

Illinois	7.6 per cent
California	14.5 " "
New York	28.5 " "
New Hampshire	44.4 " "
Laboratory	94.0 " "

The amount of DDT evolved during these four hour exposure periods ranged from 0.146 grs. to 0.249 grs. with a mean average weight of 0.198 grs. dispersed.

The mortality produced in the laboratory strain was taken as 100 per cent and was divided by the mortality produced in each of the resistant strains. The quotient thus obtained is an expression, in terms of the non-resistant laboratory strain, of the relative tolerance of each strain. The following values, indicating the number of times greater the resistance of a particular strain to DDT is than that of the laboratory strain, were thus obtained:

Illinois	12.4
California	6.4
New York	3.3
New Hampshire	2.1

The foregoing values are at considerable variance with those reported by other investigators. Bruce (1950) reported the resistance of the Illinois strain (designated Multi-strain I by Bruce) to be much greater than his laboratory strain to both DDT and methoxychlor. In personal correspondence (letter dated February 23, 1950) he indicated this level to be approximately 1000 times greater than that of his laboratory strain for DDT. March and Metcalf (1949) reported the California strain (referred to as the Bellflower strain by March and Metcalf) as being in excess of 300 times more resistant than the laboratory strain. Comparable data are not available for the New York strain. The DDT resistance of the New Hampshire benzenehexachloride resistant strain was reported by Blickle (1948) to be twice that of his laboratory strain.

The variance between the magnitude of resistance reported by the foregoing workers and the degree of resistance herein observed may be ascribed to the complex effect of several factors. Despite the prevalence of uniform and standardized techniques in house fly rearing, the variations in environmental conditions which accompany this rearing in different laboratories undoubtedly become influencing factors upon the degree of resistance expression. Temperature and moisture optima probably are specific for

individual strains. Rearing several resistant strains, selected from different parts of the country, under the same conditions as the laboratory strain, while assuring uniformity of rearing conditions, nevertheless probably introduces an element of divergence from the optimum for any one resistant strain. The optimum conditions for any one resistant strain would not be expected to be the same as those of another resistant strain nor as those of the laboratory strain. It follows then that uniformity of rearing procedure for all strains concerned may conceivably favor the expression of resistance in one strain reared under conditions most nearly coinciding with its individual optimum requirements. Contrariwise the expression of resistance may be suppressed when a second strain is reared under identical conditions since these same conditions may be somewhat removed from the optimum of this second strain.

Whether or not the effect upon resistance expression induced by such variables as temperature and moisture is direct or indirect is mere speculation. Variations in the length of the life cycles (discussed later in this study) of different resistant strains and the laboratory strain would indicate the preference of some of the strains for

conditions other than those under which they were being reared. Such variation, if induced by conditions of temperature and moisture other than optimum, might well indirectly induce a variation in resistance to DDT. Upon this premise then the degree of resistance of a particular strain as reported by the above investigators might well differ from the resistance herein observed.

A second probable source of variation is represented by the comparative resistance or susceptibility of the various laboratory strains to DDT. The degree of resistance to DDT in the various resistant strains reported by the original investigators has been expressed in terms of a multiple of that of their particular laboratory strain. While it is fully appreciated that every possible precaution is employed to standardize fly rearing throughout different laboratories, nevertheless the potential of variation in vigor of the laboratory strains at different locations is always present.

Perhaps the greatest source of variation in these resistance levels may be attributed to the testing procedures employed. In the present study levels of relative resistance have been computed from mortality produced by exposure to DDT dispersed in continuous aerosol phase. When DDT is thus evolved from the American Aerovap under

the conditions of this investigation two physical states of the compound result. The air becomes saturated with the toxicant in vapor phase and simultaneously supports liquid particles of the compound derived from condensation of that portion of the vapor phase in excess of the saturation requirements.

The level of resistance in the Illinois strain was derived (Bruce, 1950) from mortalities produced by topical application of the toxicant to the thorax. March and Metcalf (1949) computed their tolerance levels in consequence of mortalities resulting from residual deposits, topical prothoracic applications and micro-chemical cervical injections. Data are not available depicting the technique employed by Barber and Schmitt (1948, 1949a, 1949b) for determining the resistance level of the New York strain. Blickle (1948) used a modification of the insect toximeter (O'Kane et al, 1941) whereby a known volume of spray under constant air pressure was directed from an artist's air brush toward a rotating cage of flies.

Differences in testing procedure such as just described would induce variations in the comparative levels of resistance of the strains concerned. A comparison of the resistance level of one resistant strain with that of any

other resistant strain on the basis of the results reported by these workers becomes impracticable. However since the primary concern of this section of the work is to establish relative resistance levels rather than lethal dosage levels, the foregoing average mortalities effected by the four hour exposure period will serve as standards of comparison.

Paired and mass crosses. In order to detect and properly evaluate in the progeny of subsequent crosses any variation in tolerance to DDT due to selection of parental stock, a number of paired and mass crosses were carried out, reciprocal crosses being simultaneously effected.

The marked resistance to DDT displayed by the Illinois strain favored its selection as one of the parental lines in these first genetic crosses to be considered. This high degree of relative resistance afforded a better potential for evaluation of any differences in tolerance to DDT that might ensue in the filial generations in consequence of crosses and reciprocal crosses with a lesser resistant strain. The non-resistant laboratory strain was selected as the second parental line.

Individual puparia were placed in glass vials, one puparium per vial, covered and the adults allowed to emerge. Paired crosses consisted of one male and one female from the Illinois and Laboratory strains placed in tarlatan cloth cages, fed in the manner previously described and permitted to reproduce. Mass crosses consisted of 20 males and 20 females from each of the strains, processed in a similar manner. Reciprocal crosses were carried out at the same time and in a similar way. The progeny of each cross and reciprocal cross were maintained through eight successive filial generations, each such generation being reared apart from its respective parental generation.

Duplicate exposures of four hours duration were made for each of these generations, each exposure containing 4 cages of the progeny of the cross and the reciprocal cross. A like number of cages of the Illinois and Laboratory strains served as a basis with which to compare the level of tolerance displayed in the successive filial generations. The average mortalities produced in the parental Illinois and Laboratory strains and those produced in each filial generation of the progeny of the cross and reciprocal cross were plotted (Fig. 4) against the respective generations concerned.

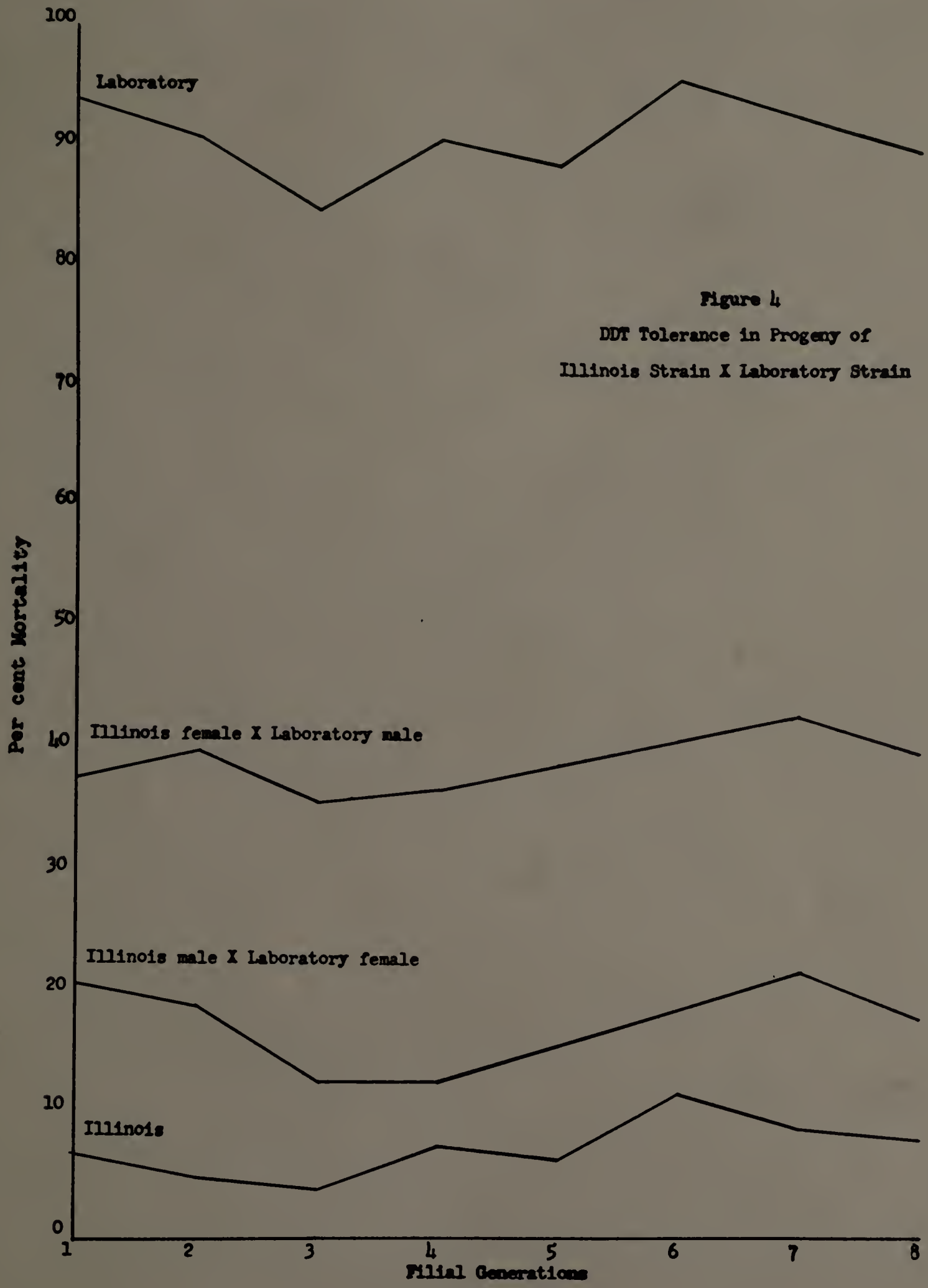


Figure 4

DDT Tolerance in Progeny of

Illinois Strain X Laboratory Strain

No difference in tolerance to DDT could be detected between the progeny of the paired crosses and the mass crosses. The data indicate that the degree of resistance displayed by the resistant Illinois strain is diluted in the progeny when that strain is crossed with the non-resistant laboratory strain.

A constant difference in the level of tolerance to DDT was observed however between the progeny of the cross and the reciprocal cross. In each instance the level of tolerance displayed by the progeny of a male resistant Illinois fly crossed with a female non-resistant laboratory fly exceeded that of the reciprocal cross (Fig. 4). Although this difference in tolerance to DDT remained constant throughout eight filial generations it is questionable whether such difference indicates any sex-linked characteristics. In consequence of similar crosses Bruce (1950) concluded that the resistance factors were carried by both the male and the female of the resistant strain. The foregoing data would support that finding. However, in view of the constantly higher tolerance to DDT exhibited by the progeny of the cross between the male resistant Illinois and female non-resistant laboratory flies, possibly this resistance factor, in addition to being carried by both

sexes of the resistant strain, is carried to a greater degree or becomes capable of greater expression in the male sex.

Crosses between resistant and laboratory strains.

The difference in the degrees of tolerance to DDT expressed by the progeny of the cross and reciprocal cross of the Illinois and laboratory strains warranted a consideration of the probable degree of tolerance that might be exhibited in the progeny of equivalent crosses between the remaining resistant strains and the laboratory strain. If the progeny of each such cross and reciprocal cross expressed a pattern of tolerance similar to that displayed by the progeny of crosses between the Illinois and laboratory strains there would be an indication of some degree of similarity in the nature of the resistance factor inherent within each of the resistant strains herein considered. Such similarity would thus become expressed regardless of the manner of origin of either resistant strain.

The resistance of the Illinois strain (Bruce, 1950) was developed by artificial selection of larvae which had been reared on DDT-impregnated media. March and Metcalf (1949) derived the California strain from resistant field populations. The New York strain was developed by Barber and Schmitt (1948) through selection from an original

resistant population encountered during the winter months at a resort hotel. This strain may be considered as having developed originally under conditions quite similar to those of a laboratory. The benzenehexachloride resistant New Hampshire strain (Blickle, 1948) was developed in consequence of the accidental contamination with benzene-hexachloride of the laboratory culture. Such divergence in the origin of these different resistant strains favored consideration of more crosses and reciprocal crosses between the resistant and laboratory strains in order to detect any possible indications of similarity or dissimilarity in the pattern of tolerance to DDT expressed in the progeny.

A. Crosses between California and Laboratory strains.

Mass crosses and reciprocal crosses between the California and Laboratory strains were effected in a manner identical to that discussed in the preceding section. Duplicate exposures of the first eight filial generations were made in conjunction with simultaneous exposures of the parental lines. The levels of mortality induced in the progeny of the cross and reciprocal cross were plotted (Fig. 5) against the respective generations concerned. The mortality level of the parental strains was plotted for comparison.

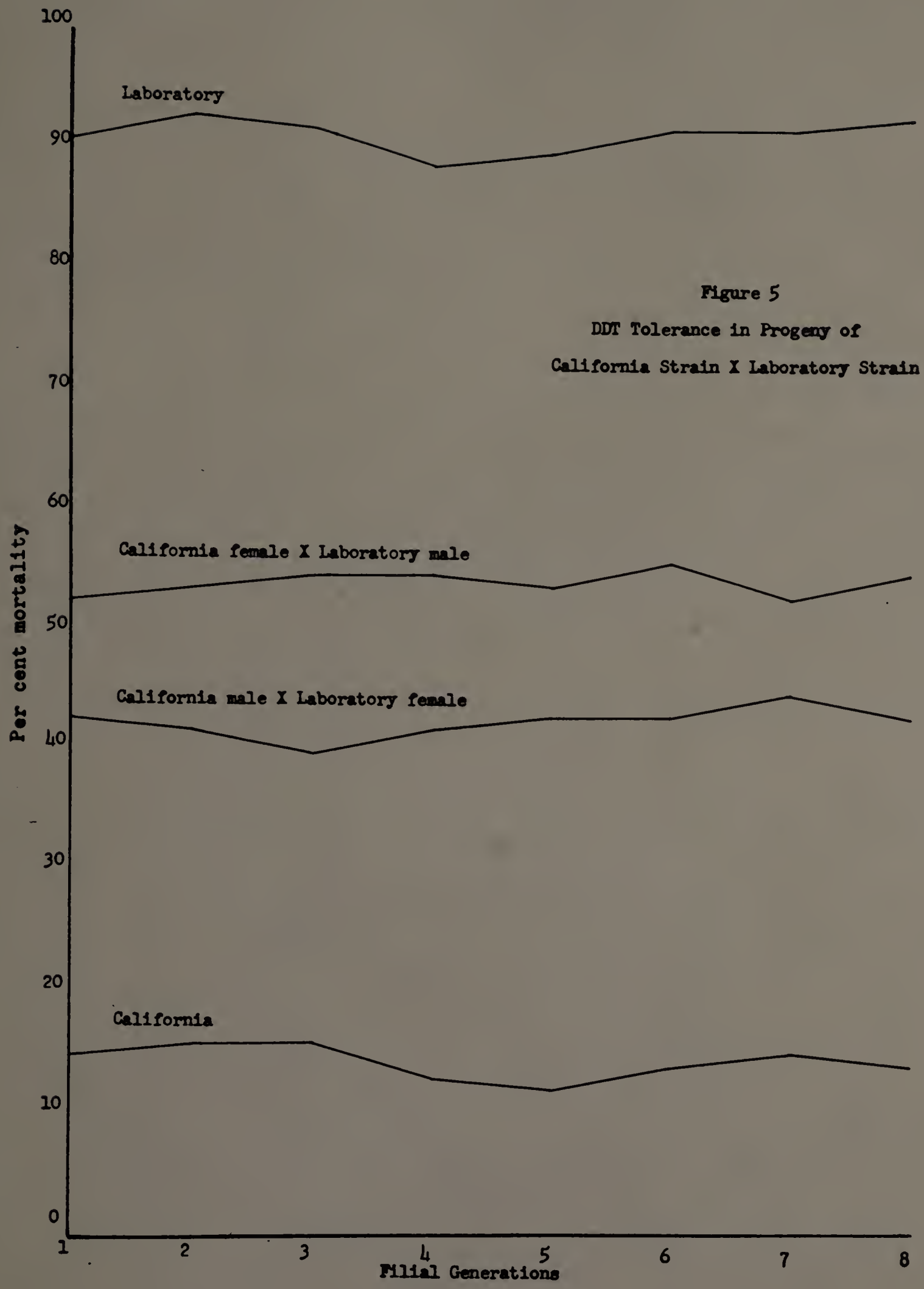


Figure 5

DDT Tolerance in Progeny of

California Strain X Laboratory Strain

The degree of tolerance expressed in the progeny of the cross and the reciprocal cross represented a dilution of the level of tolerance exhibited by the resistant California parent. The progeny of the male California and female Laboratory cross displayed a level of tolerance which exceeded that of its reciprocal cross by an average of approximately 12 per cent. The progeny of an equivalent cross between the Illinois and Laboratory strains exceeded that of its reciprocal by an average of approximately 21 per cent (Fig. 4).

