BIOMETRICAL GENETICS OF INSECTICIDE RESISTANCE - A QUANTAL RESPONSE CHARACTER

NARENDRA NATH SINGH

Thesis submitted to the Indian Statistical Institute
for the award of the degree of Doctor of Philosophy

ndian Statistical Ins itute
New Delh
1976

BIOMETRICAL GENETICS OF INSECTICIDE RESISTANCE — A QUANTAL RESPONSE CHARACTER

NARENDRA NATH SINGH

Thesis submitted to the Indian Statistical Institute
for the award of the degree of Doctor of Philosophy

Indian Statistical Institute
New Delhi
1976

ACKNOWLEDGEMENTS

This thesis contains the work done at the Indian Statistical Institute, Delhi Centre (ISI-DC) and at the National Institute of Communicable Diseases (NICD). I shall always respectfully remember my associations with the ISI-DC and the NICD.

My work at the ISI-DC became possible due to the kind interest, encouragement and guidance of Dr.C.R. Rao, Sc.D., F.N.A., F.R.S. I shall ever remain grateful and indebted to him.

Professor Sujit Kumar Mitra, M.Sc., Ph.D. of the Indian Statistical Institute was my thesis adviser and guide. For his guidance, direction and availability during my thesis work I express my sincere appreciation and gratitude to him. I shall remain indebted to him for his affections for me and interest in my work.

The experimental work was carried out at the NICD. I express my gratitude to LT.Col.Jaswant Singh, M.B., Ch.B, D.T.M.& H.; Dr.S.P. Ramakrishna, M.B.B.S., D.P.H., D.Sc. and Dr.M.I.D. Sharma, M.B.B.S., M.P.H., who at different times served as the Director of the NICD, for giving me all facilities to carryout the experimental work and for their valuable suggestions and encouragements. I would like to extend my special thanks to Dr.R.Pal, M.Sc., Ph.D., D.Sc., now Scientist Biologist of W.H.O., for his suggestions and direction in the genetical work with C.p.fatigans. I owe an abundant thanks to Shri B.N. Mohan for giving me experimental material, help and background information which I utilised for the studies presented in Chapters II to IV of this thesis.

I shall ever remain grateful to Dr.A.K. Chakrabarti,M.B.,D.P.H. for his inspiration and guidance that shaped my research carrier. The development of my interest in Bio-statistics was mainly due to him.

I sincerely record my appreciation to my friends Dr.M.Das,M.Sc., Ph.D. and Dr.R.L. Kalra, M.Sc., Ph.D for their help and suggestion in my work. I owe thanks to my wife Mrs. Aruna Singh for her moral support, concern and prayers.

My appreciations are also due to Shri C.L. Bhagat of the ISI-DC for typing the manuscript.

Finally I thank all the junior level workers of the vector control laboratory of the NICD. They remained unknown though had worked with devotion and had collectively made significant contribution towards focusing the problem of resistance and its understanding from the genetical angle. I also record my sincere thanks to Shri T.K. Aikat, M.Sc., for his encouragement in my thesis work.

C O N T E N T S

CHAPTER 1

INTRODUCTION

1.1 Geneses of the Problems

1.1.1 Resistance - a problem in the fields of public health, agriculture, and animal husbandry

Discovery of the synthetic insecticides of residual toxicity and their availability for civilian use after the World War II had presented a bright prospect of freedom from insect borne diseases. Limited field trials of chlorinated hydrocarbon insecticides in the sphere of public health had resulted in decline of the vector/pest populations by 'crisis' and consequently the incidence of vector borne diseases recorded dramatic falls. Inspired by such good results different countries and World Health Organization (WHO) embarked upon the adventure of national/global vector and disease control/eradication programmes. The malaria control/eradication, filariasis control and mosquito abatement programmes are the examples of such adventures. In the initial periods of their executions, the programmes gave impressive results, but sooner than expected they ran into troubles. All hopes turned into despair because of reversal of the downard trends of insect population and of the incidences of vector borne diseases due to development of insecticide resistance in the vector species. In India application of insecticides like DDT and BHC had to be given up for the filariasis control as the filaria vector Culex pipiens fatigans became resistant to the hydrocarbon insecticides. These insecticides also became in-effective, in some areas, against the resistant strain of important malaria vectors Anopheles culicifacies and A.stephensi. Development of resistance in these species had resulted

in recrudescence of malaria in many parts of India, which is now of great concern. Resistance in insects to insecticides is thus a challenging problem in the field of public health. It could also be a problem in the fields of animal husbandry, agriculture and food storage where insecticides were also used to control the insects. In fact resistance was first reported in insects of agricultural importance in 1908. By 1958 at least 30 species of insects were reported resistant. The review by Brown (1971) showed that 130 species of agricultural importance were resistant to insecticides. Early solution of the problem of resistance, therefore, is of great importance for prevention of diseases in man and domestic animals, for maintaining the achievements in health standards, and agricultural productions. Attempts were made to solve the problem of resistance by alternate applications of different groups of insecticides or by substitution of the obsolete insecticide by a potent one; this did not offer permanent solution since the target population of a species developed resistance also to newer insecticide(s). Resistance thus is rather a rule than exception. It is felt that successful solution of this problem, if it exists, could be found only through comprehension of the phenomenon by basic studies involving several disciplines at the same time.

1.1.2 Problem of Methodology for Genetical Studies of Resistance

In the multi-disciplinary approach genetics had a pivotal role because resistance is a heritable character. The genes for resistance could control, independently or through interactions or linear additions of their effects, the functioning of protective physiological system or behaviour pattern of the individuals of a species giving rise to the

phenomenon. The problem in the genetical study of physiological resistance, here-in-after called resistance which is distinct and separate from behaviouristic resistance, is a methodological one. Resistance is a metrical character and at times it is just not possible to assign the individuals to mutually exclusive and exhaustive phenotype classes. The classical Mendelian methods developed for genetical study of qualitative character are, therefore, not suitable for genetic studies of resistance. The models and methods of biometrical genetics, developed through the fusion of genetical and biometrical methods for the study of directly measurable gene-controlled quantitative character giving continuous variations, can not also be directly used since resistance is a quantal response character indirectly estimated through the stimulus-response data.

It is, thus, found that the applications of residual insecticides for control of insects had precipitated a new challenging problem of insecticide resistance. Resistance in its turn had surfaced out the need for developing theoretically sound genetic models and a best method for estimation of parameters involved in them.

1.2 Present Status of Knowledge and Lacunae

1.2.1 Methods and Techniques for Susceptibility Tests - Their Scope and Limitations

The first requirement of approaching the problem of insecticide resistance was of a simple, sensitive, reliable and valid method and technique for detection, characterization and quantification of resistance. The WHO had contributed significantly towards this direction; with the collaboration of scientists working in different laboratories, the WHO

has brought out standard susceptibility test kits and designed methods for estimation of susceptibility status and resistance levels in arthropods of medical importance (WHO 1960). It was expected that the results on the susceptibility of mosquito species obtained by the WHO kits and methods would be comparable with the results obtained earlier by the Busvine and Nash (1953) method. Further the results obtained by the use of WHO technique and methods by different workers would also be comparable.

The susceptibility/resistance test method was basically similar to the test methods used in bio-assay of insecticides based on quartal response; the only difference being that in the former two insect-populations are tested against one insecticide where as in the latter one population is tested against two insecticides. Resistance is estimated as ratio of mean response (in actual dose unit) of the candidate population to that of the reference population. In order to estimate the means of responses of two populations a series of doses of insecticides, spaced at equal log interval, are provided in the WHO test kits. These doses were found to cause mortalities below and above 50.0 percent in the reference (susceptible) populations. But in case of a highly resistant population even the highest dose provided in the WHO kit had failed to cause mortality even upto 50.0 percent level. Such a situation is dealt with by using higher dose of insecticide prepared locally. Alternatively, the exposure time could be increased and lethal time required to cause 50 percent mortality, (LT_{50}), at a given dose of insecticide could be estimated since the uptake of insecticide was found proportional to the duration of exposure and to the dose of the insecticide (Pennel et al 1964, Ariaratnam and Brown 1969).

It was found that the product defining the dose (concentration x time) was a constant whether it was determined from the LD_{50} or the LT_{50}. (Busvine; 1958b; Hamon 1963b; Garms and Rehm 1961). However, with a highly resistant strain the increase in the dose could give exaggerated resistance ratio as observed by Busvine (1957b) and Crow (1954). The exaggerated estimate of resistance could slightly be improved by using another test method called 'topical application method'. This method involved in application of fixed micro quantity of insecticide solution on the dorsal surface of immobilised insects. The group of insects treated with varying doses of insecticide are scored for response after 24 hrs of 'reaction period'. The topical application method was found to give consistant results. The method is more sophisticated and so not handy for field use. It minimises but does not mitigate the problem of exggerated estimate of resistance. The problem of unexaggerated estimate of resistance in a highly resistant population is, therefore, still remaining unsolved. The experience, however, showed that both the WHO standard tests method and topical application method were, usually, useful for studying the genetics of resistance (Brown and Pal 1971, Pal and Singh 1958).

1.2.2 Design of Tests for Genetical Study of Resistance

The designs of susceptibility tests used in genetical studies involved the use of 'scalar doses' and 'discriminating doses'. In the 'scalar dose' design batches of insects are tested at a series of increasing doses, usually spaced at equal log intervals, to obtain graded mortalities below and above 50.0 percent so that LD_{50} and regression

coefficient values could be precisely estimated. In case of highly resistant population such a graded mortality with available doses may not be obtained and hence this method will be of no use for precise estimations of these two statistics. The second limitation of the method is that it will also not yield data for precise estimation of the two statistics, LD_{50} and regression coefficient, for the segregating generations when the constituent genotype/phenotype classes have mutually non-overlapping tolerance distributions, since dose response curve for such a compound populations will be a nonlinear one. Such curve, however, will give informations on the presence of number of phenotype classes having non-overlapping distributions and also help in finding out the levels of doses that will separate the constitutent classes.

The use of one or two levels of doses for seperations of the phenotype classes and for scoring their frequencies in a compound population is known as the 'discriminating dose' design. The data obtained by discriminating dose, however, can be used to test the hypothesis of segregation of resistance as a character controlled by a single Mendelian gene. The discriminating doses are very high as they are LD_{100} of the different phenotype classes. Use of such a high dose(s) will not give information on the involvement of the poly-genes whose contributions towards increased tolerance are small but significant. Hence the limitation of using discriminating dose to get full information on the genetic basis of insecticide resistance.

Both the 'scalar dose' and the 'discriminating dose' designs contain inherent bias. A review by Milani (1956 and 1957) of the results of

genetical studies showed that the inference of complicated multifactorial basis of resistance were usually drawn from the studies based on 'scalar dose'. Results based on the 'discriminating'dose' were frequently associated with the conclusions of monofactorial basis of resistance.

1.2.3 Limitation of Susceptibility test in Genetical Study

Resistance can be detected only from the respose of insects to insecticide and hence susceptibility test to insecticide is unavoidable for detection, measurement and genetical study of resistance. But this method imposes restriction on some aspects of genetical studies. Susceptible insects which were killed at a given dose of insecticide cannot be scored for genotype analysis from their offsprings since it is not possible to obtain progenies from the dead insects. The surviving insects also can not be retested for estimation of their mean tolerance level as their initial susceptibility status would change due to previous exposure to insecticide.

A combination of toxicological methods with the morphological mutations as visible markers for the chromosomes is the most reliable method for genetic study of resistance (Tsukamoto 1963). Such a method is highly effective to get more accurate informations on the number of resistance factor(s)and their linkage relations with other genes. Such method would help to point the locus (loci) of the resistant gene(s) on the chromosomes and hence will help in advancement of our genetical knowledge. In most insect vectors/pests of medical or agricultural importance, however, the method can not be used since mutant markers for many of them are not available yet. Therefore this method has only limited applications.

1.2.4 Methods of Statistical Analysis, Inferences and their Limitations

Insects exposed to or treated with a given dose insecticide, following any of the methods of susceptibility tests (c.f.1.2.1), were found either to die or to survive. The quantal, response to a dose of insecticide, characterised by 'kill' or 'no kill', is not instantaneous; it is materilised after a lapse of time known as 'reaction period'. The reaction of insecticides in the physiological system of insects is completed by 24 hours as indicated by the stable slope of the dose-response regression line and hence the quantal response in the test insects is scored after 24 hours of treatment, known as holding period. Insects found dead at a given dose of insecticide, at the end of holding period, are those whose 'critical' or threshold' levels of tolerances are less than the dose. Obviously the surviving insects have the 'threshhold' level of tolerance higher than the picked up/treating dose. When a randomly selected batch of insects are exposed to/treated with a given dose, the proportion, p', of the individuals independently responding to it could be due to the toxic effect of the dose of insecticide and factors other than the 'dose' of the insecticide. The effect of dose alone is estimated, by what has come to be known as Abbott's formula, as

$$p = \frac{p' - C}{1 - C} \times 100$$

where p = estimated response due only to dose

p' = estimated response, in proportion, observed in a given dose of the insecticide treated group.

C = is the proportion responding in 'control' where all other factors except insecticide were allowed play their roles.

When values of p, obtained from experiments based on scalar dose design, are plotted against log doses a sigmoid curve is generated, suggesting normal distribution of tolerance over log doses. Number of sophiticated statistical methods are available for analysis of such data of which the probit analysis method is the one that found favour among the workers in the field. The probit analysis method for statistical treatment of the quantal response data can be attributed to Goddum (1933) and Bliss (1934a,b; 1935a,b). The former proposed the transformation of quantal response rate, p, to its normal equivalent deviation (NED) and the latter gave the tables of probit and weighting coefficients, where probit was the NED, with mean 5 and sandard deviation 1. Fisher (1935) indicated the maximum likelihood (ML) analysis for zero and 100 percent responses and showed the need for using 'working probits' for all levels of mortality. Finney (1952) in his classical book, 'Probit analysis'. has developed the ML method, and has given the computational plans for its routine use by the workers in the field. By probit analysis method the mean, m, and standard deviation, s, of a population's tolerance to insecticides are estimated from the linear relation of probits and log dose(x) as

$$m = \frac{(5 - a)}{b} \quad \text{and}$$

$$s = \frac{1}{b}$$

where a and b are the ML estimates obtained from the probit and log dose data fitted to

$$Y = a + bx, \text{ and}$$

$$a = \bar{y} - b\bar{x},$$

The estimation of m and s are valid and reliable when the tolerance of a population is normally distributed which is usually true for each of the homozygous susceptible and resistant populations and heterozygous F_1 populations. But in F_2 and backcross (BIP) progenies the normal distributions of tolerance may or may not be true since the gene(s) for resistance /susceptibility will segregate in these generations giving rise to compound populations consisting of two or more phenotype classes, the tolerance of each will be distributed normally. Hence the estimates of mean and standard deviation for the segregating generations may be of no use for genetical inference. For this purpose the pattern of probit/log dose curve, fitted by eye, is used.

The susceptible population and the resistant population, used as parents for crossing experiments in a genetical studies, are characterised by the two estimates, LD_{50} and s, The LD_{50} is the anti log of m and s is inversely proportional to regression coefficient. Similarly the F_1 populations of the reciprocal crosses of the susceptible and resistance populations are characterised. Hoskins and Gordon (1956) in their classical work had shown the effect of progress of insecticide selection on the LD_{50} and regression coefficient values. According to these authors when specific resistance is developed in a population, the probit/log dose regression line becomes flatter as LD_{50} increases. The slope of the line becomes steeper again when the population becomes homogeneous for resistance. They have characterised resistance of vigour tolerance type by slight increase in LD_{50} with increased slope of the probit/log dose regression line.

Hoskins (1959) has given a method for calculations of expected probit/log dose line for a compound population consisting of two or more phenotype classes from the lines characteristic of each class. He has also analysed the lines given by samples of a heterogenous resistant population into their constituent genotypes. Presence of plateaus on the probit/log dose line over the LD_{100} of preceeding susceptible classes and conforming with expected mortality rate based on a single factor segregation are taken as an evidence of monofactorial basis of resistance (Brown and Pal 1971). Such a pattern of dose-response metameter curve will be a necessary but not sufficient proof for monofactorial basis of resistance.

The indepth statistico-genetical study by Tsukamoto (1963) had shown that the shapes of the probit/log dose curves of the segregating generations (F_2 and BIF) are influenced not only by the number of genes but also by the level of resistance exhibited by these genes, dominancy, gene interation etc. Based on the assumption of five resistant genes each contributing 20 times resistance he showed that the probit/log dose curve of F_2 generation would have a plateau around 24 percent mortality (4.3 probit) which would give the impression that resistance was due to a monofactorial dominant system. This showed that the biometric parameters and curves of the segregating generation, by themselves, may not lead to valid genetical inference. For validity of the inferences it is necessary to examine them in the background of results of selection studies and/or of the results of repeated backcrossing (F_1 X appropriate parent genotype) and selection.

1.2.5 Genetical Information - their Convergence, Inconsistencies and Gaps

The field observations on the poor response to insecticide control measures had stimulated studies/researches on

1. technique and methodology for detection, measurement, classification and geographic delimitation of resistance (species-wise) and

2. basic aspects of physiology, biochemistry, biology, toxicology and genetics of resistance.

The results of these studies were published in scientific journals. Six hundred and fifty papers were published by 1957 and this number had increased to more than 1800 by 1975. Number of review papers, collating information on different aspects of resistance, published by 1969 were 67. The WHO monograph entitled 'Insecticide Resistance in Arthropods' by Brown and Pal (1971) and the book on 'Genetics of Insect Vector of Diseases' edited by Wright and Pal (1967) contained exhaustive information on genetics of insecticide resistance and allied fields. A critical reading of these publications and other research/review papers clearly show that resistance in insects is a stable quantal response character. It had developed as a response to the insecticide. The character was heritable one, determined by gene(s). The defence mechanisms against insecticide in a resistant strain were the actions of the resistant gene(s) at the physiological and biochemical levels.

The available information, however, were inconsistent in specific aspects like gene transmission, gene expression and gene action. Scaning of the literature showed that the information on mode inheritance had

bipolar concentrations around polygenic and monofactorial basis of resistance. The expression of resistant gene(s) was, usually, lower in the laboratory selected strain than in the resistant strain evolved in the field. In mosquitoes resistance was usually expressed in both the adult and larval stages but there were few records where its expression was only in one stage of the life cycle (Mamen as quoted by Pal 1958, Sharma and Kalra 1958). Usually resistance was expected and found in both the sexes of a species but in case of houseflies there was a record of resistance limited only to male flies; the female flies remained susceptible to insecticide (Kerr 1960, 1961; Hiroyoshi 1961). The resistant gene allele(s)in some studies was reported as completely or partially recessive to and in others as completely or partially dominant over the susceptible allele(s). There were also reports of no dominance of either types of gene alleles. The actions of resistant allele(s) (specially DDT resistant), as expressed in the physiological and biochemical defence mechanisms against an insecticide, were inconsistant even within a species as could be seen from the reports of different studies based on C.p.fatigans (see pages 192-198 of the monograph by Brown and Pal 1971). There existed a polemic on the theory of evoluation of resistance. Theories like 'induction of resistance by insecticide'(Perry 1961), 'selection of pre-existing resistant gene by insecticide' (Brown 1958), 'lingering modification' Martin 1955) were put forward to explain the evolution of resistant strain of a species due to contamination of the ecosystem with insecticide. Hence it could be concluded that the existing information

were inconsistant, they did not give a clear picture about the genetic basis of resistance. The need for theoretical work for investigation and comprehension of resistance phenomenon in its genetical perspective has also remained open.

Information on the population genetics of resistance were virtually absent. There was also a need for an indepth study of the reproductive system, breeding system, sex-drive, chromosomal system of at least the important vectors/pests. Information on these aspects were also required to understand the genetic system of a species.

1.3 Selection of The Topic, Objective And Contributions

1.3.1 Considerations for selection of the topic

The choice of the topic, Biometrical genetics of insecticide resistance - quantal response character, for this thesis has been prompted by the

1. serious problem created by development of resistance to insecticide in the control of vectors/pests (c.f.1.1.1);
2. need for methodology to study the genetics of insecticide resistance (c.f. 1.1.2);
3. necessity of theoratical comprehension of genetical basis of resistance (c.f. 1.2.5) and
4. author's direct confrontation, in professional capacity, with the problem of insecticide resistance (c.f. VITA).

1.3.2 The objective

It has been aimed in the thesis to presented the systematically collected experimental information on the genetical aspects of insecticide resistance in a meaningfully organised system which could be useful to

1. develop such methods and strategy of vector/pest control that could optimise the benefit of residual insecticide with minimum loss;
2. modify, where needed, the objectives of the use of insecticides;
3. develop improved statistical models and methods for genetical studies of a quantal response character like resistance;
4. fill in some of the gaps in our knowledge on the genetical aspects of resistance and
5. comprehend the gentical basis of resistance, as had emerged, from available information in such a way that would give direction to future work and also focus the newer areas of study.

To achieve the above objectives laboratory studies on selection and inheritance of resistance were carried out with important malaria and filaria vectors. To obtain reliable information, different methods and techniques were used in those studies.

The results of the studies on selection of resistance have been presented in Chapter II. The experimental results on inheritance of specific and non-specific resistance in a malaria vector have been described in Chapters III and IV. The chapter V contains the results of inheritance of DDT resistance in a filaria vector. In Chapter VI a synthesis of the available information has been attempted to comprehend the genetic basis of resistance. Each of the chapter containing the experimental results is divided into six broad sections viz. introduction,

material and methods, results, discussions, summary and references; some of the sections are also divided in sub-sections. The introduction of a chapter contains the need and objective(s) of the study presented in the chapter. In Chapter VI the inconsistant information are indicated, the major sources of inconsistancies and their control are suggested. An attempt has been made to explain the variability of information originating from genetical source. For clearity in communication, the concept and definition of the technical terms used are given at the end of the thesis (c.f. glossary).

1.3.3 Contributions

The results of the different genetical experiments, their statistical analysis and the inferences drawn there on have been summarised in each of first five chapters. The results of synthesis of genetical information have also been presented for comprehension in six statements (Chapter VI). The total contributions of the thesis, which could be considered significant, are briefly outlined here under.

The immediate response to DDT selection in Anopheles stephensi is the development of non-specific resistance of low magnitute (vigour tolerance). It has been followed by, with a time lag, specific high resistance in larvae and then in adults. Multi-resistance extending over BHC and dieldrin has also been indicated. Insecticide resistance has been considered as a complex phenomenon, consisting of non specific low degree of resistance, stage specific high degree of resistance and insecticide group specific high degree of resistance. Development of resistance has been inferred to be a rule rather than exception.

The speed of development of resistance has been fastest when both larvae and adults are exposed to insecticide. The degree of resistance achieved has a limit under a given eco-genetical situation; any increase of degree of resistance beyond the limiting value is not possible even by high insecticidal pressure. Selection of a given degree of resistance is dependent; among other factors, on the size of the population selected; larger the population size greater is the degree of resistance. Withdrawal of insecticidal pressure for eight generations has shown very small loss of resistance under closed breeding but under out-breeding the level of resistance may show reversion.

Insecticide resistance is a heritable character, it is, usually, developed as a response to insecticidal pressure. Insecticidal pressure has been considered to work as a directional selection on the available free-genetical variability. Free variability can however be made available from potential variability present in the population of a species. Natural selection has stabilising effect. It, however, has supported and maintained genetical variability for resistance within a limit.

Insecticide resistance is the expression of resistant gene-alleles borne by the chromosome(s). Each distinct type of resistance is the expression of distinct classes of resistant gene-allele(s). The behaviour of the dose-response curve (Chapter II) and the results of the crossing experiments (Chapter IV) have shown that the 'vigour tolerance' is polygenic. This polygenic system is neither recessive to nor dominant over the alleles for susceptibility. The polygenes are capable of integration reorganisation and segregation. Resistance due

to these gene-alleles are non-specific, they also offer protection against adverse ecological changes. Thus these gene alleles support and maintain the alleles for specific resistance. They are, therefore, of great economic value to the species.