The difference in tolerance herein expressed between the progeny of the cross and the reciprocal cross remained relatively constant throughout eight successive filial generations in comparison with the tolerance displayed by the resistant California and non-resistant Laboratory parental strains. The variation in mortality was plus or minus 3.75 per cent. The pattern of DDT tolerance herein expressed by the progeny of the crosses resembles that of the progeny of the cross between the Illinois and Laboratory strains.

These data indicate that the factor governing tolerance to DDT in the California strain, while apparently carried in both sexes, is, as in the Illinois strain, carried to a greater degree or becomes capable of greater expression in the male.

B. Crosses between New York and Laboratory strains.

In furtherance of the foregoing type of cross between resistant and laboratory strains, a series of equivalent crosses and reciprocals were effected between the New York and Laboratory strains, following the same general procedure previously described. The level of tolerance to DDT indicated by the filial generations and the parental strains was plotted (Fig. 6) against the respective generations concerned.

The level of tolerance expressed by the progeny indicates a dilution of that expressed by the resistant New York parental strain. This dilution level was below a point midway between the two parental strains. It remained relatively constant throughout eight successive filial generations compared to the tolerance expressed by the parental strains. No detectable difference in tolerance to DDT was observed between the progeny of the cross and the reciprocal cross. The variation in mortality was plus or minus 5.25 per cent.

A consideration of these data indicates that the resistance factor governing tolerance to DDT in the New York strain is carried by both sexes and is equally capable of expression therein.

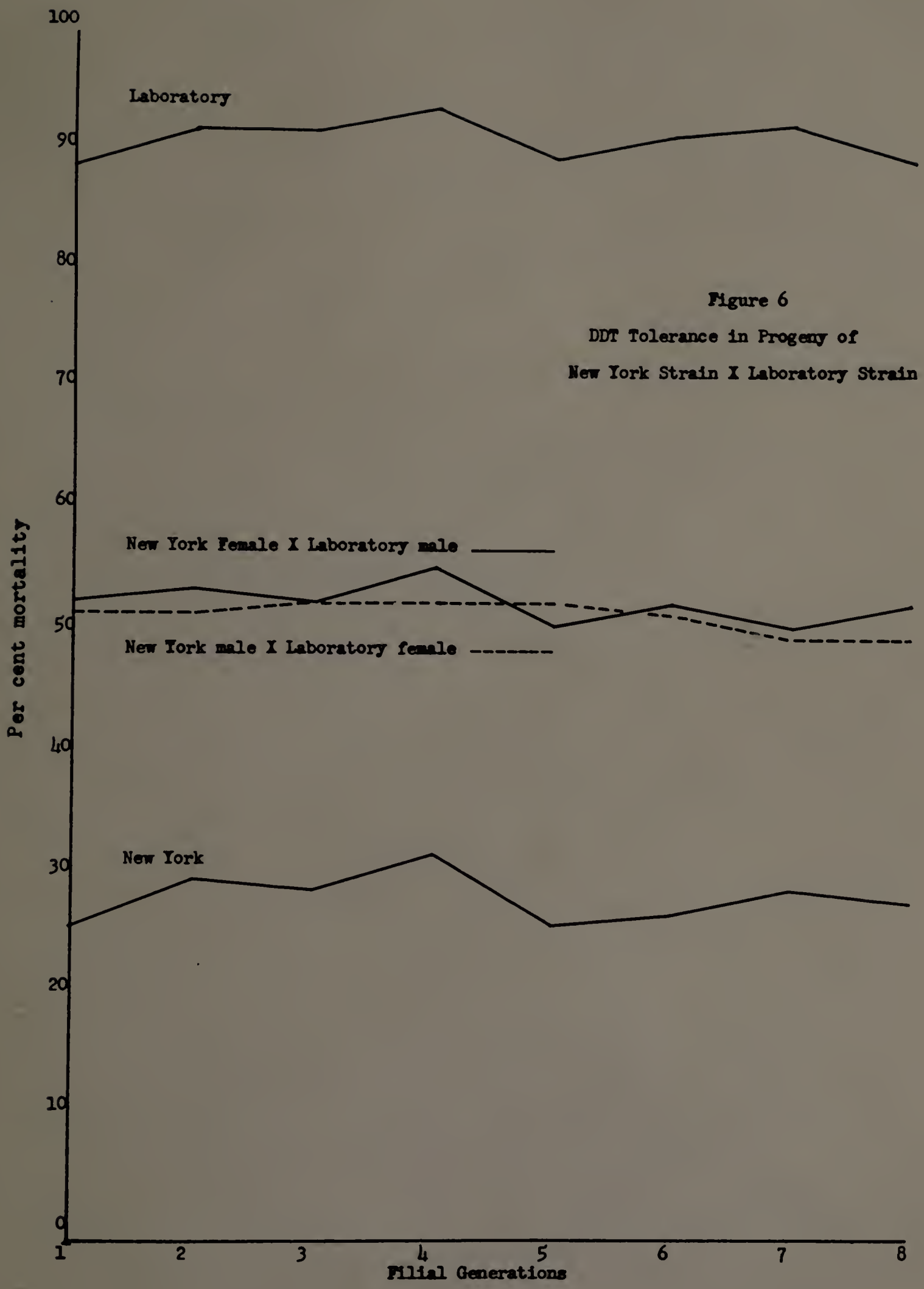


Figure 6

DDT Tolerance in Progeny of

New York Strain X Laboratory Strain

C. Crosses between New Hampshire and Laboratory strains.

As a final step in the series of crosses between resistant and non-resistant strains, equivalent crosses were carried out between the New Hampshire benzenehexachloride resistant strain and the Laboratory strain. The average mortality induced by duplicate exposures of the progeny was plotted against the respective generations concerned. The mortality of the parental strains was plotted for comparison (Fig. 7).

The degree of tolerance to DDT expressed in the progeny represented a dilution of the level of tolerance inherent in the resistant New Hampshire parent. As in the preceding cross between the New York and Laboratory strains, this level was below a midpoint between the levels of tolerance expressed by the parental strains. When compared to the degree of tolerance displayed by the parental strains, the tolerance of the progeny was relatively constant throughout eight successive filial generations. No difference in degree of tolerance to DDT could be detected between the progeny of the cross and the reciprocal cross. The variation in mortality was plus or minus 6.50 per cent. The pattern of tolerance as expressed by the progeny of the cross resembled that displayed by the progeny of the New York - Laboratory cross.

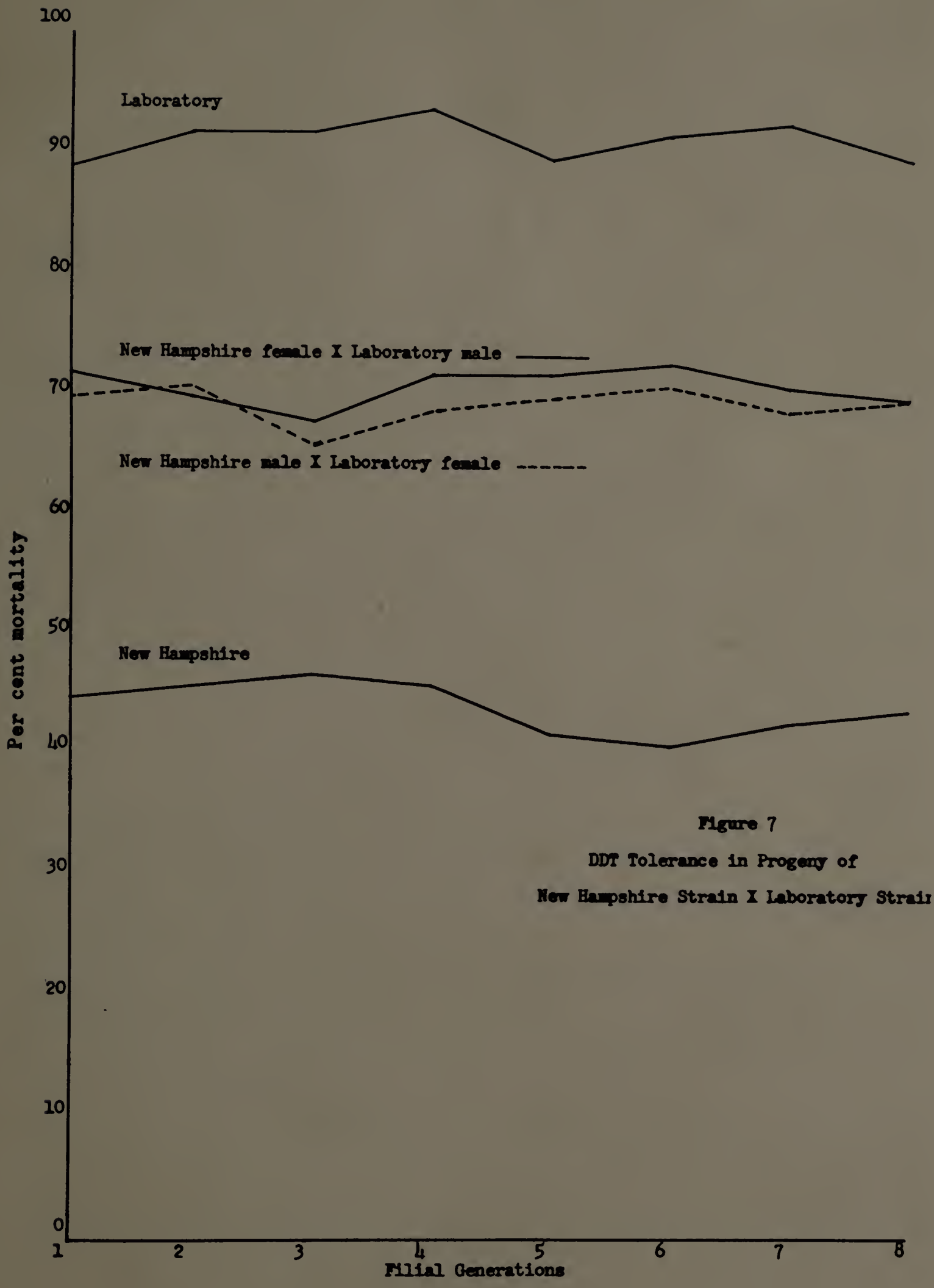


Figure 7

DDT Tolerance in Progeny of

New Hampshire Strain X Laboratory Strain

The foregoing crosses and reciprocal crosses between the various resistant strains and the laboratory strain have brought about the expression of two types of tolerance within the progeny. In each instance the degree of tolerance to DDT inherent within the various resistant strains is diluted in the progeny when either resistant strain is crossed with the laboratory strain. In all instances this level of dilution has been below that of an intermediate between the level of tolerance displayed by each parental strain.

A cross between males of either the Illinois or California strain and females of the Laboratory strain induces a higher degree of expression of DDT tolerance in the progeny than does the reciprocal cross. An equivalent cross between the New York or New Hampshire strains and the Laboratory strain apparently does not give rise to this same difference of expression of tolerance in the progeny. If differences in tolerance are present in the progeny it has not been possible to detect them by the technique employed.

The relative magnitude of DDT tolerance displayed by both the Illinois and California strains (Figs. 2 and 3) as compared to that of the New York and New Hampshire

strains may conceivably be sufficiently greater to allow detection of differences in tolerance in the progeny and to defeat its detection in the progeny of the New York and New Hampshire strains.

Inter-resistant strain crosses. Brief reference has been made in the preceding section to the apparent mode of inception of resistance within the various strains being considered. The extent to which the manner of origin of this resistance might influence the expression of resistance in the progeny of crosses between two resistant strains is, in the absence of any conclusive evidence, a matter of conjecture. The data presented in conjunction with tolerance levels exhibited by the progeny of crosses between resistant and non-resistant strains (Figs. 4 - 7) however indicate that the influence of some factor (or factors) is being expressed to some degree by reason of the differant levels of tolerance within the progeny of crosses between the Illinois or California strain and the Laboratory strain.

Crosses between two strains possessing resistance to a common toxicant such as DDT might conceivably nullify the degree of resistance presumably contributed by each resistant parental strain and thus give rise to progeny devoid of DDT resistance. Contrariwise the progeny could

exhibit an increase in tolerance in excess of that previously expressed by either parental strain. In view of the work of Hough (1928, 1929, 1934, 1943), Dickson (1941), and others it would be more logical to anticipate a dilution of the level of tolerance displayed by the more resistant strain and a simultaneous increase in the level of tolerance inherent in the less resistant strain, with the resultant composite level evolving into a level approximately midway between those of the two parental strains.

The matter of anticipating the effect upon a resistance level in the progeny of a cross between two strains, each with a resistance to a different chemical compound, does not afford such definitive background information upon which to theorize. It therefore becomes a question of trial and error in determining the degree of tolerance to DDT or to benzenehexachloride that would be expressed by the progeny of a cross between a DDT resistant strain and a BHC resistant strain.

In view of the above the objective of the present phase of this study becomes threefold: (1) to determine the average degree of tolerance to DDT that is represented by the progeny of crosses between two DDT resistant strains; (2) to ascertain whether the higher level of filial

tolerance induced by the male Illinois or California fly crossed with females of the Laboratory strain is in any way modified when these strains are similarly crossed with each other or with other resistant strains; (3) to obtain some indication of the relative degree of DDT tolerance that might be expected in the progeny of a cross between a DDT resistant strain and a BHC resistant strain. Because of the problems involved in decontamination subsequent to BHC or lindane treatments, it was not feasible to assay the level of tolerance to BHC that would be found within these progeny.