Different types of high specific resistance are the expression of different class of polygenes occuring in clusters. These clusters are formed through the integration and organisation of the available minor gene alleles under insecticidal pressure. (Chapter II). A cluster of resistance may show high variability with respect to the number and/or classes of its constituent members and their organisations. A given resistant cluser slowly develops into a stable state (Chapter II). Such a state or organisation of gene alleles offer better survival chance in an insecticidal contaminated enviourment. A stable cluster of DDT resistance was found to be transmitted like a single Mendelian factor (Chapters III & IV). An unstable resistance cluster would give evidence of polygenic basis of resistance in its transmission. Multi-stage and multiple resistance to different groups of insecticides are due to linkage of different classes of genes in a resistance cluster. The magnitude of genetical variability and the state of dominance of resistance were due to segregation of the constituents of the resistant cluster and cumulative and/or interactive effect of their (sub-units of the cluster) number and dominance respectively. An optimum organisation of the resistant gene-alleles has a better survival value, in an insecticidal contaminated eco-system, than their existance in an unorganised state..

The resistant gene alleles constituting the resistance cluster occupy some space in the chromosome(s). The space is likely to contain other genes also and so they could be linked with each other and one another. The correlated response of other characters, to DDT selection, was due to such allelic linkage. The concept of resistance cluster its composition, expression and function may be considered a reasonable one in view of the theoretical and emperical work on chromosome structure, gene cluster by James Bonner & Jung-Rung (1973), and Gillie & Peto (1969) and others.

The statistical models developed to test the hypothesis of selection of DDT resistance due to single major factor could also be considered a small contribution in the field of population genetics of resistance.

The statements, based on synthesis of the available information, on the

1. Course of development of insecticide resistance;
2. Composition and function of the system/cluster of resistance gene alleles;
3. Chromosomal mechanism and development of resistance;
4. position effect of resistance cluster;
5. reversion and re-selection of resistance and
6. mechanism of evolution insecticide resistant strain.

have been made for comprehension of the genetical basis of insecticide resistance phenomenon in its totality (c.f. Chapter VI).

1.4 Reference

1. Ariaratnam, V. and Brown, A.W.A. (1969) : Bull.Wld.Hlth.Org.40, 561-567

2. Bliss, C.I. (1934) : Science, 79, 409-10

3. Bliss, C.I. (1935) : Ann.Appl.Biol. 22, 134-67

4. Brown, A.W.A. (1958) : Insecticide Resistance in arthropods, Geneva (World Health Organization: Monograph series No.38.

5. Brown, A.W.A. (1971) : Post resistance to pesticides. In: White Stevens, R.H., ed. Pesticides, New York, Dekker Vol.I Ch.7.

6. Brown, A.W.A. and Pal, R. (1971) : Insecticide Resistance in arthropods, Geneva (World Health Organization: Monograph Series No.38(Second Edition)

7. Busvine, J.R. and Nash, R (1953) : Bull.ent.Res. 44, 371-376.

8. Busvine, J.R. (1957) : Bull.Wld Hlth.Org. 16, 206-208

9. Busvine, J.R. (1958) : Bull.Wld Hlth.Org. 19, 1041-1052.

10. Crow, J.F. (1954) : J.econ.Ent., 47, 393-398.

11. Finney, D.J. (1952) : Probit Analysis, 2nd Edition Cambridge University Press.

12. Fisher, R. (1935) : Appendix to Bliss, C.I. Ann.Appl. Biol. 22, 164-165

13. Garms, R and Rehn, W.F. (1961) : Z.angew. Zool. 48, 385-403.

14. Gillie, O.J. and Peto, R. (1969) : Genetics 63, 329.

15. Gaddum, J.H. (1933) : Spec. Rep.Ser. Med. Res. Con. Lond., no.183.

16. Haron, J. (1963) : Bull.Soc. ent.Fr., 68, 225-232.

17. Hiroyoshi, T. (1961) : Genetics, 46, 1373-1380.

18. Hoskins, W.M. (1959) : Mosquito News, 19, 52-62.

19. Hoskins, W.M. and Gordon, H.T. (1956) : Ann. Rev.Ent. 1, 89-122.

20.	James Bonner and Jung-Rung WU	(1973) :	Proc.Nat.Acad.Scie. USA 70, 535-537.
21.	Kerr, R.W.	(1960) :	Nature (London) 185, 868.
22.	Kerr, R.W.	(1961) :	Aust.J.biol. Sci., 14, 605-619.
23.	Marmon as quoted by Pal	(1958) :	Indian J.Malar 12, 539-546.
24.	Martin	(1955) :	In: Origin of Resistance to toxic agent Ed: Orre E Reynold,Academic Press Inc. New York.
25.	Milani,R.	(1956) :	Riv.Parassit; 17, 233-246
26.	Milani,R.	(1957) :	Riv.Parassit; 18, 43-60
27.	Pal,R. and Singh,N.N.	(1958) :	Indian J.Malar. 12, 499-516
28.	Perry, A.S.	(1966) :	Mosquito News, 26, 301-309.
29.	Pennell, J.T., Miskus,R and Graig, R.	(1964) :	Bull.Wld.Hlth.Org., 30, 91-95
30.	Sharma,M.I.D. and Kalra, R.L.	(1958) :	Indian J.Malar., 12, 93-100
3	Tsukamoto, M.	(1963) :	Botyu-Kagaku, 28, 91-98.
32	Wright,J.W. and Pal, R. (ed.)	(1967) :	Genetics of insect vectors of disease, Amsterdam, Elsevier.
33.	WHO	(1960) :	Expert Committee on Insecticides: Tenth Report, Geneva (Wld. Hlth.Org. Rep. Sr. No.191.).

CHAPTER II

SELECTION OF DDT RESISTANCE IN ANOPHELES STEPHENSI - LISTON IN LABORATORY

2.1. Introduction

Information on the genetics of insecticide resistance in insects are largely based on inheritance studies. Such information alone are not adequate to comprehend the genetical basis of insecticide resistance. Complementary information, based on selection studies, are necessary for comprehension of resistance in its totality. Reports on selection and inheritance studies on the same species originating from same geographical area are few and many of the selection studies were, generally, not extended over large number of generations. Further the selection pressures were not directed to different stages of lifecycle of a species. Therefore, to collect adequate information studies on DDT selection directed against adult and larval stages of Anopheles stephensi for large number of generations and on inheritance of DDT resistance in A.stephensi were carried out. Strains of A.stephensi used for both the selection and inheritance studies had originated from same geographical area - Tamilnadu state of India. It was expected that information obtained from the selection studies would be of immediate applied value as well, since they would be needed to optimise the vector control measures with minimum loss and hazards. The results of selection studies are presented in this chapter.

Material and Methods

2.2.1 Material

Considering the vectorial importance, length of generations, amenability to colonizations of the species in laboratory and readily

available stock colony with its history sheet, A.stephensi type was chosen as an animal for the study.

The stock colony of A.stephensi was established* with 25 larvae collected from house wells in Madras city in 1938. The colony was maintained as a self-propagating one from 1938 to 1940 at Guindy,Madras; from 1940 to 1942 at Coonoor in a tropical room and from 1943 onward at the field laboratory of the National Institute of Communicable Diseases at Mettupalayam. This colony is referred to as strain M. To study the effect, if any, of the different types of selection on the speed of the development of resistance, three sub-colonies were raised from the stock colony by selecting with DDT at adult, larval and both adult and larval stages. The three selected strains are referred to, here-in-after, as strains A,L,AL respectively.

2.2.2 Method of Selection

Strain A was maintained by selecting the male and female mosquitoes with DDT before mating, the male and female mosquitoes being seperated with-in 24 hours of hatching. For DDT selection, three days old glucose fed male and blood fed female mosquitoes were exposed in each generation to varying doses of DDT, and the survivors at LD_{50} and above were used for raising the subsequent generations. In earlier phase of the study, mosquitoes were exposed to DDT crystals (25 mg/sq.ft) and in later phase they were exposed to filterpapers treated with varying concentrations of DDT solution in non-volatile Resilla oil.

---

* The colony was established and maintained by the malaria investigation centre of the Rockefeller Foundation under Dr.P.F.Russel. It has been maintained by the Malaria Institute of India (now National Institute of Communicable Diseases) since 1943 at its South India branch Coonnor.

Strain L was selected by exposing fourth instar larvae of each generation to varying doses of DDT in alcohol-water mixture for 24 hours. The survivors at doses giving fifty percent and above mortality were used for raising the subsequent generations.

Strain AL was selected both at adult and larval stages following the methods used for selecting the A and L strains.

To study the effect of very high doses of DDT on a field selected highly resistant strain another colony of A.stephensi was established with larvae collected from wells of Erode Town. A.stephensi larvae of Erode were highly resistant to DDT and tolerant to BHC and dieldrin (Rajagopalan et al 1956 and Mammen as quoted by Pal (1958)). The colony was maintained under high DDT selection pressure (60.00 ppm) at larval stage. This strain, hereafter is referred to as Strain E.

2.2.3 Method of Rearing

Adult mosquitoes were colonized in 1 c.ft. wooden cages and fed with 10 percent glucose water. The adult females were periodically also fed on rabbit blood for production of eggs.

Eggs were floated on tap water in enamal basin (40 cm diameter). Approximately 500-600 larvae were reared in each basin. The quantity of larval food (yeast powder) was kept constant for each basin. Fourth stage larvae and pupae were obtained on seventh day and ninth day, respectively.

To avoid inadvertent mixing of strains, the adult colony of each strain was maintained in a seperate room and the larval cultures were kept covered with cloth.

2.2.4 Method of testing

To evaluate the effect of DDT selection both the adults and larvae of different strains were tested for their susceptibility to DDT. They were also tested against gamma BHC and dieldrin to observe correlated response of DDT selection, if any, as expressed by increased or decreased susceptibility to BHC and dieldrin. Adult mosquitoes were tested following Busvine-Nash technique (Busvine and Nash 1953) or WHO technique (WHO 1960). For this purpose four to six days old glucose fed or blood fed female mosquitoes were used. Larval tests were carried out on late third or early fourth instar larvae following different testing methods suggested by WHO (Wright 1958) WHO 1960). In case of highly resistant larvae higher concentrations of DDT than those supplied with WHO test kit had to be used for obtaining mortality rates above and below 50 percent. These higher concentrations of DDT were locally prepared using p,p' DDT and absolute alcohol. In developing the WHO testing technique due care was taken to ensure that the results obtained would be consistent with those obtained by Busvine-Nash Technique (Wright 1958).

Adult mosquitoes or/and larvae of each generation were primarily utilized for selection studies and more often than not the material in adequate number were not available for susceptibility tests. Due to this constraint the progress of selection could not be evaluated in each generation of selection. Insects once exposed to insecticide cannot be retested and hence due to this constraint tests could not also be carried out to estimate the mean susceptibility level of the set of insects surviving the selection pressure.

2.2.5 Statistical Analysis and Interpretation

Dose and response data collected from the susceptibility tests were transformed to log dose and probit metameters. Using these metamctors LD_{50} and regression coefficient, b, were estimated by 'cyo' estimation method as suggested by Finney (1952). These statistics as well as the Probit/log dose regression lines were interpreted following Hoskins and Gordon (1956); Singh (1961) and Brown and Pal (1971). In addition response to selection, R, was estimated following Mather(1963) as $R=m_s - m_b$ where m_s and m_b are log LD_{50} of the selected generation and base line values, respectively. R in the dose units is $LD_{50S} \div LD_{50B}$ where LD_{50S} and LD_{50_B} are mean response rates of selected generation and M-strain, respectively.

2.3 RESULTS

2.3.1 Baseline Statistics

The three selected strains had originated from the M-strain and hence the LD_{50} and regression coefficient, b, estimated from the dose-response data of M-strain were used as the base-line i.e. zero generation statistics to evaluate the progress of different types of DDT selection.

The M-strain was tested at different times and the LD_{50} of DDT for larvae were 0.04, 0.06, 0.016 parts per million (ppm) and the regression coefficients, b, were 3.3, 2.5 and 2.7. LD_{50} for adult females were 1.0, 0.7 and 0.46 percent concentrations of DDT and b values were 5.3, 4.2 and 5.5 (Table 2.1). This shows the existence of variations in statistics obtained from different independent tests

Table 2.1

The base line estimates - LD_{50} of DDT-and regression coefficient values for A.stephensi -M-strain tested* at different times.

Tests at different times during selection experiment	Adults		Larvae	
	LD_{50} (in percent)	b	LD_{50} (in ppm)	b
Initial test	1.0(a)	5.3	0.04(c)	3.3
Mid test	0.7	4.2	0.06(c)	2.5
Closing test	0.46	5.5	0.016	2.7

* Unless otherwise stated the estimates were based on susceptibility tests following WHO methods and using WHO test kits and DDT solutions.

(a)- DDT solutions were locally prepared using BOC white oil and technical DDT. Busvine-Nash Technique was used.