In the following series of inter-resistant strain crosses the same procedure has been followed as was employed in the preceding section. Crosses and their reciprocals were effected between the various resistant strains. The progeny were reared separately from their respective parental generations. Duplicate exposures were made of the first eight filial generations, the parental strains being simultaneously exposed in order to derive a basis of comparison of the relative tolerance levels expressed. The average per cent mortality produced in each filial generation was plotted (Figs. 8 - 13) against the individual generation concerned, the mortality induced in the parental lines being comparatively plotted.

A. Crosses between Illinois and California strains.

The tolerance of the more resistant Illinois parental strain was somewhat diluted (Fig. 8) in the progeny of this cross. The degree of tolerance assumed by the F_1 generation was slightly below a level midway between the tolerance levels of the parental Illinois and California strains. This level appears to have become well established in the F_1 generation since no detectable fluctuation in tolerance was observed from generation to generation. This level remained relatively constant throughout eight successive generations. A slight difference was observed in the degree of tolerance to DDT expressed by the progeny of the cross and reciprocal cross. The male Illinois and female California cross fostered a slightly higher level of tolerance in its progeny than did its reciprocal cross. The degree to which this level exceeded that of its reciprocal progeny however was considerably less than noted in earlier crosses between the Illinois and Laboratory strains. The variation in mortality was plus or minus 4.25 per cent.

B. Crosses between Illinois and New York strains.

The pattern of tolerance exhibited by the progeny of this cross (Fig. 9) resembled that of the progeny of the preceding cross. The filial tolerance was below that of

Figure 8

DDT Tolerance in Progeny of

Illinois Strain X California Strain

Illinois male X California female ————————

Illinois female X California male - - - - - - - -

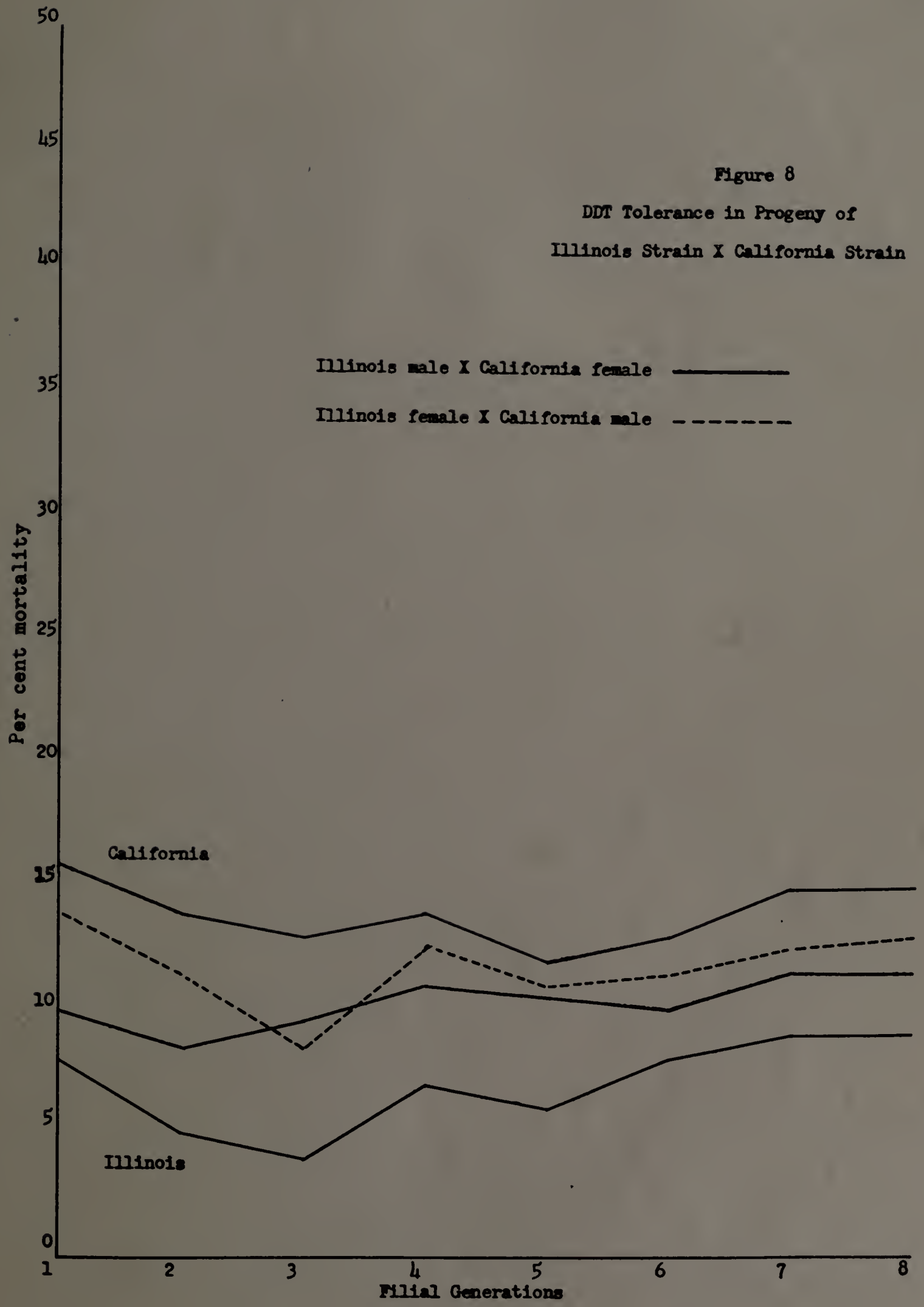
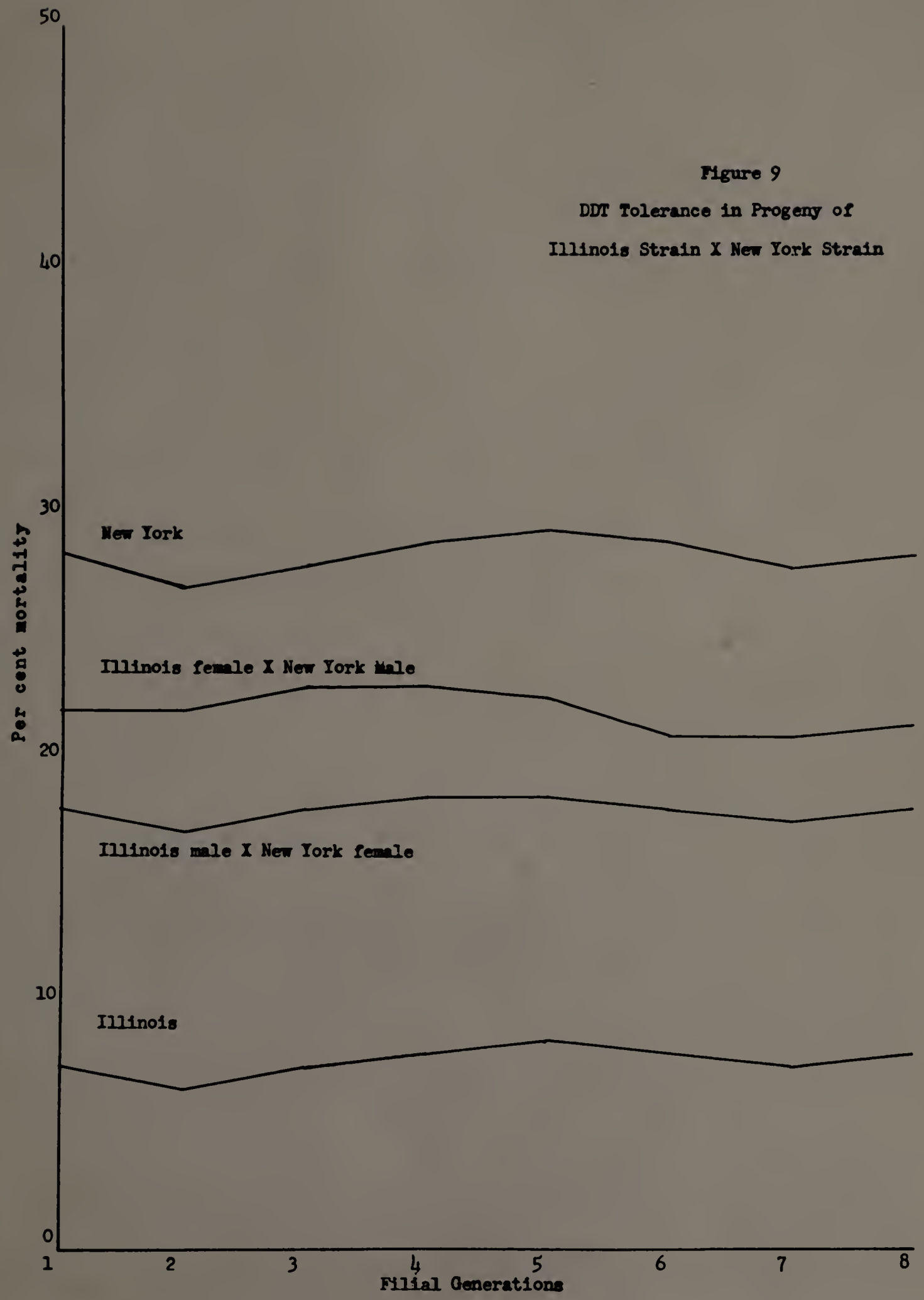


Figure 9

DDT Tolerance in Progeny of

Illinois Strain X New York Strain



the more resistant Illinois parent and in excess of that of the less resistant New York parent. This level, somewhat below a point midway between the resistance inherent in each strain, ensued throughout eight successive generations in a relatively constant plane. A slightly higher level of tolerance was observed in the progeny of the male Illinois and female New York cross. Variation in mortality was plus or minus 5.50 per cent.

C. Crosses between Illinois and New Hampshire strains.

As in each of the preceding Illinois crosses the filial tolerance of this cross expressed itself (Fig. 10) at a point below that of the Illinois strain and above that of the New York strain, remaining relatively constant throughout eight filial generations at a point just below midway between both strains. A somewhat greater difference in tolerance was observed between the progeny of the cross and reciprocal cross than was indicated above in the progeny of equivalent crosses between either the Illinois or California strain and the New York strain. Approximately 10 per cent greater resistance to DDT was indicated by the offspring of the male Illinois and female New Hampshire cross than by those progeny of the reciprocal cross. Variation in mortality was plus or minus 7.25 per cent.

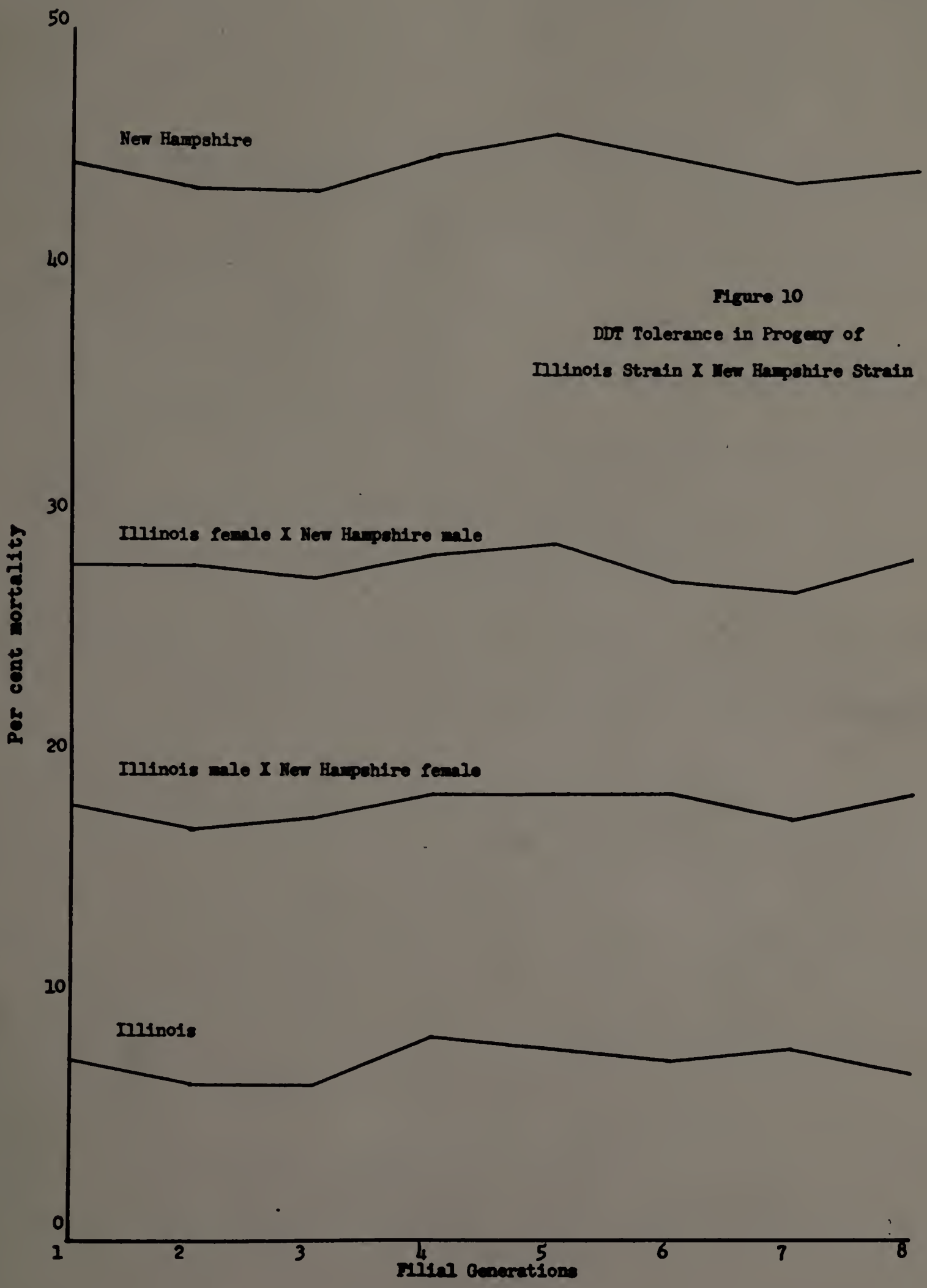


Figure 10

DDT Tolerance in Progeny of

Illinois Strain X New Hampshire Strain

D. Crosses between California and New York strains.

The progeny of this cross displayed the same comparative level of tolerance to DDT (Fig. 11) as indicated in preceding crosses between resistant strains. The F_1 assumed an intermediate degree of tolerance and successive generations did not fluctuate appreciably therefrom. In contrast to the differing levels of tolerance displayed by the progeny of crosses and reciprocal crosses between the Illinois and New Hampshire strains no divergent tolerance was herein detected between the progeny of the cross and reciprocal cross. Variation in mortality was plus or minus 5 per cent.