(c)- DDT solutions were locally prepared using p,p' DDT and alcohol

carried out at different times on the same strain. Therefore, to minimise the errors in accepting a case of resistance when it is not or in over estimating the magnitude of response to selection, the highest LD_{50}, 0.06 ppm and 1.0 percent, and the lowest values of b, 2.5 and 4.2 for the larvae and adults, respectively, were considered as the baseline statistics. The lowest estimate of b was preferred because it is inversely proportional to the variance of tolerance distribution; which is of genetical importance in selection studies.

2.3.2 Response to DDT selection at adult stage

LD_{50} of DDT and the regression coefficient, b, values along with the estimates of response to selection, R, of the female adults and larvae of A.stephensi A-strain are given in Table 2.8. The results of susceptibility tests with adult mosquitoes showed that the LD_{50} had increased to 1.5 percent in the 10th generation to 1.8 percent with 15th generation from the baseline value of 1.0 percent. The LD_{50} values from 16th generation to 46th generation was found to fluctuate around the value of 1.8 percent. The increase in LD_{50} values in the 10th and 15th generations was found to be associated with the increase in the b values whence the values were 7.6 and 8.0, respectively. The values of b, thereafter, declined to a stable level of 4.0 in the 37th generation. The results of parallel tests with M-strain and A-strain, using the WHO technique and DDT solution, showed that even after 57th generation of selection followed by eight generations of culture without DDT selection (57/8th generation) the LD_{50} of A-strain was 1.4 percent against 0.46 percent of M-strain. The values of b were 5.0 and 5.5 for A and M strains, respectively, (Table 2.7). The estimate of response to selection, R, was consistently higher than one for all generations tested. It, however, had fluctuated between 1.4 and 2.6 (Table 2.2). The parallel test with adults of (56/8)th. generation gave the value of R, as 3.0 (Table 2.7). As LD_{50} is the mean of tolerance distribution, b is inversely proportional to variance of tolerance and R is the estimate of response to selection interpretations of information supplied by the three parameters

Data and estimate of response to DDT selection (R) of adults and larvae of A.stephensi A-strain

Adults				Larvae			
Generation tested*	LD_{50} (in percent)	b	R	Generation tested*	LD_{50} (in ppm)	b	R
0	1.0(a)	4.2	1.0	0	0.06(r)	2.5	1.0
10	1.5(a)	7.6	1.5	15	0.12(r)	3.3	2.0
15	1.8( )	8.0	1.8	28	0.15(r)	5.5	2.5
16	1.6(c)	6.6	1.6	41	0.12(r)	1.3	2.0
20	1.8(c)	5.5	1.8	44	0.16(r)	2.1	2.7
37	2.6(c)	4.0	2.6	47	0.12(r)	- **	2.0
40	1.6	4.2	1.6	56	1.12	1.5	18.7
43	2.0	4.0	2.0	57	1.58	- **	26.3
44	1.6	4.2	1.6				
45	1.4	4.0	1.4				
46	1.7	4.0	1.7				

* Unless otherwise stated the susceptibility tests were carried out following WHO technique and using WHO test kit and DDT solutions.

(a) Estimates are based on suceptibility tests carried out following Busvine-Nash technique. DDT solutions were locally prepared using p,p' DDT and BOC white oil.

(c) Estimates are based on susceptibility tests carried out following Busvine-Nash technique. DDT solutions were locally prepared using technical DDT.

(r) Estimates are based on susceptibility tests carried out following WHO technique. DDT solutions were locally prepared using p,p' DDT and alcohal.

** Plateau was suggested on the probit/log dose regression line. The estimate of b will, therefore, be less reliable and hence was not calculated.

are that DDT pressure at adult stage had resulted in selection of low degree of resistance (1.5 to 3.0 times) in adult mosquitoes. The variance of resistance was reduced in the earlier generations under selection due to elemination of susceptible individuals. Subsequent increase in variance was due to segregation of genes in large number of genotype classes each with different level of resistance. The low level of resistance with variance of distribution of resistance over log doses less than or equal to that of base line value and the continuous nature of distribution of resistance over log doses indicated by the linear probit/log dose regression lines could be attributed to large number of genes, occuring in a system, each with small contribution towards resistance. Hence following Hoskins and Gordon (1956) it could be said that low degree of resistance selected in A.strain was of vigour tolerance type (VT). The DDT selection is a directional one; it acts against susceptible phenotypes and favours the resistant ones. Therefore, the stability of VT could be attributed to elemination of susceptible phenotype and absence of high resistant type. The susceptible alleles could survive the selection through their linkage with the resistant genes alleles. Through crossing over when they were released from such linkage bond and become homozygous, they were elemineted by DDT selection. Absence of high resistant type was probably due to non-availability of resistant genotypes. The genetic contribution towards fluctuations of mean and variance of VT could be due to release and recombination of VT genes at varying level (dose).

The statistical summary of the dose-response data of the larvae of different generations of A-strain (Table 2.2) showed that the LD_{50} had increased to 0.12 ppm in the 15th generation from the base line value of 0.06, the response to selection was 2.0. The value of regression coefficient was 3.3 against 2.5 of base line. These suggest that the DDT pressure at adult stage had resulted in VT in larvae as well. The LD_{50} after the 15th generation to 47th generations showed small fluctuation around 0.12 indicating stability. The lower variance observed upto 28th generation was due to elemination of susceptible phenotypes released from the linkage bond. The stability of mean and increase in variance in generations 41 to 47 was due to segregations of VT genes in number of groups and also appearance of high resistant phenotype in a proportion that did not affect the mean. The hypothesis of evolution of classes having pronounced phenotypic difference under prolonged DDT pressure could be supported by the plateau on probit/log dose regression line.

The dose-response curve in metametric unit of the 47th generation showed plateau. Continued selection resulted in higher LD_{50} as well as variance of distribution of resistance over log dosages in the 56th generation. Further selection in 56th generation resulted in still higher LD_{50} giving response to selection a value as high as 26.3 in the 57th generation. The probit/log dose regression line of the 57th generation also showed a plateau. Plateau on the probit/log dose line suggested a change from unimodal to multimodal distribution of tolerance indicating evolution of classes each with distinct log normal distribution

of tolerance. This quantitative to qualitative change could be due to clustering of genes and alleles at varying dose and states of organization. The genes are held together in a cluster by the linkage bond, and hence the state of its stability is proportional to the strength of linkage. A resistant cluster in its earlier phase of evolution could be unstable and hence likely to be disintegrated, through crossing over, in its transmission. The absence of plateau on the probit/log regression line of the 56th generation was probably due to disintegration, through crossingover, of the newly evolving cluster. The re-appearance of the plateau on the probit/log dosage line of 57th generations, when R was 26.3, indicated that clustering of the genes and alleles was the best response to ecological challenge as it contributed towards economy and efficiency of the physiological functionings needed for survival. An unstable cluster, under continued selection, could become a stable one which will not disintegrate in transmission and hence will behave like a single factor.

The results of parallel tests, using the same (WHO) technique showed that the LD_{50} at the $(57/8)^{th.}$ generation was 0.55 ppm against 0.016 ppm of A.stephensi M-strain. Here also the regression coefficient value, 1.9, for the selected strain was lower than the value of b, 2.7, for the strain M (Table 2.7). The response to selection, R, was 2.0 in the 15th generation which increased, through fluctuations, to 26.3 in the 57th generation (Table 2.2). The estimate of R based on parallel tests was 34.4 in the $(57/8)^{th.}$ generation (Table 2.7). Thus the results

indicated that the DDT pressure at adult stage first selected resistance of the type of vigour tolerance and then, after a long quiescent period (in generation), selected also resistance of high order, 34.4 times, in the larval stage.

A comparision of the pattern of behaviour of mean and varaince of resistance expressed in the adults and larvae of A.stephensi A-strain showed that the mean expression of resistance in adults was lower than that in the larvae and both the stages showed vigour tolerance in the earlier generations of selection. The variance of the distribution of resistance in adults of the earlier generations was less than the base line value. It almost reverted back to base line level in the latter generations. In the larvae the variance in each of the earlier generations was also less than the base line value but in latter generations it increased progressively and remained greater than the reference strain even after 8th generation of withdrawal of selection pressure. These information indicate that DDT selection pressure at adult stage had selected a system of large number of genes controlling vigour tolerance (VT) which was expressed both at adult and larval stages. These genes are considered to contribute also towards better fitness to survive under enviornmental stress and strain (Hoskins and Gordon 1956) so their frequency in the stock population is usually high since the population experienced and survived ecological strains. It is due to this high frequency of the system of VT genes that vigour tolerance was first selected by DDT selection .

of A.stephensi L-strain

Adults				Larvae			
Generation tested*	LD_{50} (in percent)	b*	R	Generation tested*	LD^{*}_{50} (in ppm)	b	R
0	1.0(c)	4.2	1.0	0	0.06(r)	2.5	1.0
12	1.5(a)	7.7	1.5	27	0.1(r)	2.7	1.7
17	1.5(c)	4.7	1.5	30	0.2(r)	4.0	3.3
20	1.9(c)	6.6	1.9	33	0.2(r)	2.2	3.3
27	2.1(a)	5.3	2.1	34	6(r)	3.0	10.0
41	1.7	5.7	1.7	58	1.6	1.6	16.7
43	1.8	4.5	1.8				
45	1.8	5.6	1.8				
46	1.2	5.0	1.2				
47	1.2	5.0	1.2				

* Unless otherwise stated the susceptibility tests were carried out following WHO technique and using WHO test kit and DDT solutions.

(a) Estimates are based on susceptibility tests carried out following Busvine-Nash technique. DDT solutions were locally prepared using p,p' DDT and BOC white oil.

(c) Estimates are based on susceptibility tests carried out following Busine-Nash technique. DDT solutions were locally prepared using technical DDT and BOC white oil.

(r) Estimates are base on susceptibility tests carried out following WHO techniques. DDT solutions were locally prepared using p,p' DDT and alcohal.

The results showed that DDT pressure at larval stage had selected low degree of resistance (1.2-3.0 times) of VT type in the adult mosquitoes. The lower variance of resistance in 12th generation could be due to elimination of non-vigourous individuals. Increase of variance in subsequent generations as well as in the $(59/8)^{th}$ generation indicated existance of large number of classes with varying degree of resistance. The log dose-probit regression lines for each generation was linear. From these information it could be inferred that the VT was due to large numbers of genes each contributing in a small way towards resistance of VT type. The vigour tolerance in adults was observed by 12th generation of L-strain against 10th generation of A-strain.

The results of larval test showed that the LD_{50} increased to 0.1 ppm in 27th generation from the base line value of 0.06 ppm (Table 2.3). From this table it could also be seen that the response to selection increased from 3.3 in 33rd generation to 10.0 and 16.7 in 34th and 58th generations respectively. The parallel tests in $(59/8)^{th}$ generation showed that LD_{50} was 0.89 ppm against LD_{50}, 0.016 ppm, of M-strain. The variance of resistance, however, was almost equal to that of the M-strain (Table 2.7). From this Table it could also be seen that the value of R_1 was 55.6 in the $(59/8)^{th}$ generation. Such a high value of R indicated that the resistance was not of vigour tolerance type.

A comparative review of the information obtained from the selection studies with A.stephensi A-strain and L-strain showed that high resistance in larvae alone was selected by DDT pressure on adults as well as on

larvae. This suggested that for successful selection of high resistance the stage of lifecycle subjected to selection is of marginal importance; what matters more is the intensity of selection pressure and the availability of free genetic variance upon which selection could act. Failure to select resistance in the adults of both A-strain and L-strain was probably due to non-availability of genetic components, in free state, for selection. If this be true then the genetic components for adult and larval resistance are not the same. Further, selection of larval resistance through adult selection suggest that physiological defence mechanism common with both the adult and larval stages but having relevance with larval resistance alone is of greater importance than the stage specific external and anatomical properties.

2.3.4 Response to DDT Selection at Adult and Larval Stages

The statistical summary of the results of susceptibility tests of different generations under DDT selection of AL-strain is given in Table 2.4 . The LD_{50} for the adult mosquitoes of the 10th generation was 2.2 percent against 1.0 percent of the initial generation. In the 30th generation LD_{50} was 4.0 percent which declined to the lowest level of 2.8 percent in the 41st generation and thereafter again increased to a level greater than 4.0 percent in the 55th generation. In $(57/8)^{th}$ generation LD_{50} was 1.6 percent against 0.46 percent of the M-strain (Table 2.7). The response to selection, R, was 2.2 in the 10th generation which was associated with the highest value of b, 9.0. This suggested the selection of vigour tolerance by DDT pressure extended over 10 or

Table 2.4

Summary of dose-response data and estimate of response to DDT selection(R) of adults and larvae of A.stephensi AL-strain.

Adults				Larvae			
Generation tested*	LD_{50}	b	R	Generation	LD_{50}	b	R
0	1.0(c)	4.2	1.0	0	0.06(r)	2.5	1.0
10	2.2(a)	9.0	2.2	28	0.2 (r)	3.7	3.3
25	2.2(c)	3.6	2.2	37	0.2(r)	4.8	3.3
30	4.0(c)	-**	4.0	38	0.4(r)	3.1	6.6
40	3.2	3.0	3.2	39	0.6(r)	2.0	10.0
41	2.8	3.7	2.8	40	0.8(r)	2.8	13.3
44	3.2	3.0	3.2	44	1.0(r)	2.1	16.7
45	3.2	2.6	3.2	46	0.5(r)	2.1	8.3
55 more than	4.0	-*** more than	4.0	47	1.0(r)	3.6	16.7
				56	0.9(r)	1.6	15.0

* Unless otherwise stated the susceptibility tests were carried out following WHO technique and using WHO test kit and DDT solutions.

(a) Estimates based on the susceptibility tests carried out following Busvine-Nash technique. DDT solution were locally prepared using p,p' DDT and BOC white oil

(c) Estimates based on the susceptibility tests carried out following Busvine-Nash technique. DDT solutions were locally prepared using technical DDT and BOC white oil.

(r) Estimates based on the susceptibility tests carried out following WHO technique. DDT solutions were locally prepared using p,p' DDT and alcohal.

** Due to high heterogeneity of response the values could not be precise and hence was not estimated.

*** Mortality rate at 4.0 percent was less than 50 percent and hence the estimate of b will not be reliable.

less generations. The high response to selection, 4.0, observed in 30th generation was associated with high heterogeneity of response which suggested increase in the frequency of high resistant genotypes resulting from the concentration of alleles for resistance at varying levels. The alleles not strongly bound by linkage bond disintegrated during transmission. This was indicated by low values of R, 2.8-3.2, associated with low values of b, 2.6-3.7, during 40th to 45th generation. Continued selection of such a variable population resulted in selection of high resistance again in the 55th generation. In view of high variability of response to testing dosages of DDT the resistance selected could be considered unstable.

Selection at LD_{50} of both larvae and adults of each generation constituted higher selection pressure, than selection at LD_{50} of any one of the stages. Due to such high pressure of selection sufficient material for susceptibility test were not available. Hence larval tests were carried out after 27th generation of selection. The LD_{50} of larvae for 28th generation was 0.2 against 0.06 giving a response rate of 3.3. The regression coefficient value was 3.7 which was not as high as observed when vigour was selected; infact the statistics are similar to those found in post-vigour selection period whence LD_{50} and R values are 2-3 times higher but the b values are lower than the base line values. Hence it is not possible, in this case, to find the minimum time needed for selections of resistance of VT type.

High resistance started building up in 38th generation as indicated by high values of R, 6.6, and low value of b, 3.1.

The development of high resistance reached to a maximum level, 16.7 times as high as the base line value, in the 44th generation. This level was attained through fluctuations and also followed by fluctuations in the values of LD_{50}, b and R. (Table 2.4). This showed that large number of genes were involved which through the mechanism of segregation and linkage contributed towards means and variance of the distribution of resistance in different generations. Response rate as high as 51.9 of M-strain was observed in $(57/8)^{th}$ generation; whence b value, 2.7, was the same as that of the M-strain (Table 2.7).

From the result it could be concluded that higher selection pressure of DDT resulting from selection of both the adults and larvae at LD_{50} levels than the selection either at adult or larval stages had resulted in development of low resistance of VT type and probably had initiated the development of high resistance in the adults. The probit/log dose regression lines were linear which again showed that the VT was polygenic. Due to experimental constraint VT in larvae could not be observed. However development of high resistance to DDT in the larvae was found.

2.3.5 Trends of R and regression coefficient during selection

The assessment of response to DDT pressure made so far was based on comparision of LD_{50}, b and R of the three selected strains with similar base line values and at the end with the estimates of parallel test results of the stock colony. The estimates of the three parameters for different generations were subject to sampling and non-sampling

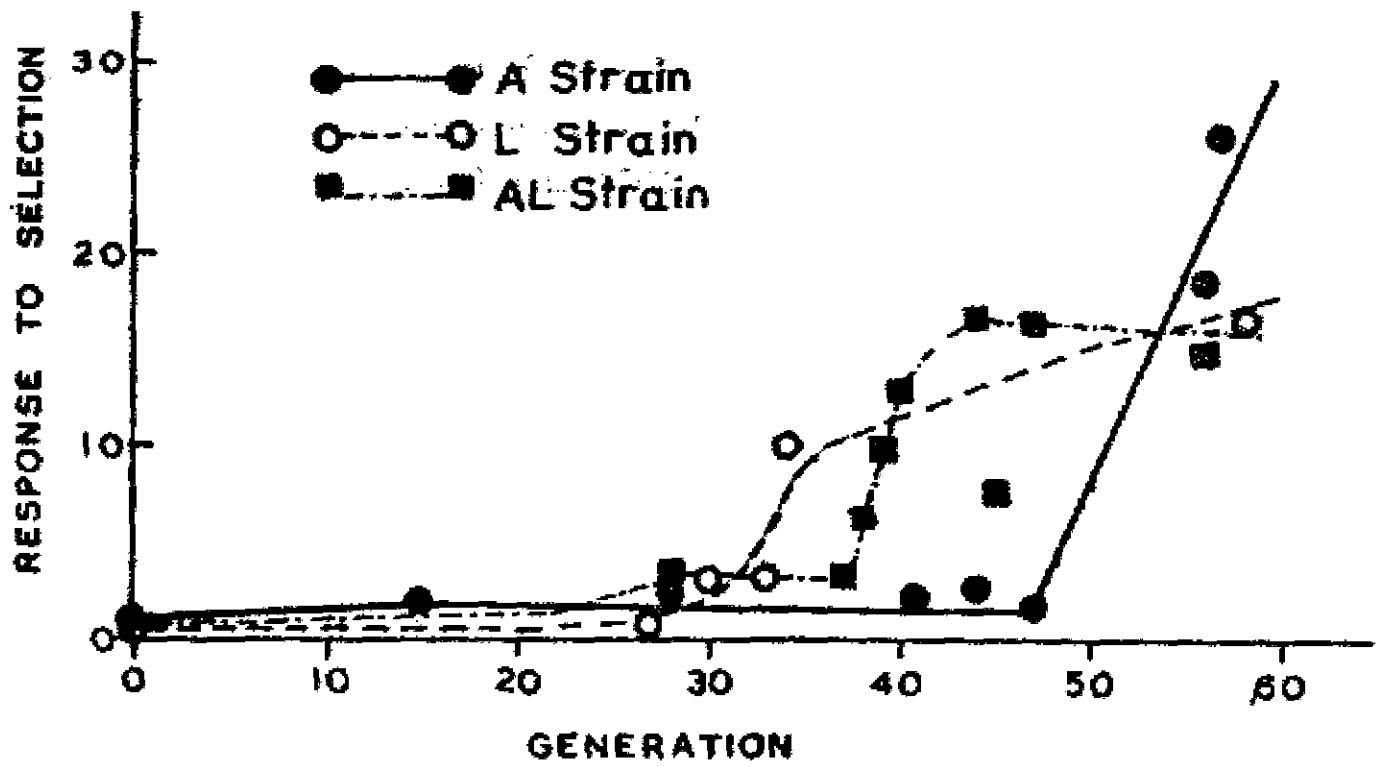

Fig. 2.1. RESPONSE TO DDT SELECTION IN LARVAE OF *A.stephensi*

errors originating from testing technique, quality of DDT, other experimental and ecological factors. The effects of these factors are likely to exaggerate or mask the true status of resistance. Therefore, there is the need to see the trend of R in the selected strains. This was done by graphic method. It is seen that the curve of response to selection, R, in larvae of A-strain, L-strain and AL-strain over generations are like growth-curves (Fig.2.1). In AL-strain all the three phases viz wer asymptosic phase (lap) exponential phase (ep) and upper asymptotic phase (uap) were observed.

The results of the selection studies by Decker and Bruce (1952) showed that the progress of evolution of specific resistance in NAIDM-strain of housefly under the pressure of methoxychlor, DDT, gamma HCH, chlordane and dieldrin had also followed the growth curve, each curve exhibited the lap, ep and the uap. The growth curves observed with the A-strain, L-strain and AL-strain of A.stephensi could, therefore, be considered as characterstic curves of response to selections. Hence the information collected from the susceptibility tests of the larvae of erent generations of A-strain, L-strain and AL-strain of A.stephensi s used to assign meaning and significance to the different phases of the growth curve representing the response to selection.

The lower asymptotic phase, lap, represented the period in generations) when VT-type resistance was selected (c.f. 2.3.2, 2.3.3 2.3.4). The altitude of this phase measured from x-axis gives the tude of expression of VT-resistance which was R $\leq$ 3.5 with A.stephensi.

Therefore $1.0 < R \leq 4.0$ could be considered as characteristic mean response to selection of the VT-genes. The lap is the procrusor the development of high specific resistance. The genes controlling VT-type resistance being hardy, and vigorous provided opportunity, through some protection against ecological hazards and accidents, to the genotypes having potential genic variance for specific resistance. They also give such genetypes time to break the chromosomal inertia for releasing the genetic variance in 'free state' under the stimulus of DDT pressure. Thus lap is preparatory phase for the development of high specific resistance. Milani (1956,1957) also considered the first few generations of selections to constitute a period when the ancillary gene-alleles are accumulated, the incompatible ones are eliminated and genotype as a whole remodelled to receive new resistant gene alleles.

The lower asymptotic phase could also be observed under DDT selection of a pre-existing single gene of major effect (c.f.2.3.11, Fig.2.4. According to Crow(1957) the initial latent period represented by the lap could arise either as an artifact from the method of testing, and its arithmetical expression or from the exponential interrelations between genotypes and genefrequency or both. The credibility or otherwise of the two explanations for the lap could be tested by comparing the time (in generations) needed for the 'take off' of the observed and expected growth curves as done in sections 2.3.11 and observing the behaviour of LD_{50} and b in different generation under selection (c.f. 2.3.2- 2.3.4).

The lap is followed by the exponential phase representing fast rate of selection of resistant genes and alleles. The effect of DDT selection is porceptible at this phase because of availability of much genetic variance in free state. The rate of increase in resistance decreased as the free variance was progressively utilized by selection. During this phase the genes for specific high resistance could go for new linkage group to meet the challenge of DDT selection. Such linkage of resistant genes/alleles, if and when materializes, would result in formation of resistant cluster(s) having expression of high resistance. In case of pre-existing single major factor for resistance the population under this phase also become highly heterogonous due to pre-ponderance of the homozygous resistant and heterozygous genotypes. Therefore the probit/log dose curve obtained for this phase would show low slope or plateau or even eratic behaviour.

The upper asymptotic phase, uap, indicated that the population has become homogenous with respect to resistant genotype. The free genetic variance available with the population was utilized completely or almost completely by selection. During this phase, if polygenes or multigenes are involved, the selected genes are also organized in cluster to achieve optimum level of average fitness for survival as demanded by insecticide selections pressure. For efficient and economic functioning of the complex defence mechanism controlled by the genes alleles the cluster(s) must evolve to a 'stable' state so that it is not disintegrated during transmission from generation to generation. Stability of the cluster of resistant genes could evolve through the development of strong

linkage bond or due to Heterozygous chromosomal inversion. The map could, therefore be considered to represent the evolution of stability of resistance cluster(s). The fluctuations or otherwise of LD_{50} and regression coefficient values during the generations under the map could be attributed, among other factors, to the state of stability of the cluster; the higher the fluctuations in mean and variance of resistance the weaker the state of stability. Fluctuations in the values of LD_{50} and b observed in the generations under the map of the AL-strain of A.stephensi (c.f. 2.3.4) and also with the highly resistant 'Erode' - strain (c.f. 2.3.10) are attributed to the weak state of stability of the resistant cluster. A highly stable resistance cluster, matured over generations of insecticide pressure or its correlated pressure, will be have like a major gene (super gene of Mather) having pronounced expression of resistance as was observed with A.stephensi E-strain (c.f. 2.3.10 and chapter III). The information obtained from the LD_{50} and b values of different generations of A.stephensi under laboratory selection by DDT clearly undermine the confidence on the hypothesis of evolution of resistent-strain due to selection by insecticide of a pre-existing macro-mutant gene. It may, however, happen that a given type of ecological pressure having effect similar to a given type of insecticide, could also result in evolution of resistant - cluster even in absence of the use of that insecticide. Sometimes the resistant cluster could evolve due to linkage with genes of other characters favoured by a given ecological selection. In such

situations the selections of resistance will conform to the theory of selection by insecticide of a pre-existing macro-mutant gene for resistance.