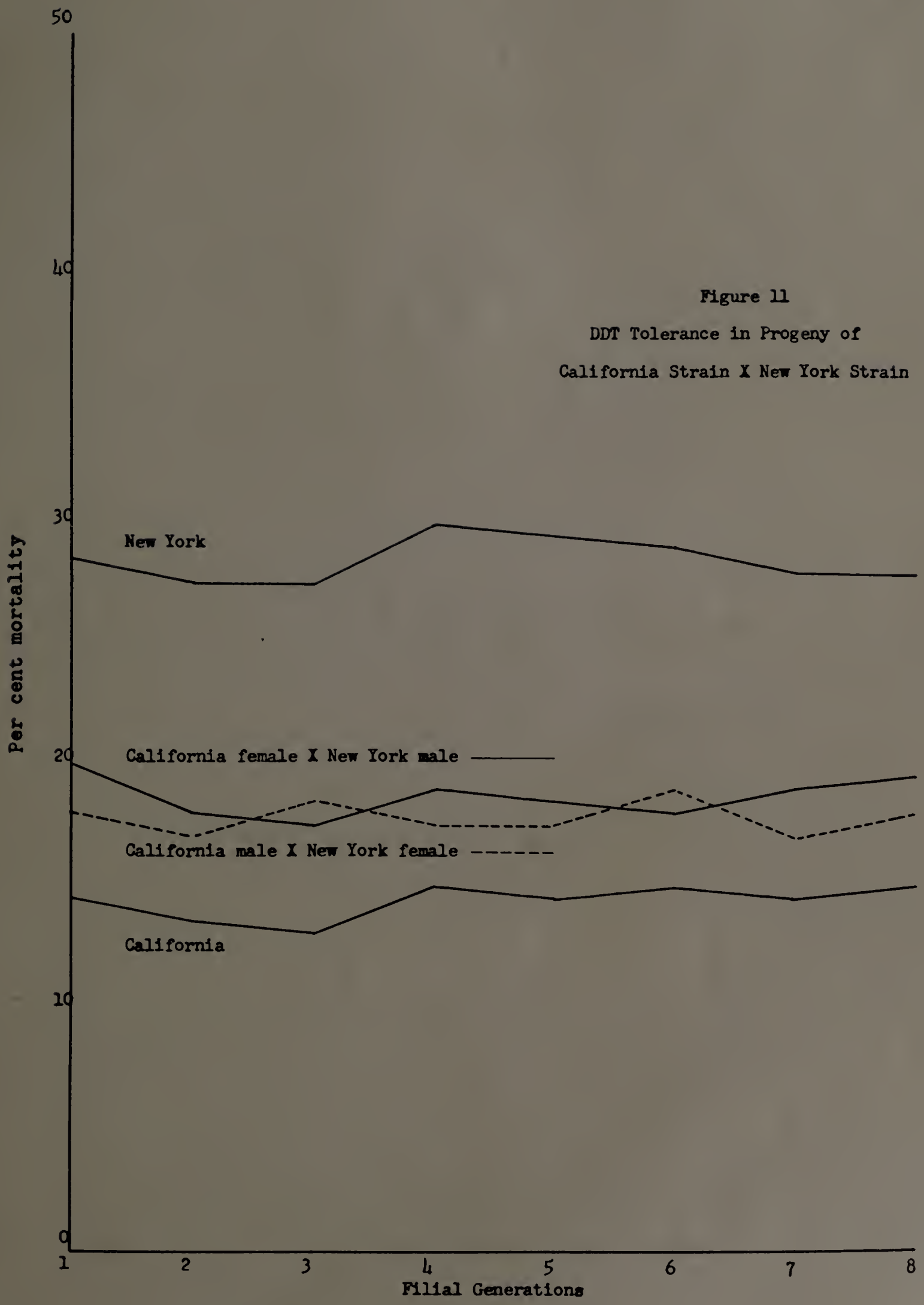
E. Crosses between California and New Hampshire strains.

The progeny of this cross expressed a level of tolerance (Fig. 12) somewhat less than that displayed by the more resistant parent and in excess of that represented by the less resistant parent. Although the New Hampshire strain is essentially a BHC resistant strain, having been conditioned thereto by accidental exposure to BHC, the level of tolerance exhibited by the respective filial generations of this cross followed the same general pattern of the preceding crosses. Resistance to DDT in the New Hampshire strain may be termed incidental whereas resistance to DDT in the Illinois, California, and New York strains

Figure 11

DDT Tolerance in Progeny of

California Strain X New York Strain



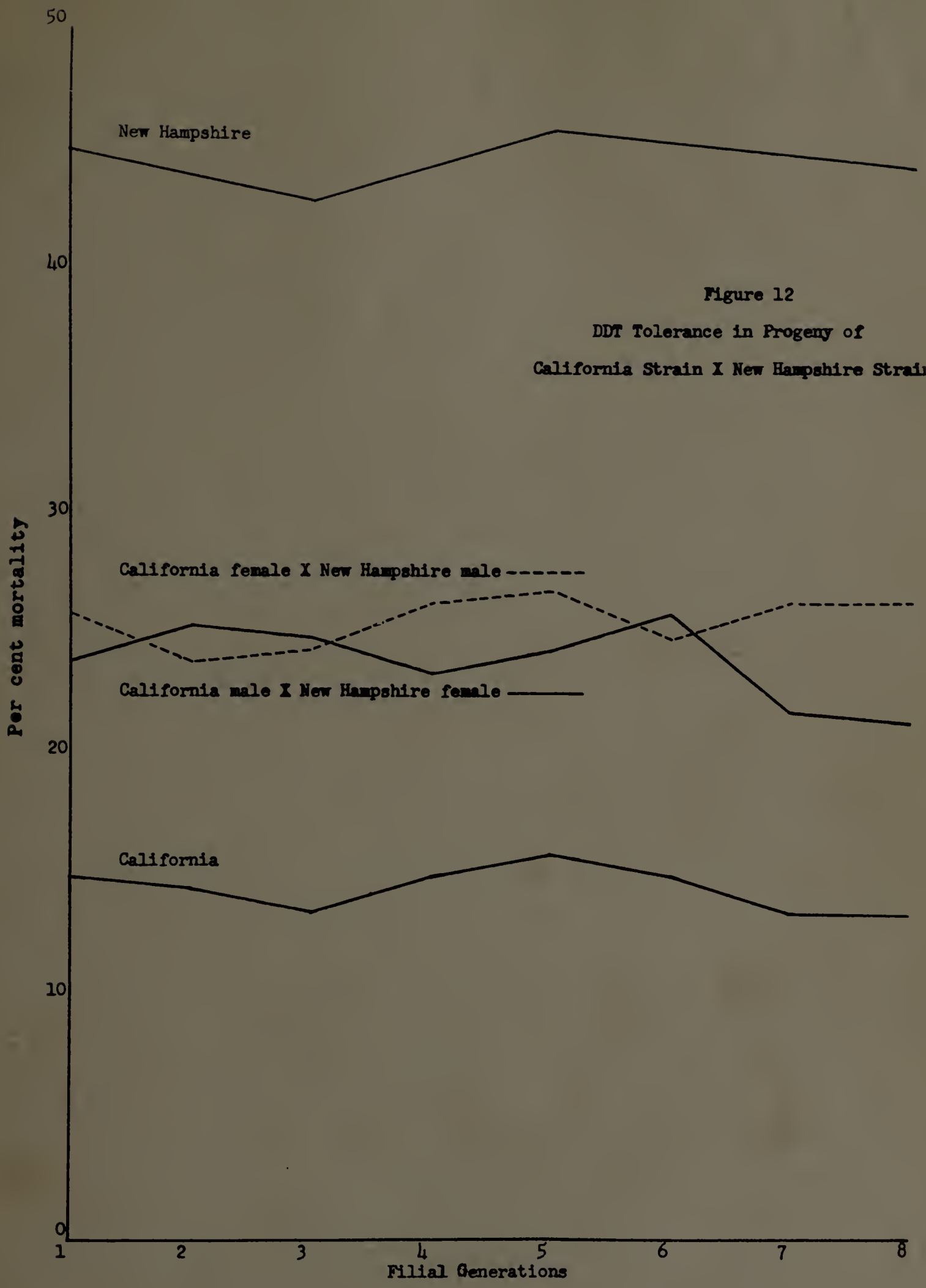


Figure 12

DDT Tolerance in Progeny of

California Strain X New Hampshire Strain

has apparently been induced by conditioning to the DDT compound itself. No detectable difference in tolerance was observed between the progeny of the cross and the reciprocal cross. Variation in mortality was plus or minus 5 per cent.

F. Crosses between New York and New Hampshire strains.

The level of tolerance to DDT displayed (Fig. 13) by the progeny of this cross represented a dilution of that of the more resistant strain to a point somewhat below midway between both parents. This degree of tolerance remained relatively constant throughout eight successive generations. The progeny of the cross and reciprocal cross were approximately equivalent in ability to withstand DDT. Variation in mortality was plus or minus 5 per cent.

An appraisal of the data presented in the above section (Figs. 8 - 13) indicates a rather uniform pattern of behavior of the resistance factor. The progeny of all inter-resistant strain crosses have expressed a tolerance to DDT somewhat below a level midway between that of both parental strains. On the basis of the results presented the degree of tolerance assumed by the F_1 generation of an inter-resistant strain cross appears to become well established within that generation and to be capable of

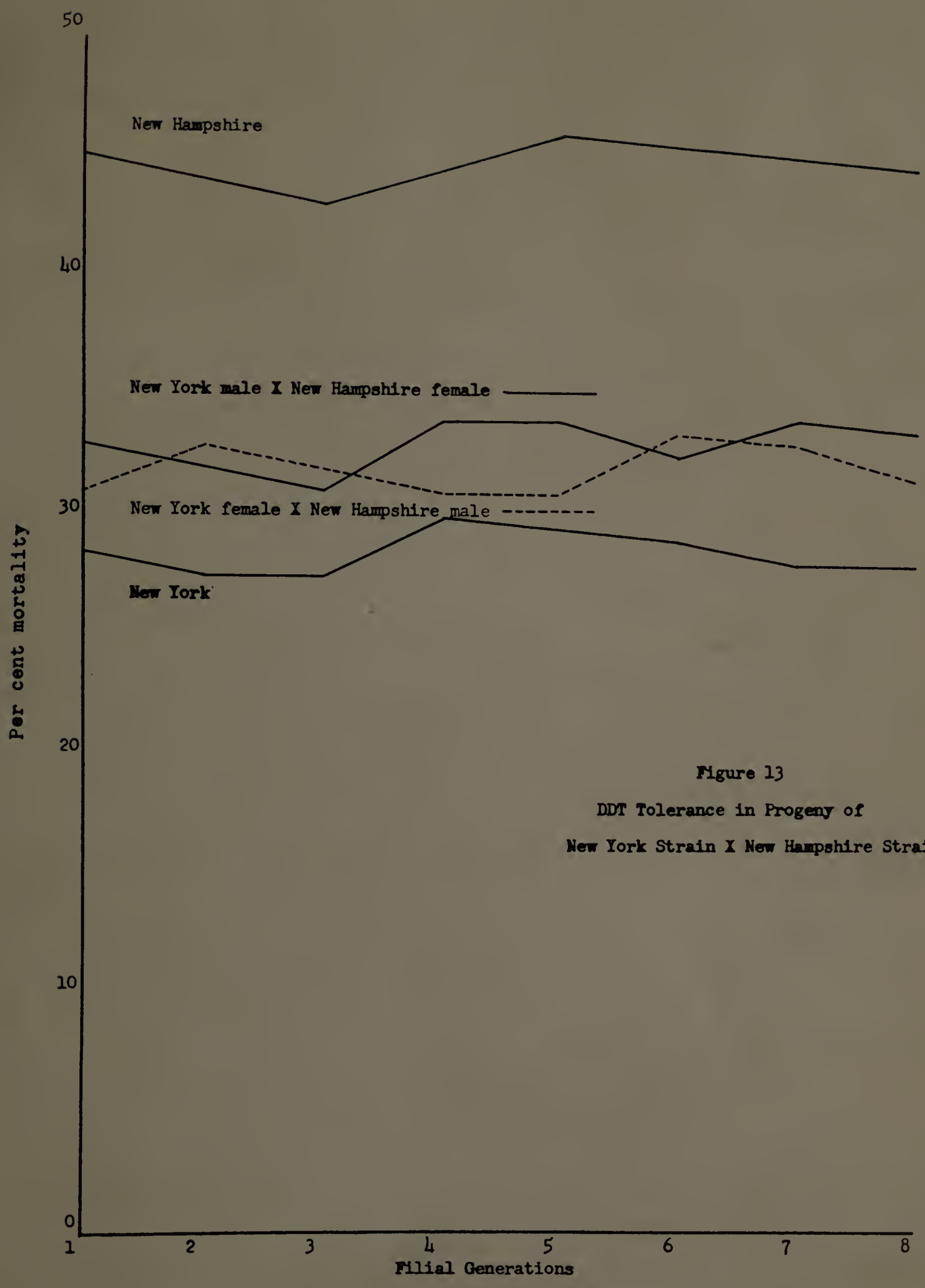


Figure 13

DDT Tolerance in Progeny of

New York Strain X New Hampshire Strain

expression at a constant equivalent level in successive filial generations.

The progeny of the Illinois or California strain crossed with other resistant strains express the same differences in tolerance between the cross and reciprocal cross as displayed by the progeny of the Illinois or California strains crossed with the Laboratory strain. As in the resistant-laboratory series of crosses, the progeny of crosses between the New York and New Hampshire strains showed no difference in tolerance between the cross and reciprocal cross.

Although conditioned to BHC and apparently having concurrently developed a lesser resistance to DDT, the New Hampshire strain apparently is capable of transmitting this DDT resistance in greater or lesser amounts to successive generations whether crossed with a DDT resistant strain or with a non-resistant strain.

A compilation of average mortalities effected in the foregoing section (Figs. 4 - 7) of crosses between resistant and laboratory strains and those produced by crosses between resistant strains (Figs. 8 - 13) is presented in Table V. All averages are based on mortalities herein observed. Direct comparison may be made between the relative tolerance of each of the strains as well as between the progeny of crosses and reciprocal crosses.

Table V

Per cent Mortality induced by DDT in

Progeny of Crosses and Reciprocal Crosses

	Males				
Strains	Ill.	Calif.	N.Y.	N.H.	Lab.
Illinois	7.6	12	18	28	39
California	10	14.5	19	24	40
New York	22	18	28.5	32	51
New Hampshire	18	26	33	44.4	71
Laboratory	17	42	53	69	94

Maximum variation 7.25 per cent

Females

Tolerance induced by successive backcrosses.

At about the same time that Bruce's (1950) comprehensive work on house fly resistance to insecticides was reported, an editorial by Decker (1950) presented a rather extreme point of view on house fly resistance to DDT. According to Decker if resistant flies are permitted to interbreed with susceptible flies the degree of tolerance is progressively reduced with each generation of cross breeding. While such decrease in tolerance is not unreasonable to anticipate, in view of a complete lack of data, this statement must be viewed cautiously. It has been suggested earlier in this study that resistance might well be the effect of several factors expressed as a composite or summation effect. Any prediction then as to the effect on a multi-factor tolerance complex induced by cross breeding with susceptible flies which is not supported with adequate data can hardly be justified.

Commenting upon the seriousness of DDT resistance Decker further states, "Apparently in the short span of two to three years we have established and intensified resistance to a point where DDT is rapidly losing its usefulness against flies. We may be approaching the stage of rapid intensification of resistance, and if DDT is used for one more year we may quickly develop a strain of flies that would be essentially immune to DDT. Further, if in

three years we have moved from zero to 87 per cent resistance in the field, we might very easily in one more year eliminate the last of the DDT-susceptible strains. With the passing of this last strain we might lose our one hope for ever getting out of this embarrassing predicament."

A critical evaluation of this comment reveals certain pseudo-facts with which exceptions are taken. Possibly data not included by Decker have been procured by or rendered available to him. In such instance a portion of the foregoing may be justified. However on the basis of the information presented several points of disagreement evolve. The development of a strain of flies essentially immune to DDT by the use of this material throughout an additional year is hardly plausible. Admittedly the current level of resistance might be increased. The incidence of resistance might conceivably be intensified. However since it is highly improbable that all flies throughout the area concerned or throughout any area would assume tolerance to DDT, not to mention intensified resistance to the toxicant, it is reasonably logical to anticipate that many flies would retain their susceptibility to DDT. As stated by Decker then, interbreeding with susceptible flies would progressively reduce the tolerance with each successive generation of cross breeding.

The allegation that there has been a move from zero to 87 per cent resistance in the field is impossible to reconcile with the information presented. However the similarity of these figures to those of W. N. Bruce (1950) leaves the alternative that reference to the work of Bruce is inferred. If such inference is assumed however, again there appears no justification for the statement. The following quotation from the work of Bruce is relevant. "Of particular interest to the PCO should be the results obtained from a field survey conducted to determine the prevalence of DDT tolerance among flies on farms in Illinois. There was only 12.8 per cent of the farms with normal or susceptible strains of flies. The remainder or 87.2 per cent of the farms had flies which showed from four to sixty times the normal amount of tolerance for DDT." Bruce has clearly stated that 87.2 per cent of the farms examined had flies which showed some degree of resistance. In no sense has he implied that an increase of from zero to 87 per cent resistance has been encountered in the field.

The further allegation that the last of the DDT susceptible strains might be very easily eliminated in one more year, while not impossible, is highly improbable. Of a total of 94 farms examined 87.2 per cent had flies which showed from four to sixty times the normal amount of

tolerance for DDT. With such a small portion of the total number of farms having been examined and these same farms showing flies with a range of tolerance from four to sixty times that of the normal, the potential for cross breeding with non-resistant flies appears extremely high. In the absence of contradictory data it appears equally logical to assume that cross breeding with susceptible flies would gradually serve to reduce this tolerance and, in the absence of factors contributing solely to the expression of tolerance, might even nullify it. If the level of tolerance were not so markedly reduced as to nullify its expression, it is possible that an equilibrium might become established in consequence of interbreeding with the susceptible flies. Such a balance could result in a static state of the tolerance complex in spite of additional exposure to DDT.

The reference by Decker to progressive loss of tolerance induced by permitting resistant flies to cross breed with susceptible flies is relevant to observations made earlier in this study. When the Illinois strain was crossed with the Laboratory strain (Fig. 4) the level of tolerance expressed by the progeny was more or less midway between that of both parental lines. A constant difference in tolerance was noted however between the progeny of the

cross and the reciprocal crosses. In conjunction with such projected crosses certain patterns of behavior of genetic factors appear pertinent.

According to Mendel's law of segregation two genes at a given locus segregate from one another during meiosis. As a result only one member of a pair of genes is present in each gamete. The genetic constitution of any organism therefore is the result of the particular gametes which unite when that organism is formed. Since the degree of expression of some characters may be clearly defined qualitatively this expression is probably dependent upon one or a very few sets of alleles. Such variations are usually discontinuous. Other characters may be expressed quantitatively and are generally continuous. Characters which vary continuously are generally dependent upon many sets of alleles which apparently express no particular dominance and are probably therefore cumulative in their influence. According to the multiple factor theory several sets of alleles may produce equal and cumulative effects on an individual character. This theory is essentially equivalent to the polymeric gene theory.

The premise that resistance to DDT is a composite effect or summation of several sets of alleles expressing

themselves without dominance may be considered. Since Decker has alleged that cross breeding with susceptible flies would result in progressive loss of tolerance, the corollary as to whether breeding susceptible flies with resistant flies would progressively raise the level of tolerance seemed worthy of consideration.

Referring back briefly, the relatively constant level of tolerance to DDT displayed (Fig. 4) by the progeny of the Illinois-Laboratory cross and reciprocal cross and by the respective parental strains indicates a probable high percentage of homozygous individuals within these parental strains. Such probability suggested the use of these strains as progenitors of the projected crosses and reciprocal crosses above discussed.

Sufficient of the F_1 generations were retained to propagate the filial progeny as separate inbred cultures, each generation being reared apart from its respective parental generation. The remainder was divided into five categories for processing as follows:

1. Exposure to DDT for tolerance level determinations;
2. Males backcrossed to females of parental Illinois strain;
3. Females backcrossed to males of parental Illinois strain;

4. Males backcrossed to females of parental Laboratory strain;
5. Females backcrossed to males of parental Laboratory strain.

The degree of tolerance expressed by each of the foregoing categories was computed, as in previous tolerance determinations, from mortalities induced by four hour exposures to DDT in continuous aerosol phase, duplicate exposures being used in each instance. In conformity with previous determinations these categories were extended through eight successive filial generations. Variation in mortality was not in excess of 6.50 per cent.

A. Backcrosses with progeny of Illinois female X Laboratory male.

The F_1 generation of a cross between resistant Illinois females and susceptible Laboratory males was used as the starting point in this series of backcrosses.

1. Tolerance of inbred filial culture

The tolerance expressed by successive filial generations of this cross, propagated as an inbred culture, followed the same general pattern (Fig. 14) displayed by the progeny of an equivalent cross discussed earlier (Fig. 4) and remained relatively constant throughout.

2. Filial males backcrossed to parental Illinois females

A number of males selected from the F_1 generation were backcrossed to parental Illinois females. The offspring of this cross and similarly of subsequent equivalent crosses were divided into two groups. One group was exposed to aerosol DDT in order to secure tolerance data while the other group was backcrossed to parental Illinois females. This process of exposing a portion of the progeny of each cross to aerosol DDT and of backcrossing the other portion with parental Illinois females was continued throughout eight successive generations.

The degree of tolerance expressed by the progeny of these successive backcrosses (Fig. 14) indicates a progressive but gradual increase in tolerance with each successive backcross.

3. Filial females backcrossed to parental Illinois males

Following the same general procedure discussed above, a portion of the F_1 females were backcrossed to males of the parental Illinois strain. Successively a portion of the progeny of this backcross and of subsequent equivalent backcrosses were exposed to the toxicant while the respective remaining portions were backcrossed to males of the parental Illinois strain.

The pattern of tolerance exhibited by the progeny of these successive backcrosses (Fig. 14) is observed to be slightly higher than that expressed by the progeny of equivalent backcrosses to the parental Illinois females. Whereas the same progressive increase in tolerance is observed with each successive backcross, a higher level is fostered initially and continued throughout by the present series of backcrosses.

4. Filial males backcrossed to parental Laboratory females

In this series of backcrosses filial males were backcrossed to parental Laboratory females. With each successive backcross (Fig. 14) the level of tolerance was progressively reduced, the rate of reduction appearing more abrupt in the earlier generations and then becoming more gradual in succeeding generations.

5. Filial females backcrossed to parental Laboratory males

In consequence of backcrosses similar to the foregoing the level of tolerance expressed by the respective progeny (Fig. 14) was nearly identical to the tolerance indicated by the progeny of the reciprocal series of backcrosses, i.e., between the filial males and the parental laboratory females. Although variation is to be noted

between the two lines of tolerance as expressed by any one generation, the mathematical average of tolerance expressed throughout eight generations by both lines is about equivalent.

The patterns of tolerance expressed by the two foregoing series of backcrosses, i.e., to the resistant parental strain and to the susceptible parental strain, are similar. With each successive back cross to the resistant parental strain the level of tolerance is progressively increased. Likewise with each successive backcross to the susceptible parental strain the level of tolerance is progressively reduced. The rate at which tolerance is reduced by backcrossing to the susceptible strain however is more rapid than the rate at which tolerance is increased by backcrossing to the resistant strain. In neither the increased nor the decreased tolerance pattern is the tolerance level of the progeny intermediate between that of the parents. In both instances this level is far below the intermediate, beimg much lower in the progeny of the backcrosses to the resistant parent than to the susceptible parent. In the increased pattern of tolerance a slightly higher level is observed in consequence of backcrossing to the male resistant parent.

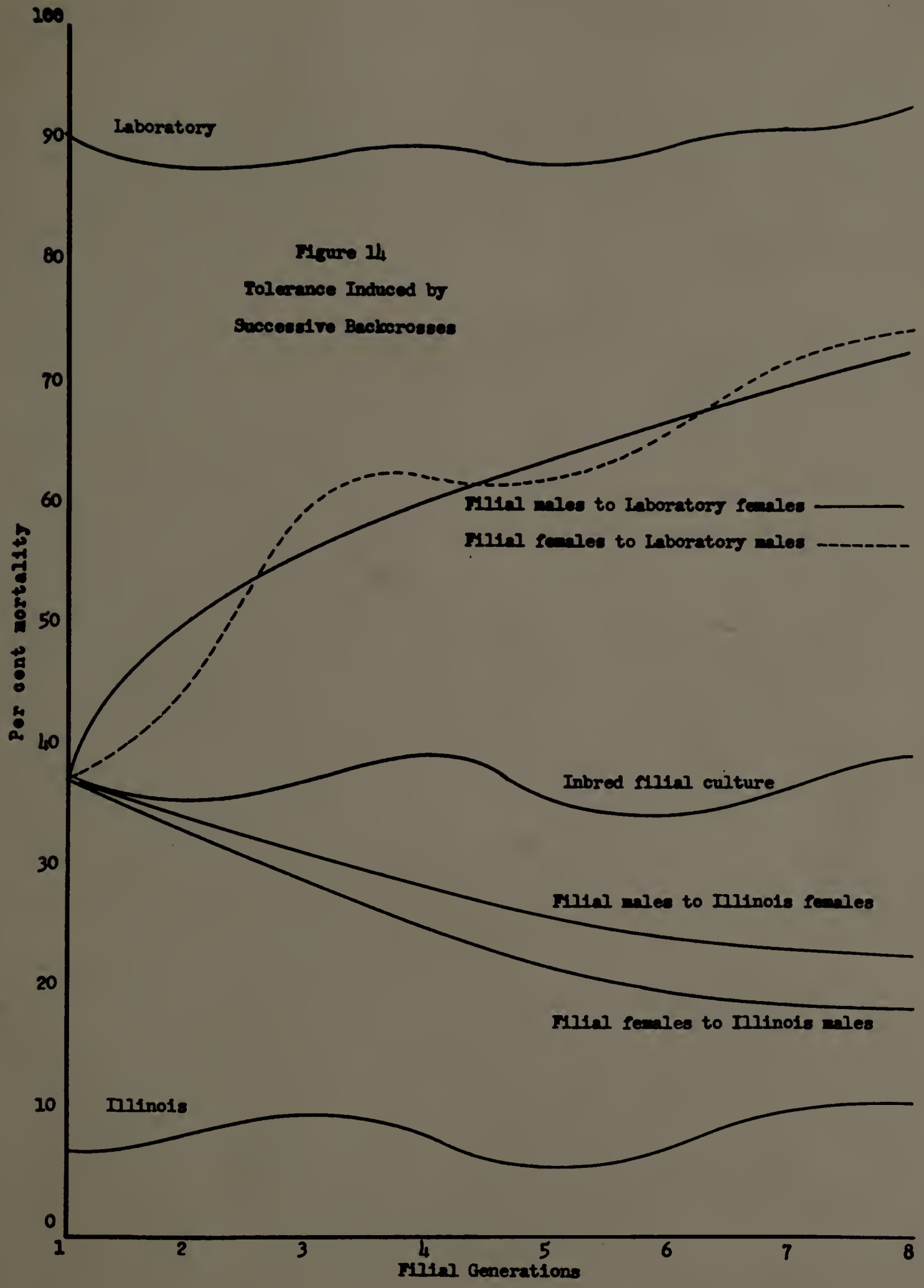


Figure 14

Tolerance Induced by

Successive Backcrosses

B. Backcrosses with progeny of Illinois male X Laboratory female

In contrast to the above series of backcrosses (A, 1-5; Fig. 14) the F_1 generation of a cross between resistant Illinois males and susceptible Laboratory females was used as the starting point in this series of backcrosses. The same procedure was used as in the preceding series, the same sequence of exposures and equivalent successive backcrosses both to resistant and susceptible parental strains being carried out. An inbred filial culture was maintained, successive generations being reared apart from their respective parental generations and exposed to DDT simultaneously with the progeny of respective backcrosses. Variation in mortality was not in excess of 4.75 per cent.

1. Tolerance of inbred filial culture

The level of tolerance expressed by eight successive filial generations of the male Illinois-female Laboratory cross (Fig. 15) was about the same as that indicated earlier (Fig. 4) by an equivalent cross. As previously indicated (Fig. 4) this level of tolerance (Fig. 15) was somewhat higher than that of the reciprocal progeny.

2. Filial males backcrossed to parental Illinois females

The pattern of tolerance indicated by the progeny of these successive backcrosses (Fig. 15) resembled that displayed by the progeny of the backcross between filial males and parental Illinois females in the preceding series of backcrosses (Fig. 14). Whereas the actual level of tolerance is somewhat higher in the present progeny than indicated by the progeny of an equivalent cross in the previous series of backcrosses (Fig. 14) this is attributed to the higher level of tolerance inherent in the filial males serving as parental males in the backcross. A gradual increase in tolerance is noted.

3. Filial females backcrossed to parental Illinois males

The level of tolerance expressed by the progeny of these backcrosses was somewhat higher than indicated by the reciprocal backcross progeny. The same general pattern of increased tolerance with each successive backcross as indicated in the reciprocal backcross was observed (Fig. 15). As in the progeny of similar crosses above (A-3) the initial level of tolerance was higher than in the progeny of the reciprocal backcross (B-2) and continued at that higher level throughout.

4. Filial males backcrossed to parental Laboratory females

Apart from initiating at a higher level of tolerance in consequence of higher parental tolerance, the progeny of this series of backcrosses followed the same pattern (Fig. 15) as the progeny of the backcross between the first F_1 group and the parental laboratory females (A-4; Fig. 14). The rates of progressive loss in both instances are about equivalent.

5. Filial females backcrossed to parental Laboratory males

The level of tolerance expressed by the progeny of this backcross (Fig. 15) was about the same as observed in the progeny of the preceding backcross between filial males and laboratory females, no appreciable difference being detected between the tolerance of the two.

The foregoing series of backcrosses (B, 1-5; Fig. 15) initiated with the F_1 of a male Illinois-female Laboratory cross shows the same general tendencies in expression of tolerance as indicated by the backcross series initiated with the F_1 of a reciprocal mating, i.e., between female Illinois and male Laboratory flies (A, 1-5; Fig. 14). The progressive increase in tolerance by backcrossing to the resistant parent and progressive loss in tolerance by backcrossing to the susceptible parent (Fig. 15) is about the same as observed in the preceding series (Fig. 14).