From the above considerations it could, therefore, be concluded that the polygenes/multigenes for specific high resistance could evolve to a major factor (single gene of pronounced expression) for specific high resistance under prolonged specific insecticidal pressure.

The regression coefficient values, estimated from the larval tests, of the different generations of A-strain, L-strain and AL-strain did not show any trend (Fig.2&2) The values of b increased in the earlier generation of selection when VT-type of resistence was evolved and then it decreased, through fluctuations, to a level close to the base line one, though the magnitude of VT-type resistance did not change. In the latter phase of selection low value of b, lying in the neighbourhood of similar base line value, was associated with evolution of high resistance. This suggested that there exist a tendency to conserve variance within characteristic limits. Supply of genetic variance when it is less than the lower characteristic limit and draining it out when it is more than the upper limit are controlled by the genetical and ecological factors. Release of free genetic variance from and transformations of free variance to potential one, as suggested by Mather (1973), could be considered as the genetical machanism of regulations of variance. The stabilizing selection pressure eliminated extreme phenotypes and hence could be considered to constitute the ecological source of regulation. Lower variance of resistance than that of base line one in the earlier generations of selection was

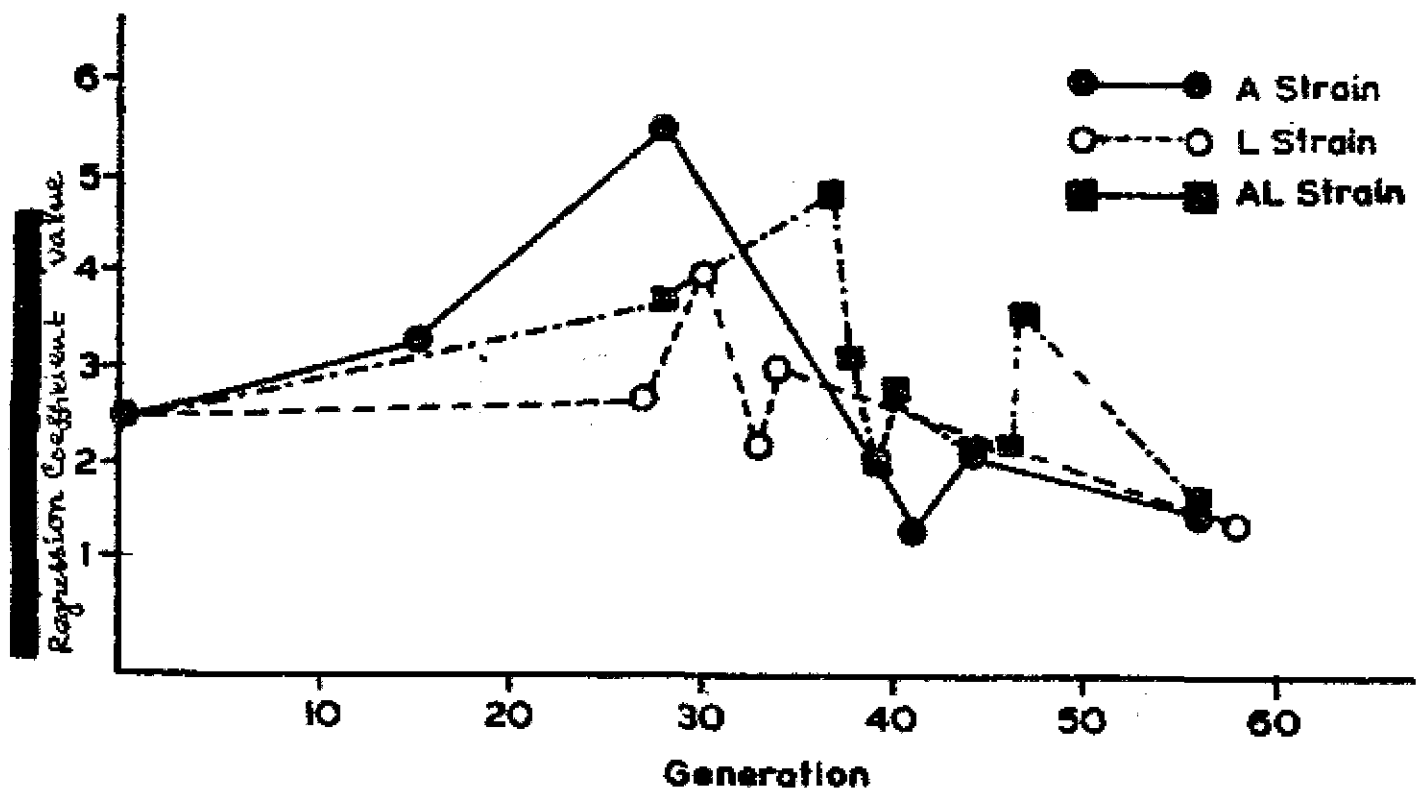

Fig.2·2 BEHAVIOUR OF REGRESSION COEFFICIENT IN DIFFERENT SELECTED GENERATIONS OF A.stephensi.

compensated by release of free variance through chromosomal activities. Further the excess variance are finally controlled through adverse selection and re-transformation of free variance to potential variance. The induced reduction or inflation of variance beyond the characteristic lower and upper limits generate the force for conservation which when equal to or greater than a thresh hold value primes the regulatory gene-system for suitable action.

2.3.6 Speed of development and intensity of expression of Resistance

Resistance of VT-type ($R \leqslant 4.0$) was found to develop in adults by 10, 12 and 10 generations whence the values of R_{α} were 1.5, 1.5 and 2.2, in female adults of A-strain, L-strain and AL-strain, respectively of A.stephensi. The maximum expressions of VT, as estimated by R_{β}, were 3.0, 3.0 and 3.3 in A-strain L-strain and AL-strain, respectively (Table 2.5). VT-type resistance in larvae was developed by 15, 27 and 28 generations, whence the responses to selection were, (R_Y) 2.0, 1.7 and 3.3 in A-strain, L-strain and AL-strain, respectively. Maximum expressions of VT, as estimated by R_{β}, were 2.7, 3.3 and 3.3 in A, L, and AL strains respectively. This suggested that the expressions of VT genes, irrespective of mode of selections, were at both adult and larval stages of the life cycle. Further the intensity of expression of genes for VT-type resistance in larvae and adults were practically of same magnitude even when the modes of selections were different.

High resistance ($R > 4.0$) in adults were observed in AL-strain (Table 2.5) alone. It was selected by DDT pressure, at both adult and larval stages, of 30 generations. It was of low order, say 4 times

Table 2.5

(Data showing speed of development and intensity of expression of DDT resistance in adult and larvae of A.stephensi due to different mode of selection at a level $\geq LD_{50}$)

Type of resistance	Parameters for evaluation	Strain evaluated					
		Female adults			Forth instar larvae		
		A-strain	I-strain	M-strain	A-strain	I strain	M-strain
Vigour tolerance (VT)	Generation which first observed	10	12	13	15	27	23
	Degree of expression (R_α)	1.5	1.5	2.2	2.0	1.7	3.3
	Max.level of expression observed (R_β)	3.0	3.0	3.5	2.7	3.3	3.3
Specific high resistance	Generation on which first observed	*	*	30	56	34	38
	Degree of expression (R_α)	*	*	4.0	18.7	10.0	6.6
	Max. level of expression observed (R_β)	*	*	>4.0	34.4	55.6	51.9

* high resistance was not developed.

R_α a = given LD_{50}/Basc line-LD_{50}

R_β b = given LD_{50}/LD_{50} of M-strain, both the LD_{50} estimated from parallel tests.

as high as M-strain. High resistance in larvae of A-strain, L-strain and AL-strain were developed by 56,34 and 38 generations, respectively whence the magnitude of expression were 18.7,10.0 and 6.6 times as high as the base line susceptibility. The maximum levels of resistance attained were 34.4, 55.6 and 51.9 due to DDT pressure at adult, larval and both adult and larval stages. These suggested that speed of development and intensity of expression of high-resistance were maximum when DDT pressure was exerted on larval stage.

2.3.7 Susceptibility of DDT Selected Strains to gamma BHC and dieldrin

To study the effect of DDT pressure on the susceptibility to other groups of chlorinated hydro carbon insecticides the A-strain,L-strain and AL-strain of A.stephensi were tested in the 58th, 60th and 58th generation of DDT selection against dieldrin and gamma BHC. Statistical summary of the test-results are given in Table 2.6. It may be seen from the table that the LD_{50} of dieldrin for adults of A-strain,L-strain and AL-strain were 0.21 percent, 0.18 percent and 0.36 percent, respectively. A comparision of these estimates with LD_{50} of the M-strain showed that the strains A,L and AL were 1.6,1.4 and 2.8 times as resistant as the M-strain. The regression coefficient values were higher than or equal to that of M-strain. In case of gamma BHC the A-strain, L-strain and AL-strain were also 1.6,1.6 and 2.7 times as resistant as the M-strain. The regression coefficient values were higher than or equal to that of the M-strain. This low degree of multiple resistance is therefore non-specific and hence is of VT type of resistance. It was controlled by polygenes which got selected

...ptibility status of selected strains of A.stephensi to dieldrin and

Strains	Generation	Adult						Larvae					
		Dieldrin			gamma BHC			Dieldrin			gamma BHC		
		LD_{50} in percent	R*	b	LD_{50} in percent	R*	b	LD_{50} in ppm	R*	b	LD_{50} in ppm	R*	b
A.strain	58	0.21	1.6	6.7	0.014	1.6	6.3	0.0032	2.3	3	0.14	4.7	7.1
L-strain	60	0.18	1.4	6.0	0.014	1.6	5.0	0.01	7.1	-	0.20	6.6	9.0
AL-strain	58	0.36	2.8	4.6	0.024	2.7	3.3	0.0039	6.4	3.3	0.13	4.3	7.0
M-strain	-	0.13	1.0	4.5	0.009	1.0	4.2	0.0014	1.0	4.2	0.03	1.0	3.9

$$*R = \frac{LD_{50} \text{ of selected strain,}}{LD_{50} \text{ of M-strain}}$$ R is 1 for M-strain

under DDT pressure. The expression of polygenic non-specific VT type of resistance was also observed in the larval stage. This was evinced by the 2.3, more than 7.1 and 6.4 times higher tolerance to dieldrin and 4.7, 6.6 and 4.3 times higher tolerance to gamma BHC of the A-strain, L-strain and AL-strain respectively. The regression coefficient values of the selected strains for BHC were higher than that of M-strain but they were lower for dieldrin. This suggested the possibility also of development of specific dieldrin resistance. Such a phenomenon, considered as a correlated response to DDT selection, could occur due to linkage of some dieldrin specific resistant alleles with those of alleles for VT or/and DDT-specific resistance and hence the possibility of being selected even when its selection was not aimed at.

2.3.8 Effect of Withdrawal of DDT Selection Pressure on the Susceptibility Status of the Selected Strains.

To study of the effect, if any, of withdrawal of DDT pressure on the different types of resistance developed, the three selected strains of A.stephensi were cultured for eight generations without DDT pressure. The 57th, 59th and 57th generations, under DDT pressure, of A-strain, L-strain and AL-strain respectively were the commencing point of the study and hence they were taken as W_0 generation. The eighth generation under withdrawal of DDT pressure of each strain is referred to as W_8th generations. The susceptibility status of the adults and larvae against DDT and dieldrin (DL) were assessed in the W_8th generation. The estimates obtained from the parallel tests with M-strain were used to calculate the response to natural selection, R at W_0 and W_8. A statistical summary of the test results is given in Table 2.7.