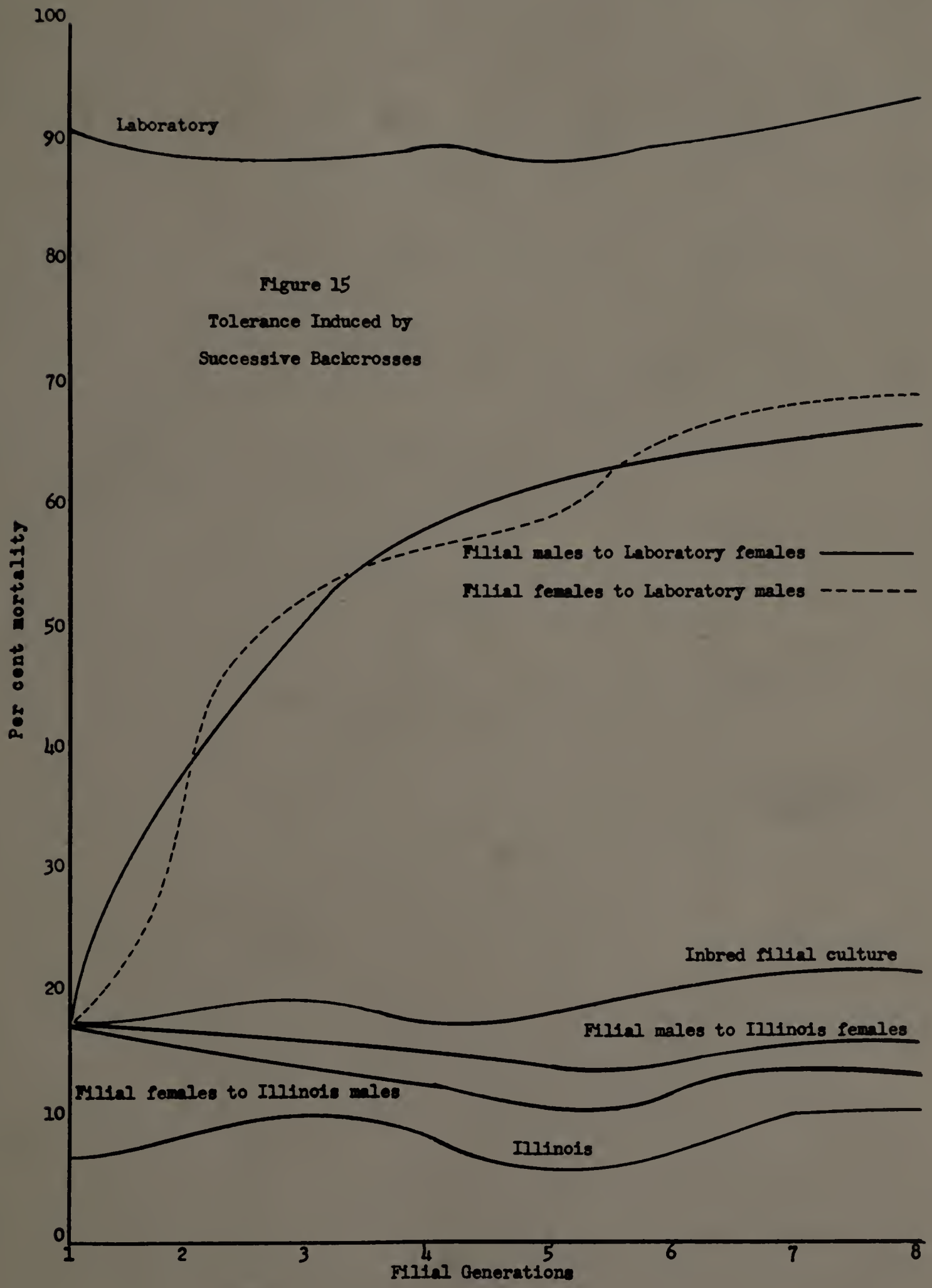


Figure 15

Tolerance Induced by

Successive Backcrosses

The tolerance induced by backcrossing to the male resistant parent is slightly greater than induced by backcrossing to the female resistant parent. The rate of loss of tolerance is greater than the rate of increase of tolerance. The general level of tolerance is somewhat greater because of the higher level of tolerance inherent in the F_1 generation from which this series of backcrosses was developed.

A consideration of the pattern of tolerance expressed in the foregoing series of backcrosses (Figs. 14 and 15) indicates a similarity in the behavior of the resistance complex whether backcrossing to the resistant or susceptible parent. The loss of tolerance by cross breeding with susceptible flies is conjoined with a concomitant increase in tolerance in consequence of cross breeding with resistant flies.

Variation from this general pattern is recognized in the relatively slower rate at which backcrossing induces increased tolerance. The rate at which tolerance is decreased by backcrossing to susceptible flies is more rapid in the early generations than in succeeding generations. The overall rate of loss of tolerance however is more rapid than the rate of increase. These data would indicate that interbreeding with susceptible flies progressively reduces the level of tolerance with each successive generation of cross breeding. These data further indicate that the

expression of insecticide resistance, in this instance resistance to DDT, is dependent upon a multi-factor complex rather than a solitary pair of genes.

It seems probable that the factors governing the expression of resistance are present in both resistant and susceptible strains. Because of certain ecological aspects to which the now resistant strain had at some time previously been exposed, this strain is now capable of resistance expression. Contrariwise and along the same line of reasoning, the susceptible strain, devoid of these particular ecological exposures, has not had the opportunity to become adapted to these ecological variants and therefore is not now capable of resistance expression. This might infer that all strains of flies are equally capable of becoming adapted to the expression of resistance if provided with the proper ecological stimulus. However it is very probable that there are flies or strains of flies which, regardless of the stimuli to which exposed, would never become adapted to resistance expression.

Whether or not the conditioning toxicant, DDT in the present study, is the sole contributing factor in the establishment of this ecological niche favorable for the adaptation to the toxicant is problematical. However since the benzenehexachloride resistant New Hampshire strain was

presumably conditioned by and to benzenehexachloride this same strain has nevertheless developed a lesser resistance to DDT. In several instances the expression of resistance to a particular toxicant has reportedly been accompanied by a simultaneous adaptation of lesser resistance to certain other toxicants. It would seem then that any toxicant is capable of inducing a progressive cumulative expression of factors which, having quantitatively attained an equilibrium with the ecological stimuli in the niche to which exposed, are able to progressively adapt themselves and eventually express themselves as a composite or aggregate complex which results in increased tolerance and eventual intensified resistance to that particular toxicant. The fact that greater resistance to hydrocyanic acid gas was observed (Quayle, 1922) in the red scale on trees that had been fumigated regularly and that greater resistance to DDT has been similarly observed where this compound has been repeatedly used would appear to support this line of reasoning.

Although not included in Figures 14 and 15 the foregoing series of successive backcrosses and exposures to DDT were extended through 10 generations. No variation from the general pattern established in the first eight generations was noted.

VARIATION IN LIFE CYCLES.

During the early part of this study it became apparent that the length of the life cycles of the different resistant strains varied from each other and from the Laboratory strain. Although reared under uniform conditions these differences had apparently persisted over a period of three months prior to the present observations. Since a study of life cycles did not fall within the scope of this problem limited observations only were made.

Procedure. Uniform volumes of randomized samples of eggs, all less than twelve hours old, were taken from each of the strains and reared in the manner previously described. Forty-eight hours after the eggs had been seeded in the rearing medium, the sand surrounding each of the jars was removed, covered, placed in the constant temperature and relative humidity chamber and replaced with fresh sand. This removal and replacement of sand was repeated every twelve hours for nine days. All larvae that crawled out of the rearing jar preparatory to pupation during any twelve hour period were thus grouped together. Three days after removal of the sand, the larvae had completed pupation and were screened therefrom. Counts were made of the number of pupae present in each lot.

These observations were replicated eight times at irregular intervals over a period of three months. Since variation was encountered in the number of eggs selected from each strain, the average number of pupations effected during any twelve hour period is expressed (Tables VI and VII) in percentage, as the portion of the total pupation which occurred during that particular twelve hour period.

A comparison of the rate of pupation of the different strains (Fig. 16) indicates that the peak of pupation in the Laboratory strain precedes that of the Illinois strain by approximately 24 hours. The peaks of the New York and California strains are generally intermediate to those of the Laboratory and Illinois strains, being slightly more closely aligned to that of the Laboratory strain. The peak of the New Hampshire strain precedes that of the Laboratory strain by about 12 hours and that of the Illinois strain by about 36 hours. The rate of pupation in the New Hampshire strain rises rapidly to its peak and then declines gradually. The rates of rise and fall in pupation curves of the other strains are quite uniform.

Upon the basis of these observations, under conditions obtaining, considerable variation in length of larval stage of development exists between strains. If the length of the Laboratory larval stage is taken as normal, the larval

stage of the Illinois strain is approximately 24 hours longer whereas those of the New York and California strains may be considered approximately 12 hours longer. The New Hampshire strain requires about 12 hours less than the Laboratory strain for completion of the major portion of its pupation.

Coincidental to the foregoing observations repeated exposures of adults to DDT in aerosol phase were being conducted in conjunction with relative tolerance level determinations (Figs. 2 and 3) of the different strains. The average mortalities induced by these exposures were:

Illinois	7.6 per cent
California	14.5 " "
New York	28.5 " "
New Hampshire	44.4 " "
Laboratory	94.0 " "

Table VI

Per cent Pupation per Twelve Hour Period

1st Observation Series

	Age of larvae in days									
Strain	3 1/2	4	4 1/2	5	5 1/2	6	6 1/2	7	7 1/2	8
Laboratory	5	12	25	32	15	5	3	2	1	0
Illinois	0	2	4	9	27	30	20	5	2	1
California	2 1/2	4 1/2	7 1/2	26	31	16	8	3	1 1/2	0
New Hampshire	7	12	29	23	13	8	5	3	0	0
New York	4	6	9	23	28	14	8	5	3	0

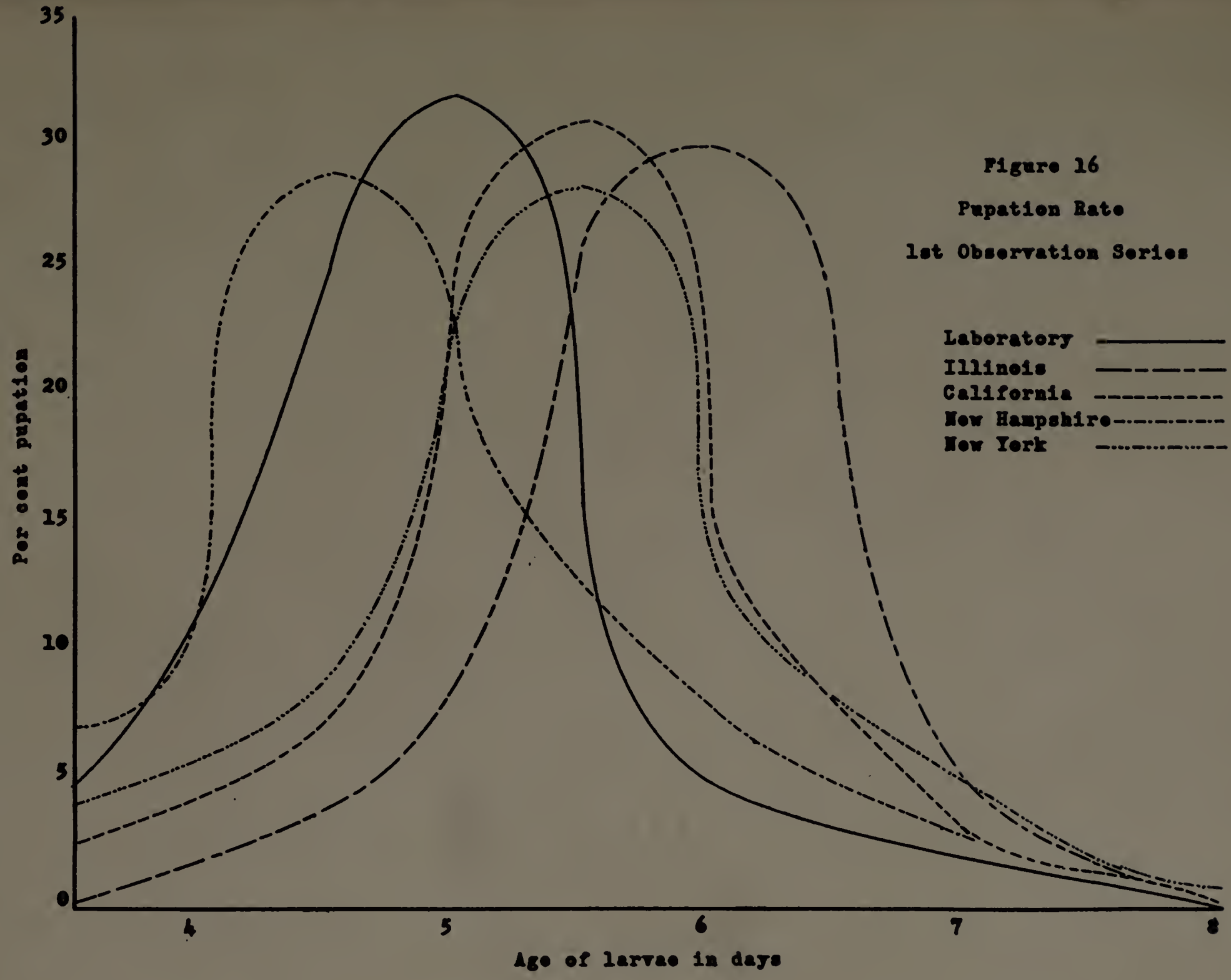


Figure 16

Pupation Rate

1st Observation Series

Approximately eight months after completion of the above observations a second series was initiated. Consideration of this latter series was prompted by a two-fold objective. From casual observation the length of the larval stages in the New York and California strains, although apparently still at some variance with that of the Laboratory strain, appeared to more nearly coincide with the Laboratory strain than they had eight months previously. Secondly the relative tolerance to DDT of these two strains, i.e., the New York and California strains, appeared somewhat lower than in previous determinations (Figs. 2 and 3). In view thereof a consideration of further larval observations and tolerance level determinations seemed indicated.

A series of observations replicated eight times and extended irregularly over a three months period, as above, was carried out. The average number of pupations during each twelve hour period was noted (Table VII) for each strain. From these data the pattern of pupation and length of larval stage for each strain was directly compared (Fig. 17).

The larval stage of development has remained relatively constant in both the Illinois and Laboratory strains. The peak of the New Hampshire curve has shifted slightly toward an increase in length of larval stage. The greatest

Table VII

Per cent Pupation per Twelve Hour Period

2nd Observation Series

	Age of larvae in days											
Strain	3 1/2	4	4 1/2	5	5 1/2	6	6 1/2	7	7 1/2	8		
Laboratory	5	10	25	32 1/2	15	5	3	2 1/2	1	1		
Illinois	0	3	4	7	26	32	20	5	2	1		
California	2	4 1/2	11	30	25	16	8	3	1 1/2	0		
New Hampshire	5	11	28	29	15	8	3	1	0	0		
New York	3	6	20	31	21 1/2	10	5	2 1/2	1	0		

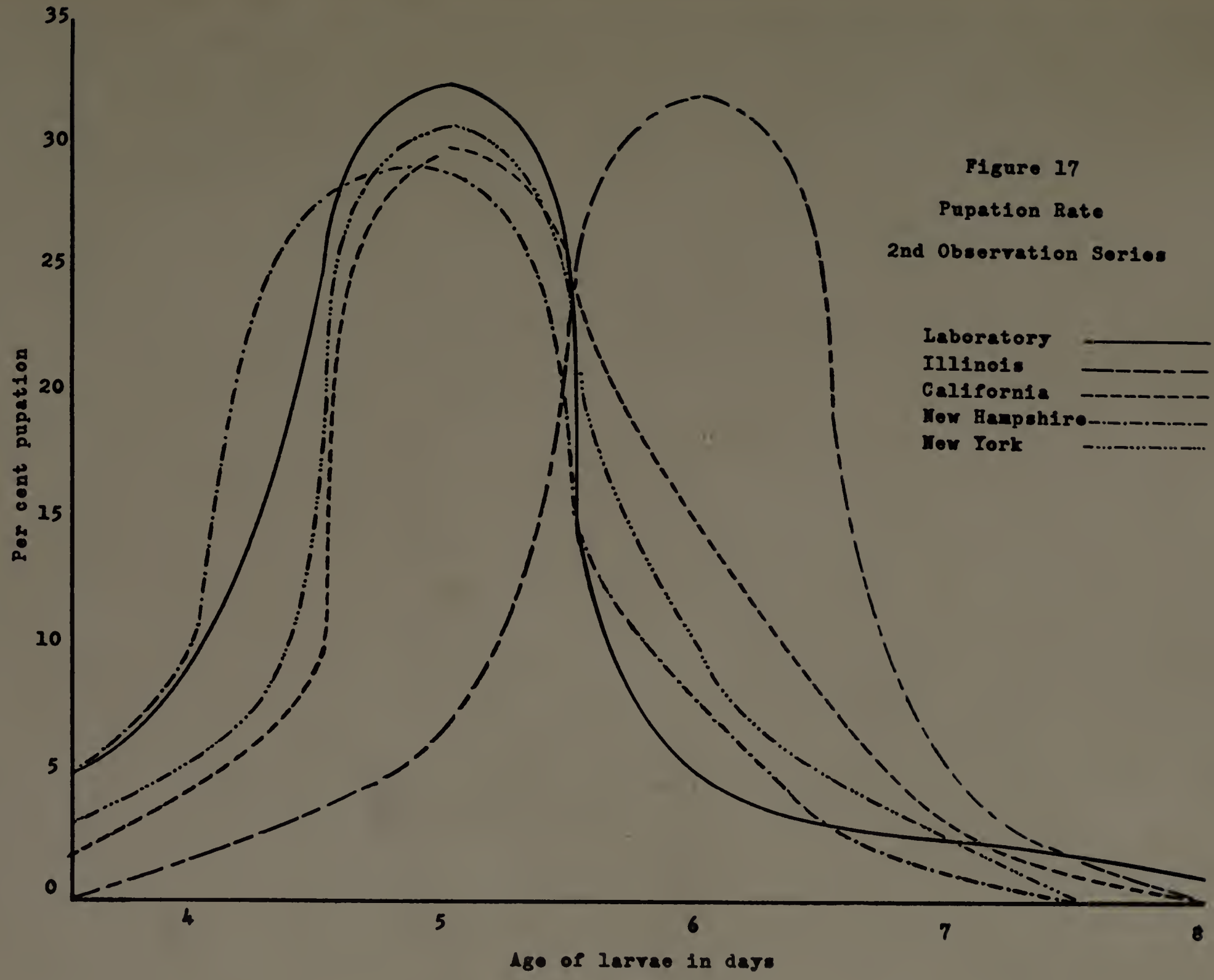


Figure 17

Pupation Rate

2nd Observation Series

change indicated however from the foregoing series of observations (Fig. 16) has been in the comparatively marked reduction in length of larval stage in both the New York and California strains. The peak of pupation in both strains occurs about 12 hours earlier and coincides approximately with that of the Laboratory strain.

In view of the above shortening of life cycles in the New York and California strains and concomitant increase in length in the New Hampshire strain and of the suspected lower level of tolerance in the New York and California strains tolerance level determinations were carried out. A series of fifteen exposures effected as in previous tolerance level determinations placed the average mortality levels for each of the strains as follows:

Illinois	6.0 per cent
California	28.0 " "
New York	55.8 " "
New Hampshire	66.0 " "
Laboratory	92.0 " "

It is apparent that the tolerance level of the Illinois strain has remained relatively constant. Whereas the Laboratory strain can hardly be discussed in terms of tolerance nevertheless its susceptibility and conversely its tolerance has remained comparatively unchanged. The

tolerance of both the New York and California strains has decreased markedly, indicating an approximate doubling of the former respective susceptibility levels. The New Hampshire strain has declined in tolerance to DDT but not as markedly as the New York and California strains.

In conjunction with these two sets of observations (Figs. 16 and 17) notes were taken relative to the comparative length of the complete life cycle of each strain. No differences in length were detected between strains for either of the other metamorphic life stages. The total length of time elapsed during the completion of a life cycle, i.e., from egg to egg, varied between strains approximately only as above indicated in the respective larval stages. From these observations it would appear that variation in life cycles between resistant strains and the normal or Laboratory strain evolves essentially from differences in the length of the respective larval stages.

The variation in length of life cycle and expressed tolerance to DDT encountered between the two foregoing series of observations may be considered from several aspects. Evaluation of these differences in terms of DDT resistance however incurs consideration of factors other than life cycles and tolerance to DDT.

The inception of resistance to DDT, or to benzene-hexachloride as in the New Hampshire strain, has presumably been fostered in consequence of exposure of the flies to the toxicant concerned. Tolerance has been retained in some strains and lost in others when exposure to the conditioning material has ceased.

During the fifty-third generation of a special DDT-resistant colony of house flies developed at the U.S.D.A. Laboratory at Orlando, Florida (King and Gahan, 1949) a subcolony was started (King, 1950) in which the flies were no longer exposed to DDT. Specific indications of marked decline in resistance in the 9th and 14th generations of this side-chain colony were detected by laboratory tests. At the same time the tolerance of the original culture, continually exposed to DDT during the interim, was from 4 to 11 times greater than that of this subcolony. A considerable loss in tolerance had developed in this new group, a loss apparently induced by cessation of exposure to DDT.

The original stock of Ellenville flies (Barber and Schmitt, 1948) was not subjected to selection. The degree of resistance declined markedly in succeeding generations. Beginning with the 18th generation a new line of flies was obtained (letter dated June 21, 1949) by selecting

survivors which had withstood exposure to residual deposits of the pp' isomer from acetone on plywood at the rate of two milligrams per square inch. The tolerance expressed by this new line was equivalent to that of the original Ellenville colony and did not decline through six generations.

The development of benzenehexachloride resistance in the New Hampshire strain (Blickle et al, 1948) was brought about by accidental exposure to this compound. Continued exposure in consequence of contamination nurtured this resistance through 28 generations. No data are available other than herein presented concerning the stability of this resistance in the absence of exposure to BHC. After 20 generations had been reared without exposure at this laboratory there was a marked decline in tolerance to DDT. For reasons previously indicated, tolerance to BHC was not determined.

In contrast to the foregoing March and Metcalf (1949) found that the high degree of resistance to DDT in the Bellflower strain was maintained without exposure to DDT for over 15 generations in the laboratory. However tolerance determinations conducted in conjunction with the second series of larval stage observations (Fig. 17) indicate a marked decrease in tolerance after being reared

without exposure at this laboratory through 16 generations beyond this level.

Along the same general line of development Bruce (1949) reported that a highly purified resistant line showed no significant change in resistance through 17 generations reared in absence of DDT exposure. The same level of tolerance was retained through 22 additional generations reared without exposure at this laboratory. Bruce did find however that a less resistant strain showed a marked loss in resistance after six generations had been reared without exposure.

Two phases of the resistance complex appear to be represented in the above behavior of tolerance in the different strains. The first phase, including the New York, New Hampshire, and California strains, loses its resistance when deprived of exposure to the conditioning toxicant. The California strain maintained its resistance without exposure to DDT for 15 generations prior to rearing at this laboratory and through an additional 16 generations of rearing at this laboratory. Subsequent to 34 generations of rearing at this laboratory however a marked decline in tolerance was noted.

In line with reasoning proposed in discussion of tolerance induced by successive backcrosses, it appears

probable that the New York and New Hampshire strains, displaying relatively low initial resistance to DDT, had not become extensively adapted to DDT. In the absence of exposure to this toxicant a regressive but gradual loss of expression has taken place in the factors governing resistance expression.

This regression may likewise be applied to the California strain. In this instance however the higher initial level of tolerance would indicate that a more extensive degree of adaptation had taken place. Two aspects of tolerance must be considered however in application of this regression concept to the California strain. In the first place a marked decline in tolerance has taken place in this strain. Secondly a considerable degree of resistance still remains. The original level of resistance probably was the aggregate expression of the cumulative effect of several factors governing the expression of resistance. If a portion of these factors, because of lack of exposure to the toxicant, gradually lost their adaptiveness to the compound, an attendant loss in resistance ensues. The degree of resistance remaining is ascribed to those factors which, having become adapted to the toxicant concurrent with the original expression of tolerance, have not as yet lost this adaptation despite lack of exposure to DDT.

Upon this premise then the original level of tolerance would be the aggregate expression of the cumulative effect of those factors which had become adapted to DDT as a part of their environment. Since several factors must have become adapted in the original expression of resistance and since only a portion of this resistance has been lost, the degree of resistance now remaining probably is the cumulative effect of those adapted factors, as yet non-reverted, which express themselves collectively.

No sharp line of distinction may be drawn demarking the California strain from the second phase of resistance above indicated. Whereas the foregoing strains each represent a type in which original resistance has been gradually reduced because of relatively unstable adaptation to the toxicant concerned, the second type, exemplified by the Illinois strain, has indicated no decline in tolerance. Originally obtained as a highly purified strain with a high degree of resistance to DDT, this strain may be construed as representing a resistance complex in which a high degree of adaptation had been effected. This resistance had been progressively increased generation by generation. Attendant therewith a step by step progressive adaptation had been effected. When the peak of resistance was expressed and the most extensive degree of adaptation had been accomplished a multi-factor cumulative complex had

become established as the degree of resistance expressed. Since there are numerous genes involved in a multi-factor complex as here proposed, a reversion process concomitant to loss of resistance would be accomplished gradually through regression to the normal status of the factors concerned. Since the Illinois strain has been maintained as a pure inbred line this process of regression probably would proceed slowly. On the basis of observations herein noted no appreciable degree of loss of tolerance and presumably no appreciable degree of regression has yet occurred in this strain.

A matter of considerable interest in the problem of resistance in the house fly is the apparent longer life cycle (March and Lewallen, 1950) conjoined with resistant strains. Whereas the present portion of this study would, to some extent, support this association an evaluation of the data presented (Tables VI and VII; Figs. 16 and 17) affords consideration of two contrasting lines of reasoning. It is noted that a decrease in the length of the larval stage of development in the New York and California strains (Figs. 16 and 17) has been accompanied by a reduction in tolerance to DDT. However the length of the larval cycle in the New Hampshire strain has increased slightly and has been accompanied by a marked decline in tolerance.

It would appear that resistance may be accompanied by longer than normal life cycles. However since the converse is also true in this study, in the case of the New Hampshire strain, it is suggested that life cycles are not of necessity associated with resistance to insecticides.

A further interpretation of differences in length of larval stage and consequently of life cycles evolves from consideration of environment and its ecology. Earlier in this study it was indicated that the optimum length of life cycle for different strains was not necessarily the same. Despite uniform rearing for all strains, propagated as inbred cultures and therefore devoid as far as known of the introduction of any element of digression from this uniformity, a gradual shift in length of life cycles has nevertheless been noted. Such a shift would indicate that the rearing environment was not optimum and that preference for a life cycle other than that being experienced was being expressed by the strains concerned. It may be concluded that a longer life history associated with resistance is actually the response of the individual strain to the environmental conditions to which subjected and that association with resistance is only coincidental.

Whereas resistance and length of life cycle have herein been dissociated from any interdependence, it is recognized that certain environmental stimuli may become conjointly resolved into an ecological niche favoring a longer life cycle and retention of the level of tolerance then being expressed. Such a combination however is produced by the expression of ecological factors upon two dissociated phases which as a result appear to be interrelated.

CONCLUSIONS

No effect upon hatching of eggs of either resistant strain or laboratory strain was induced by treatment with acetone solutions of DDT, DFDT, TDE, methoxychlor or di-tolyl trichloroethane.

The relative resistance to residual deposits of DDT and its analogs expressed by larvae of the different resistant strains was directly correlated to the degree of relative resistance to DDT expressed by the adults.

Emergence from puparia and survival for 48 hours was markedly inhibited by treatment with DDT and its analogs. All resistant strains, including BHC resistant New Hampshire strain, showed greater tolerance than the laboratory strain. No appreciable difference was observed in the response of the various resistant strains to any particular treatment.

There did not appear to be any correlation between pupal weight and susceptibility of the emerging adult to residual applications of DDT and its analogs.

Relative resistance of adults to DDT dispersed as a continuous phase aerosol coincided with relative resistance reported by other techniques.

In addition to being carried by both sexes, the resistance factor in the Illinois and California strains appeared to be carried to a greater extent or to have been capable of greater expression in the male sex.

The origin of resistance, i.e., whether induced by field applications or by artificial laboratory selection, did not appear to influence the pattern of transmission of the resistance complex.

Apart from the actual level of filial tolerance expressed, there was no difference in transmission of resistance whether from crosses between resistant and non-resistant or between two resistant strains.

Secondary resistance to DDT exhibited by the BHC resistant strain and primary resistance to DDT expressed by the remaining strains appeared to be equivalently transmitted.

The degree of resistance to DDT inherent within the DDT resistant strains was progressively reduced with each generation of cross breeding with a non-resistant strain.

When the progeny of a cross between resistant and non-resistant strains were backcrossed to the resistant parent the level of tolerance in the offspring was progressively increased with each successive backcross.

Tolerance was reduced by cross breeding more rapidly than it was increased, the greatest change in tolerance in both instances occurring in the first three generations of backcrosses.

The factors governing expression of resistance appeared to be present in both resistant and susceptible strains. Because of certain ecological variants, including or followed by exposure to toxicants, to which the now resistant strain became adapted, this strain was capable of resistance expression. Devoid of such exposures and subsequent adaptation the susceptible strain remained non-resistant.

Of four resistant strains deprived of exposure to the conditioning toxicant three gradually declined in tolerance. The fourth strain to date has not so declined. Prolonged maintenance of a static level of resistance within an inbred resistant strain devoid of exposure to the conditioning toxicant does not seem probable.