Table 2.7

Estimates of susceptibility status to DDT and dieldrin of A.stephensi DDT selected strains reared for eight generations without DDT pressure and of A.stephensi M-strain

Strains of A.stephensi	Generation	Adults						Larvae					
		DDT			Dieldrin			DDT			Dieldrin		
		LD_{50} (percent)	R(a)	b	LD_{50} (percent)	R(a)	b	LD_{50} (ppm)	R(a)	b	LD_{50} (ppm)	R(a)	b
L-strain*	W_8	1.4	3.0	5.0	0.12	2.4	4.0	0.55	34.4	1.9	0.0012	1.7	4.0
	W_0	1.7	3.7	4.0	0.21	4.2	6.7	1.58	97.5	***	0.0032	4.6	3.0
L-strain**	W_8	1.4	3.0	5.0	0.09	1.8	4.3	0.89	55.6	2.1	0.0016	2.3	6.1
	W_0	1.2	2.6	5.0	0.18	3.6	6.0	1.6	100.0	1.6	>0.01	14.3	***
AL-strain*	W_8	1.6	3.5	2.1	0.12	2.4	4.0	0.83	51.9	2.7	0.0012	1.7	6.3
	W_0	>4.0	>8.7	***	0.36	7.2	4.6	0.90	86.5	1.6	0.0089	12.7	3.3
M-strain	P —	0.46	1.0	5.5	0.05	1.0	3.0	0.016	1.0	2.7	0.0007	1.0	3.3

* Strains were selected by DDT for 57 generations and then reared without DDT selection for eight generations.

** Strain was selected by DDT for 59 generations and then reared without DDT selection for eight generation.

*** Very high variance linear probit/log dose could not be fitted to the data.

(a) = Response to selection estimated as

$$R = \frac{LD_{50} \text{ of a given selected strain}}{LD_{50} \text{ of the M-strain}}$$; R for M-strain is 1.0

W_8= Eight generation of DDT-free culture

W_0= At the terminal phase of DDT-selection.

First response to DDT pressure was the development of non-specific resistance of VT-type. This was followed by specific DDT resistance of higher magnitude. Selection of specific DL resistance, with low expression was also observed. An observed level of resistance, therefore, could be the combination at least two types of resistance. Hence to study the effect of withdrawal of DDT pressure on VT-type of resistance the results of DL susceptibility to the adults of three strains are considered. The R of adults to DL at W_8 was lower than that of W_0 for each selected strain. The lower values of R were consistently associated with lower values of b at W_8 than W_0 (Table 2.7). This suggested that VT-type resistance had decreased and the variance increased due to withdrawal of DDT pressure. This could be attributed to disintegration, under the demand of insecticide free environment, of ordered polygenic system for non-specific resistance in to a system that makes the genotypes susceptible since natural selection demands such organisation.

It was earlier observed that DL resistance in larvae was also selected due to correlation of its alleles with those for DDT or VT types of resistant alleles (c.f.2.3.7). Susceptibility tests with such a material showed that R to DL and the variance of resistance were consistently lower in W_8 than W_0. This suggested the appearance of DL susceptible phenotypes in high frequency and elamination of DL-resistant phenotypes. Thus the natural selection was found to favour the DL susceptible larvae which appeared through the disintegration of the organized resistant gene system.

The adults of both the A-strain and L-strain were observed to develop VT-type resistance. The R to DDT of adults at W_8 for these two strains were almost the same as those at W_0 and so were the variances of resistance as indicated by the b values (Table 2.7). It could, therefore, be inferred that there was no perceptible effect of the absence of DDT pressure for 8 generations on the VT type resistance in adult. The adults of AL-strain were found to develop VT-type of resistance as well as low specific resistance to DDT at the 55th generation under DDT-selection. The R and the variance of resistance to DDT of this strain at W_8 was less than that at W_0. This suggested that in the absence of DDT pressure the natural selection had favoured the susceptible phenotypes appearing from the disintegration of the resistance gene-system. It had also eliminated the few stable resistant clusters evolved. Due to the confounding expression of VT-gene alleles and specific DDT gene alleles the effect of natural selections on each of the gene-system could not be evaluated seperately.

The larvae of all the three strains were highly resistant to DDT. This was the combined effect of the non-specific VT alleles and specific DDT-resistant alleles; the effect of latter being more than the former. The values of R and the variances of resistance in the three strains were, lower in W_8 than W_0 (Table 2.7). These suggested that the natural selection resulted in the disintegration of the resistant cluster. This resulted in the release of the susceptible genes which were favoured by the natural selection. Simultaneously the natural selection could, have, eliminated the resistant individuals which contained relatively more stable resistant gene cluster. Thus the natural selection was found to work in the reverse direction to DDT selection.

2.3.9 Effect of High DDT Pressure on the Resistance level of A.stephensi Erode Strain

To study the effect, if any, of high insecticidal pressure on highly resistant strain, the A.stephensi E-strain was cultured for 29 generations, subjecting larvae of each generation to high pressure (60 ppm x 24 hrs.) of DDT. Fluctuations in the susceptibility of larvae in different generations were observed during the selection. Adults and larvae of the 29th generation, when subjected to susceptibility tests against DDT gave LD_{50} as 4.0 percent and>60 ppm respectively. Larval mortality rates (in percent) at varying doses of DDT (in ppm) were 6.0, 37.0, 17.0, 23.0 at 10,20,40 and 60, respectively. This type of larval response to different doses of DDT was very close to that observed at the initial generation. The adults of 29th generation, however, showed slight increase in LD_{50}, 4.0 from less than 4.0. Thus the results showed that resistance cannot be induced ad infinitum by even high pressure of insecticide. There exists a limit of genetical resource which when fully utilized under selection pressure will not contribute towards further increase in the expression of resistance no matter how intense and prolonged the pressure be.. The fluctuations observed in the level of resistance in different generations under selections could partly be due the environmental effect and partly genetical. The genetical contribution is due to integration and disintegration of resistant/ susceptible genes and alleles of the unstable resistant cluster.

2.3.10 Comparision of the Pattern of Evolution of Resistance under DDT selection in Laboratory and Field

Insecticides like DDT and BHC were used to control A.stephensi under the malaria control programme. This resulted in evolutions of strains resistant to DDT, BHC and dieldrin. The field selected resistant strain was colonized in the laboratory and tested for its susceptibility to DDT, BHC and dieldrin. The results are summarized in Table 2.8. It is seen from the Table that the larvae of A.stephensi Erode strain (Strain E) were highly resistant to DDT; the LD_{50} being more than 60.0 ppm as against 0.04 ppm of M-strain. The larval mortality rates were 0,2,6,12 and 13 percent at 5,10,20, 40 and 60 ppm of DDT. The LD_{50} of DDT and b values for adult female mosquitoes of E-strain were 1.5 and 3.3 against 0.7 and 4.2 respectively for adult females of M-strain. This suggested that adults of E-strain were only twice as resistant as the M-strain.

The LD_{50} of gamma BHC for the adults of E-strain was 0.019 against 0.009 for the M-strain. This indicated that the level of resistance to gamma BHC in adults of E-strain was only twice as high as that of the M-strain. The value of b of E-strain was greater than that of the M-strain.These suggested that the adult resistance to gamma BHC in E-strain was of VT-type. The VT-type resistance in larvae was also indicated by about 4 times resistance in E-strain associated with higher value of b (Table 2.8).

The adults of E-strain was about 1.5 times resistant to DL

nce LD_{50} values of E-strain and M-strain were 0.19 percent and 0.13

Table 2.8

Summary of the susceptibility test results of A.stephensi Madras strain and Field selected A.stephensi Erode strain.

Strain of A.stephensi	DDT				Gamma HCH				Dieldrin			
	Adults		Larvae		Adults		Larvae		Adults		Larvae	
	LD_{50} (percent)	b	LD_{50} (ppm)	b	LD_{50} (percent)	b	LD_{50} (ppm)	b	LD_{50} (percent)	b	LD_{50} (ppm)	b
Erode Strain (E)	1.5	3.3	>60.0	-	0.019	5.0	0.11	5.0	0.19	5.0	0.01	4.3
Madras Strain (M)	0.7	4.2	0.04	3.3	0.009	4.2	0.03	3.9	0.13	4.5	0.014	4.2

percent of DL, respectively. The b value for E-strain was 5.0 against 4.5 of M-strain. The larvae of both the strains were equally susceptible (Table 2.8).

From these results it could be concluded that the larvae of E-strain were highly resistant to DDT but the adults were slightly tolerant. The evolution of this type of resistance was due to the selection by DDT, which was used for vector control purpose. Use of DDT in field had also selected VT-type non-specific resistance which was expressed in low intensity in both adults and larvae against DL. Subsequent information showed that both adults and larvae of A.stephensi were resistant to DDT and tolerant to BHC (Bhonbere, Roy and Samson, 1963 and Bhonbere at al 1964) in the field.

The pattern of response to DDT pressure in laboratory was evolution of non-specific VT-type resistance expressed in both adult and larval stages followed by evolution of specific high resistance to DDT expressed first in larval stage alone and then extended to adult stage, and in advertant development of DL-resistance of low order. It is interesting to observe that the pattern of evolution of resistance brought about by DDT pressure on A.stephensi in laboratory was similar to that brought about by DDT-spraying under malaria control programme. The intensity of expression of resistance developed in field was higher than that developed in the laboratory. This could be due to the large size of the field population, random mating and out-breeding which provided much more genetical resources for integration into resistant cluster(s)

than that will be a expected from a small laboratory population under closed breeding.

2.3.11 Test of the hypothesis of evolution of resistance due to selection of Pre-existing single specific Gene of Major effect.

The reviews and the reference books on the insecticide resistance claim that the evolution of resistant strain of an arthropod species is due to selections, by insecticide, of the pre-existing genetical factor in its population. From the results of the inheritance studies, the genetical factor was considered to be, mainly, a specific nuclear gene of major effect (Brown 1967, Brown and Pal 1971 and the appropriate references quoted by them). The hypothesis of selection by DDT of pre-existing single gene of major effect present in the stock colony of A.stephensi (strain-M) was, therefore, tested for its credibility or otherwise. For this purpose simple biometric models are first developed to estimate the resistant gene frequency, its equilibrium level in the stock colony and the time (in generations) needed for evolution of a homozygous population under a given level of selection. The last expected estimate was then compared with the emperical results. It is hoped that the resultsof this test will help in assigning due confidence on the alternative model of evolution of resistant strain developed in the previous sections. (2.3.2, 2.3.4).

3.3.12 Model I: Estimation of resistant gene frequency

Let R and S be the two alleles of a single autosomal locus determining specific high resistance and susceptibility to a given insecticide. Let p and q be the frequencies of R and S alleles and P,H and Q be the frequencies of homozygous resistant (RR), heterozygous (RS/SR) and homozygous susceptible (SS) genotypes, respectively, such that

$$p+q = 1 \quad \text{and} \quad P+H+Q = 1$$

A.stephensi is a deploid species. Therefore following Mather (1963), the frequencies of R and S alleles could be estimated as

$$\hat{p} = P+\tfrac{1}{2}H$$
$$\text{and} \quad \hat{q} = Q+\tfrac{1}{2}H \qquad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

The equations in (1) are based on the assumption that both the males and the females have the same genotype frequencies. But in a small sample taken, from field population having small value of either p or q, for establishing colony it is likely that the genotype frequencies in males and females are not the same. Under such situation the genotype mating frequencies in the first laboratory mating is obtained as

Genotype Mating	Frequencies	
female RR x male RR	$P_f \times P_m = f_o\,(R \times R)$	(2)
female RR x male (RS/SR)	$(P_f \times H_m)+(H_f \times P_m) = f_o\,(R \times H_y)$	
female RR x male SS	$(P_f \times Q_m)+(Q_f \times P_m) = f_o\,(R \times S)$	
female (RS/SR) x male (RS/SR)	$(H_f \times H_m) = f_o\,(H_y \times H_y)$	
female (RS/SR) x male SS	$(H_f \times Q_m)+(Q_f \times H_m) = f_o\,(H_y \times S)$	
female SS x male SS	$Q_f \times Q_m = f_o\,(S \times S)$	

Where P_f and P_m are the frequencies of resistant male and female individuals; similarly H_f, H_m, Q_f and Q_m are the sex-wise frequencies of hybrid heterozygous and susceptible homozygous individuals.