The levels of resistance expressed by the resistant strains appeared to be the composite expression of several factors, individually but cumulatively adapted to environmental stimuli, which in quantitative equilibrium with the ecological niche to which exposed, were resolved

into an aggregate complex capable of transmission. Devoid of exposure to a conditioning toxicant the quantitative adaptation in excess of equilibrium retrogressed to normal with an accompanying decline in tolerance.

Longer than normal life cycles in certain resistant strains were derived from longer larval stages of development and appeared to be responses of the strains to environmental conditions to which exposed rather than indispensible phases of the resistance complex.

SUMMARY

The relative tolerance to DDT and its analogs has been compared in the immature life stages of three DDT resistant, one BHC resistant, and one laboratory strain of house flies. Comparative resistance of adults to DDT has been determined.

Eggs were immersed in 10 per cent solutions in acetone of DDT, DFDT, TDE, methoxychlor, and di-tolyl trichloroethane. Percentage hatching did not vary appreciably between strains nor between treatments. No degree of resistance was detected in eggs of either resistant strain.

Larvae were subjected to residual deposits of each of the foregoing solutions. Effectiveness was computed in terms of per cent emergence from the puparia. DFDT was the most toxic while DDT appeared the least so. The remaining analogs were intermediate in effectiveness. Tolerance to these residual deposits exhibited by the larvae followed the same pattern expressed by the respective adults to DDT in continuous aerosol phase. Larvae of the Illinois strain were the most tolerant, followed in decreasing order by the California, New York, New Hampshire, and Laboratory strains.

Pupae from each of the strains were immersed in each of the above solutions for 15 to 30 seconds and then placed on moistened filter paper in petri dishes. Effectiveness of treatments was computed from the per cent emergence and survival for 48 hours of adults. A marked toxicity to the pupal stage of all strains was displayed by all compounds, a greater susceptibility being indicated by the laboratory strain than by either resistant strain. The response of each resistant strain to any particular treatment was approximately equivalent, the BHC resistant New Hampshire strain achieving a rate of emergence equal to that of either DDT resistant strain. DFDT was far more toxic than any other compound to the pupal stage. No difference in toxicity was noted between the remaining treatments. The average puparial weights of the different strains varied from 17.6 mg. to 22.3 mg. per puparium. No correlation could be detected between the puparial weight of either strain and percentage emergence of adults.

Levels of resistance in terms of multiples of the laboratory strain currently reported for resistant populations in various parts of the world did not lend themselves to direct comparison with each other nor with the laboratory strain. The relative mortality levels

consequently established from mortality induced by exposure to DDT in continuous aerosol phase were Illinois 7.6, California 14.5, New York 28.5, New Hampshire 44.4, and Laboratory 94.0 per cent.

The progeny of crosses between male Illinois or California flies and females from either of the remaining strains expressed a greater tolerance for DDT than progeny of the reciprocal crosses. Maintained as an inbred filial culture, this level of resistance remained constant throughout eight filial generations. This difference between the tolerance expressed by progeny of the cross and reciprocal cross was expressed to a lesser degree in consequence of crosses between the Illinois and California strains.

With the exception of the foregoing, the pattern of transmission of tolerance was similar whether evolving from crosses between a resistant strain and laboratory strain or between two resistant strains. The tolerance of the F_1 generation was below a level midway between the parental levels. All strains, regardless of the apparent mode of inception of DDT resistance, conformed to this pattern. The actual level of tolerance expressed by the F_1 generation appeared to be dependent only on the level of tolerance inherent in the parental strains. The BHC

resistant New Hampshire strain, with a secondary lesser resistance to DDT, transmitted its tolerance for DDT in the same manner as other strains primarily resistant to DDT.

When the Illinois strain was crossed with the Laboratory strain the tolerance of the F_1 generation was just below the midpoint level. By successively back-crossing the progeny with the Laboratory strain the level of tolerance was progressively reduced. The greatest change in level of tolerance was noted in the first three generations, followed by a more gradual loss with each successive backcross. By reciprocal backcrosses of the F_1 generation to the Illinois strain the level of tolerance was gradually increased, the greatest change in tolerance being noted in the first three generations. The loss of tolerance in consequence of backcrosses seemed to proceed at a more rapid rate than the increase in tolerance induced by equivalent reciprocal backcrosses.

During the early part of this study the length of the life cycle of the Illinois, California, and New York strains was observed to be longer than that of the Laboratory, while the New Hampshire strain was considerably shorter. This difference was attributed to longer larval

stages of development, there being no differences in length of the other developmental stages noted. Approximately eight months later the length of the life cycle of the California and New York strains had shortened to approximately coincide with that of the Laboratory strain. The New Hampshire strain meanwhile had increased slightly in length. Relative levels of resistance determined at this time indicated a marked reduction in tolerance in the California, New York, and New Hampshire strains. The length of the life cycle of the Illinois strain had remained constant as had its tolerance to DDT.

LITERATURE CITED

Anonymous

1947 Peet-Grady method.
Blue Book, Soap and Sanitary Chemicals: 207-210.

Abbott, W. S.

1925 A method of computing the effectiveness of an insecticide. Jour. Econ. Ent. 18:265-7.

Babers, Frank H.

1949 Development of insect resistance to insecticides.
U.S.D.A., Agr. Res. Adm., B.E.P.Q., E-776, 31 pp.

Barber, George W. and John B. Schmitt

1948 House flies resistant to DDT residual sprays.
N. J. Agr. Expt. Sta. Bul. 742, 8 pp.

1949a Further studies on resistance to DDT in the housefly. Jour. Econ. Ent. 42:287-292.

1949b A line of houseflies resistant to methoxychlor.
Jour. Econ. Ent. 42:844-5.

Belding, David L.

1942 Textbook of clinical parasitology.
Appleton-Century-Crofts, Inc. New York. 888 pp.

Bishopp, F. C.

1946 The insecticide situation.
Jour. Econ. Ent. 39:449-59.

Blickle, Robert L. et al

1948 Insecticide resistant houseflies.
Soap and Sanitary Chemicals 24 (8):139,141,149.

Boyce, A. M. and C. A. Persing

1939 Tartar emetic in the control of citrus thrips on lemons. Jour. Econ. Ent. 32:153.

Breakey, E. P. and A. C. Miller

1935 A method for comparing the ovicidal properties of contact insecticides.
Jour. Econ. Ent. 28:353-8.

Bruce, W. N.

1949 Latest report on fly control.
Pests and their Control 17 (6):7, 28.

1950 Current report on housefly resistance.
Pest Control 18 (4):9-10, 19.

Chandler, Asa C.

1949 Introduction to parasitology.
John Wiley and Sons, Inc., New York. 756 pp., refr. p. 231.

Davidson, W. M. and A. Jones

1931 Change in toxicity of rotenone in solution and suspension. Jour. Econ. Ent. 24:257-262.

Decker, George C.

1950 "Hell-fire" on fly resistance.
Pest Control 18 (4):11, 16.

Dickson, R. C.

1941 Inheritance of resistance to hydrocyanic acid fumigation in the California red scale.
Hilgardia 13:515-21.

Ebeling, Walter

1950 Subtropical entomology. Lithotype Process Co., San Francisco, Calif. 747 pp., refr. p. 147.

Ferguson, J.

1939 The use of chemical potentials as indices of toxicity.
Proc. Roy. Soc. (London) B. 127:387-404.

Fraenkel, G. and K. M. Rudall

1940 A study of the physical and chemical properties of the insect cuticle.
Proc. Roy. Soc. (London) B. 129:1-35.

Frear, D. E. H.

1948 Chemistry of insecticides, fungicides and herbicides. 2nd Ed., D. Van Nostrand Co., New York. 417 pp., refr. p. 75.

Gough, H. C.

1939 Factors affecting the resistance of the flour beetle Tribolium confusum Duv. to HCN. Ann. Appl. Biol. 26:533-571.

Hansens, Elton J. et al

1948 Resistance of houseflies to residual applications of DDT in New Jersey. Jour. Econ.Ent. 41:302.

Hansens, Elton J. and A. H. Godin

1949 Reaction of certain fly strains to DDT and methoxychlor deposits. Jour. Econ.Ent. 42:843.

Haseman, Leonard and Virgil F. Burk

1929 A determination of the lethal dosage of arsenic for Missouri and Colorado codling moth larvae. Jour. Econ. Ent. 22:655-6.

Haseman, Leonard and R. L. Meffert

1933 Are we developing strains of codling moths resistant to arsenic? Missouri Agr. Expt. Sta. Res. Bul. 202, 11 pp.

Hough, Walter S.

1928 Relative resistance to arsenical poisoning of two codling moth strains. Jour. Econ. Ent. 21:325-9.

1929 Studies of the relative resistance to arsenical poisoning of different strains of codling moth larvae. Jour. Agr. Res. 38:245-56.

1934 Colorado and Virginia strains of the codling moth in relation to their ability to enter sprayed and unsprayed apples. Jour. Agr. Res. 48:533-53.

1943 Development and characteristics of vigorous or resistant strains of codling moth. Virginia Agr. Expt. Sta. Tech. Bul. 91:32 pp.

Johnstone, H. F.

1950 Properties and behavior of air contaminants. Ind. Med. and Surg. 19:106-15.

Jones, H. A. and W. M. Davidson

1931 Preparations containing rotenone for use as insecticides. I. Aqueous suspensions. Jour. Econ. Ent. 24:244-57.

Kalmus, H.

1942 Differences in resistance to toxic substances shown by different body color mutants in Drosophila (Diptera).
Proc. R. Ent. Soc. (A) 17. pts. 10-12:127-34.

King, W. V. and J. B. Gahan

1949 Failure of DDT to control house flies.
Jour. Econ. Ent. 42:405-9.

King, W. V.

1950 DDT resistant house flies and mosquitos.
Jour. Econ. Ent. 43:527-32.

Knipling, E. F.

1942 Acquired resistance to phenothiazene by larvae of the primary screw worm.
Jour. Econ. Ent. 35:63-64.

Läuger, P., H. Martin, and P. Müller

1944 Uber Konstitution and toxische Wirkung von naturlichen und neuen synthetischen insectentotenden Stoffen. Helv. Chem. Acta. 27:892-928.
Translated by Geigy Co., New York. 1945.
(Translation seen)

Lindquist, A. H. and H. G. Wilson
1948 Development of a strain of houseflies resistant to DDT. Sci. 107:276.

McDuffie, W. C. et al
1946 Control of fly larvae in simulated pit latrines and in carcasses. Jour.Econ. Ent. 39:743-9.

McGovran, E. R. et al
1939 Resistance to insecticides. The effect of knock-down on the resistance to house flies to pyrethrum sprays.
Soap and Sanit. Chem. 15 (8):88-90.
1944 Toxicity of DDT to bedbugs, cockroaches, the Mexican bean beetle and house fly larvae.
Jour. Econ. Ent. 37:139-40.

McGregor, E. A.
1944 Notes on the resistance of citrus thrips to tartar emetic.
Calif. Citrog. 29 (3):62. (Original not seen)

March, Ralph B. and R. L. Metcalf
1949 Laboratory and field studies of DDT resistant houseflies in Southern California.
Bull. Dept. Agr., Sta. Calif. 38:93-101.

March, Ralph B. and Lawrence L. Lewallen

1950 A comparison of DDT-resistant and non-resistant house flies. Jour. Econ. Ent. 43:721-2.

Martin, Hubert

1946 Insecticides: chemical constitution and toxicity. Jour. Soc. Chem. Ind. (London) 65:402-45.

Martin, H. and R. L. Wain

1944 Insecticidal action of DDT. Nature 154:512-3.

Matheson, Robert

1950 Medical entomology. Comstock Publishing Co., Inc., Ithaca, New York. 2nd Ed. 612 pp., refr. 459-93.

Melander, A. L.

1914 Can insects become resistant to sprays? Jour. Econ. Ent. 7:167-72.

Metcalf, Robert L.

1948a Some insecticidal properties of fluorine analogs of DDT. Jour. Econ. Ent. 41:416-21.

1948b The mode of action of organic insecticides. Chemical-Biological Coordination Center, Rev. No. 1. National Research Council, Wash., D. C. 47-64.

Meyer, Kurt and H. Hemmi

1935 Beiträge zur Theorie der Narkose. III. Biochem. Z. 277:39.

Missiroli, A.

1947 Riduzione o eradicagione degli anofeli. Riv. di Parassitol. 8 (2/3):141-69.

Mosna, E.

1947 Su una caratteristica biologica del Culez pipiens autogenicus di Latina. Riv. di Parassitol. 8 (2/3):125-6.

O'Kane, W. C. et al

1941 An insect toximeter. Studies of contact insecticides XV. N. H. Agr. Expt. Sta. Tech, Bul. 76: 10 pp.

Olson, Theodore and Raymond G. Dahms

1945 Control of housefly breeding in partly digested sewage sludge. Jour. Econ. Ent. 38:602-4.

Prill, E. A. et al

1945 Insecticidal activity of some alkoxy analogs of DDT. Science 101:464-5.

Quayle, H. J.

1916 Are scales becoming resistant to fumigation? Calif. Univ., Jour. Agr. 3:333-58.

1922 Resistance of certain scale insects in certain localities to hydrocyanic acid fumigation. Jour. Econ. Ent. 15:400-4.

1938 The development of resistance to hydrocyanic acid in certain scale insects. Hilgardia 11:183-210.

1942 A physiological difference in the two races of red scale and its relation in tolerance to HCN. Jour. Econ. Ent. 35;813-6.

1943 The increase in resistance in insects to insecticides. Jour. Econ. Ent. 36:493-500.

Richardson, Henry H.

1943 Toxicity of derris, nicotine, and other insecticides to eggs of the house fly and the Angoumois grain moth. Jour. Econ. Ent. 36:729-731.

Roark, R. C.

1942 Definition of aerosol. Jour. Econ. Ent. 45:105-6.

Sacca, Giuseppe

1947 Sull' esistenza di mosche domestiche resistenti al DDT. Riv. di Parassitol. 8 (2/3):127-8. Nota preliminare. 1st super. di Sanit.Rend. 10:923-4.

Shaw, Frank R. and A. I. Bourne

1946 The abundance and control of flies on a college campus. Jour. Econ. Ent. 39:543-4.

Siegler, E. H. and S. I. Gertler

1944 Toxicity of diaryl trichloroethanes and dichloroethylenes to codling moth. Jour. Econ. Ent. 39:845.

Simmons, S. W. and M. Wright

1944 The use of DDT in the treatment of manure for fly control. Jour. Econ. Ent. 37:135.

Spear, Philip J.

1950 Laboratory tests with insecticides dispersed from the American Aerovap. Unpublished thesis, Univ. of Massachusetts. 94 pp. May, 1950.

Welsh, John H. and Harold T. Gordon

1947 The mode of action of certain insecticides on the arthropod nerve axon. Jour. Cell. and Comp. Physiol. 30:147-72.

Wiesmann, R. von

1947 Untersuchungen über das physiologische Verhalten von Musca domestica L. verschiedener Provenienzen. Schweiz. Ent. Gesell. Mitt. 20:484-504. (Original not seen)

Wilson, H. G. and J. B. Gahan

1948 Susceptibility of DDT-resistant houseflies to other insecticidal sprays. Science 107:276-7.

Woglum, R. S.

1925 Observations on insects developing immunity to insecticides. Jour. Econ. Ent. 18:593-7.

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