Using the first laboratory mating frequencies given in (2) the genotype frequencies of the off springs of the first generation in laboratory will be

Genotype of ofspring	Frequencies
RR	$f_o(RxR)+\frac{1}{2} f_o(RxH_y)+\frac{1}{4} f_o(H_yxH_y) = P_1$
RS/SR	$\frac{1}{2} f_o(RxH_y)+f_o(RxS)+\frac{1}{2} f_o(H_yxH_y)+\frac{1}{2} f_o(H_yxS)=H_1$
SS	$\frac{1}{4} f_o(H_yxH_y) + \frac{1}{2} f_o(H_yxS)+ f_o(SxS) = Q_1$

(3)

Assuming absence of meiotic drive, differential zygotic lethality, unequal survival in either sexes of each genotype and non-random mating, the frequencies of R and S alleles in the first generations of culture could now be estimated by (1) as

$$\hat{p}_1 = P_1 + \tfrac{1}{2} H_1$$
$$\hat{q}_1 = Q_1 + \tfrac{1}{2} H_1.$$

under the assumptions of Hardy-Weinberg law the frequencies of R and S alleles in different generations will remain stable at the value of $\hat{p}_1$ and $\hat{q}_1$. The stable genotype frequencies will be

$$P = \hat{p}_1^2$$
$$H = 2\hat{p}_1\hat{q}_1$$
$$Q = \hat{q}^2$$

(4)

2.3.13 Model II: Selection at LD_{50} in each Generation

The laboratory population cultured for years contained the R-alleles at a stable frequency, $\hat{p}_1$. A sample of this population with $\hat{p}_1$ frequency of R allele is subjected to selection, in each generation, at LD_{50} level of the population. It is assumed that under the

selection scheme first the susceptible genotypes will be killed and then the heterozygous genotypes.

Accordingly models are developed for two cases: Case I: $Pop^n S \geq \frac{1}{2} Pop^n T$ and case II: $Pop^n S < \frac{1}{2} Pop^n T$; $Pop^n S$ and $Pop^n T$ denotes the population of homozygous susceptible genotype and $Pop^n T$ denotes the sum of the population of all the three genotypes.

Case I: When $Pop^n S \gg 0.5\ Pop^n T$

The impact of DDT selection on gene frequency is seen thus:

	RR	RS/SR	SS	Total
Population at the commencement of DDT Selection	p_o^2	$2p_oq_o$	q_o^2	1
Proportion killed	0	0	s_o	-
Relative fitness	1	1	$1-s_o$	
After selection	p_o^2	$2p_oq_o$	$q_o^2(1-s_o)$	$1-s_oq_o^2$

$$\therefore p_1 = \frac{p_o^2 + p_oq_o}{1 - s_oq_o^2} = 2p_o \quad \text{- - - - - - - - - - (5)}$$

$$\text{then } s_oq_o^2 = \tfrac{1}{2} \quad \text{- - - - - - (6)}$$

$$q_1 = \frac{q_o^2(1-s_o) + p_oq_o}{1 - s_oq_o^2} \quad \text{- - - - - - - - - (7)}$$

$$\text{or} \quad \frac{q_o^2 + p_oq_o + \frac{1}{2}}{\frac{1}{2}}$$

$$= 2q_o - 1 \quad \text{- - - - - - - - - - (8)}$$

$$\text{then } s_oq_o^2 = \tfrac{1}{2}.$$

If genotype frequencies of first generation after selection are

$$P_1,\ H_1,\ Q_1$$

then $$P_1 = p_1^2,\ H_1 = 2p_1q_2 \text{ and } Q_1 = q_1^2$$

In general at the end of n^{th} selection we have, if $Q_1 = q_n^2 \geq \frac{1}{2}$,

$$\left.\begin{aligned} p_{n+1} &= q_n \\ \text{and } q_{n+1} &= 2q_{n-1} \\ \text{and hence } P_{n+1} &= p_{n+1}^2 \\ H_{n+1} &= 2p_{n+1}\,q_{n+1} \\ \text{and } Q_{n+1} &= q_{n+1}^2 \end{aligned}\right\} \quad \text{- - - - - (9)}$$

Case II: When $Pop^n S < 0.5$ pop^n

When (say in N^{th} generation) relative frequency of SS = $Q_n = q_n^2 < 0.5$ all the SS and some of the (RS/SR) will also be killed. Let s_N, h_N be the proportion of SS and (RS/SS) that will be killed in 50 percent selection. In this case we have

$$s_N = 1$$

and $$h_N = \frac{0.5 - q_n^2}{2p_nq_n}$$

The impact of selection on gene frequency is, now, seen. Thus:

	RR	RS/SR	SS	Total
N^{th} Selection	p_N^2	$2p_Nq_N$	$q_N^2 < 0.5$	1
Proportion killed	0	h_N	$s_N = 1$	
Relative fitness	1	$(1-h_N)$	0	
After selection	p_N^2	$2p_Nq_N(1-h_N)$	0	$1-q_N^2-2h_Np_Nq_N$

$$p_{N+1} = \frac{p_N^2 + p_N q_N (1-h_N)}{1 - q_N^2 - 2h_N p_N q_N} \quad - - - - - - - (10)$$

$$q_{N+1} = \frac{p_N q_N (1-h_N)}{1-q_N^2-2h_N p_N q_N} \quad - - - - - - - - - (11)$$

Genotype frequencies after selection are

$$\left.\begin{aligned} P_{N+1} &= p_{N+1}^2 \\ H_{N+1} &= 2p_{N+1}\, q_{N+1} \\ \text{and} \quad q_{N+1} &= q_{N+1}^2 \end{aligned}\right\} - - - - (12)$$

In general after $(N+K)^{th}$ selection

$$p_{N+K+1} = \frac{p_{N+K}^2 + p_{N+K}\, q_{N+K} (1 - h_{N+K})}{1-q_{N+K}^2 - 2h_{N+K}\, p_{N+K}\, q_{N+K}} \quad - - - - - (13)$$

and $$q_{N+K+1} = \frac{p_{N+K}\, q_{N+K} (1-h_{N+K})}{1 - q_{N+K}^2 - 2h_{N+K}\, p_{N+K}\, q_{N+K}} \quad - - - - - (14)$$

and hence the genotype frequencies of RR, RS/SR and SS are obtained as

$$\left.\begin{aligned} P_{N+K+1} &= p_{N+K+1}^2 \\ H_{N+K+1} &= 2p_{N+K+1}\, q_{N+K+1} \\ \text{and} \quad q_{N+K+1} &= q_{N+K+1}^2 \end{aligned}\right\} - - - - - - (15)$$

$K = 0, 1, 2, \ldots\ldots$

The selection process ends at $(N+K)^{th}$ generation if

$$(H_{N+K} + q_{N+K}) \ll \tfrac{1}{2}$$

i.e. $$(2p_{N+K}\, q_{N+K} + q_{N+K}^2) \lesssim \tfrac{1}{2}$$

P_{N+K+1} will then be 1, and it will remain stable at this level for $P_{N+K+1+i}$ where i= 0,1,2.....

2.3.14 Estimation of resistant gene frequency in stock colony

The stock colony of A.stephensi (Madras strain) was started with 25 larvae collected from field. Assuming the frequency of male and female adults at emergence was 0.52 and 0.48 the adult males and females after emergence were 13 and 12 respectively. To estimate the lowest frequency of resistant gene in the sample it is assumed that one of the 12 females was heterozygous for resistant gene and the remaining females and all the 13 males were homozygous for susceptible gene alleles. Assuming random mating between different genotypes the first mating frequencies between different genotypes are estimated by (2) as

female RR x male RR = $f_o(R \times R)$ = 0

female RRx male (RS/SR)= $f_o(R \times H_y)$ = 0

female RR x male SS = $f_o(R \times S)$ = 0

female (RS/SR)x male (RS/SR) = $f_o(H_y \times H_y)$ = 0

female (RS/SR) x male (SS) = $f_o(H_y \times S)$=0.084 x 1.00 = 0.084

female SS x male SS = $f_o(S \times S)$ = 0.916 x 1.00 = 0.916

Let P_1, H_1 and Q_1 be the frequencies of RR, RS/SR and SS genotypes in first generation of laboratory culture, the values of $_1$,H_1 and Q_1 are estimated by (3) as

$$\hat{P}_1 = f_o(RR) + \tfrac{1}{2} f_o(RxH_y) + \tfrac{1}{4} f_o(H_y x H_y)$$

$$= 0 + 0 + 0 = 0$$

$$\hat{H}_1 = \tfrac{1}{2} f_o(RxH_y) + f_o(RxS) + \tfrac{1}{2} f_o(H_y x H_y) + \tfrac{1}{2} f_o(H_y xS)$$

$$= 0 + 0 + 0 + \tfrac{1}{2}(0.084) = 0.042$$

$$\hat{Q}_1 = \tfrac{1}{4} f_o(H_y x H_y) + \tfrac{1}{2} f_o(H_y xS) + f_o(SxS)$$

$$= 0 + \tfrac{1}{2} \times 0.084 + 0.916 = 0.958$$

Hence the frequencies of resistant and susceptible alleles $\hat{p}_1$ and $\hat{q}_1$ for either sexes now are estimated by (1) as

$$\hat{p}_1 = \hat{P}_1 + \tfrac{1}{2} \hat{H}_1$$

$$= 0.021$$

$$\hat{q}_1 = 0.958 + 0.021 = 0.979$$

Assuming absence of meiotic drive, differential zygotic lethality, unequal survival of either sexes of each genetype in larval and adult stages, non-random mating and mutation to resistant (R) or susceptible (S) alleles in stock population the frequencies of R and S alleles will remain stable at the $\hat{p}_1$ and $\hat{q}_1$ values. Hence using equation (4) stable genotype frequencies in the stock colony will be

$$P = \hat{p}_1^2 = 0.000441$$

$$H = 2\hat{p}_1\hat{q}_1 = 0.041118$$

$$Q = \hat{q}_1^2 = 0.958441$$

Let each of the three sub-colonies taken at random from the stock colony for selection studies contain the 'R' and 'S' gene alleles with frequencies 0.021 and 0.979, respectively. The two sub-colonies called strain A & strain-L were subjected to selection at LD_{50} or more of DDT in each gener ation. The frequencies of 'R' allele and 'S' allele and also RR, (RS/SR) and SS genotypes were estimated by (9) for 3 selected generations and thereafter by (13), (14) and (15). The results of these estimates along with the proportions of SS and (RS/SR) killed in each generations under selection are given in Table 2.9. The frequencies of SS, (RS/SR), RR genotype classes and 'R' allele are also shown in figures 2.3 and 2.4, respectively.

It may be seen from Fig. 2.3 that the initial population is practically homogeneous which progressively becomes heterogenous due to preponderance of (RS/SR) and RR classes. The heterogeneity reached to the maximum in the generations when the frequency curves of the three genotypes interest one another. Thereafter the population progressively became homozygous and finally reached to the limit when it contain only the RR genotype. These dynamic changes in the composition of the population will also hold good for the cases when resistance is due to number of factors (genes) linked so closely as to act as a unit. The dose response curves of the different generations will reflect the dynamic states of the population. With the progress of selection the change as shown by the slope of the probit/log dose lines may be several fold less from the steep slope for the initial population, subsequently, however, the slope will

Table 2.9

Expected Progress of Selection of Resistance in *A.stephensi* (M-strain) under DDT Selection Pressure at LD_{50} in each generation.

Generation selected (n)	Proportion Killed		Frequencies				
	SS genotype (S_n)	RS/SR genotype (h_n)	Resistant alleles (P_n)	Susceptible alleles (q_n)	Homozygous Resistant genotype (P_n)	Hetero-zygous genotypes (R_n)	Homozygous Susceptible genotype (Q_n)
0	0.521680	0.000000	0.021000	0.979000	0.000441	0.041118	0.958441
1	0.544802	0.000000	0.042000	0.958000	0.001764	0.080472	0.917764
2	0.55907.	0.000000	0.084000	0.916000	0.007056	0.153888	0.839056
3	0.722309	0.000000	0.168000	0.832000	0.028224	0.279552	0.692224
4	1.000000	0.132458	0.336000	0.664000	0.112896	0.446208	0.440896
5	1.000000	0.737921	0.612896	0.387104	0.375641	0.474510	0.149849
6	1.000000	1.000000	0.875644	0.124356	0.766752	0.217782	0.015466
7	-	-	1.000000	0.000000	1.000000	0.000000	0.000000

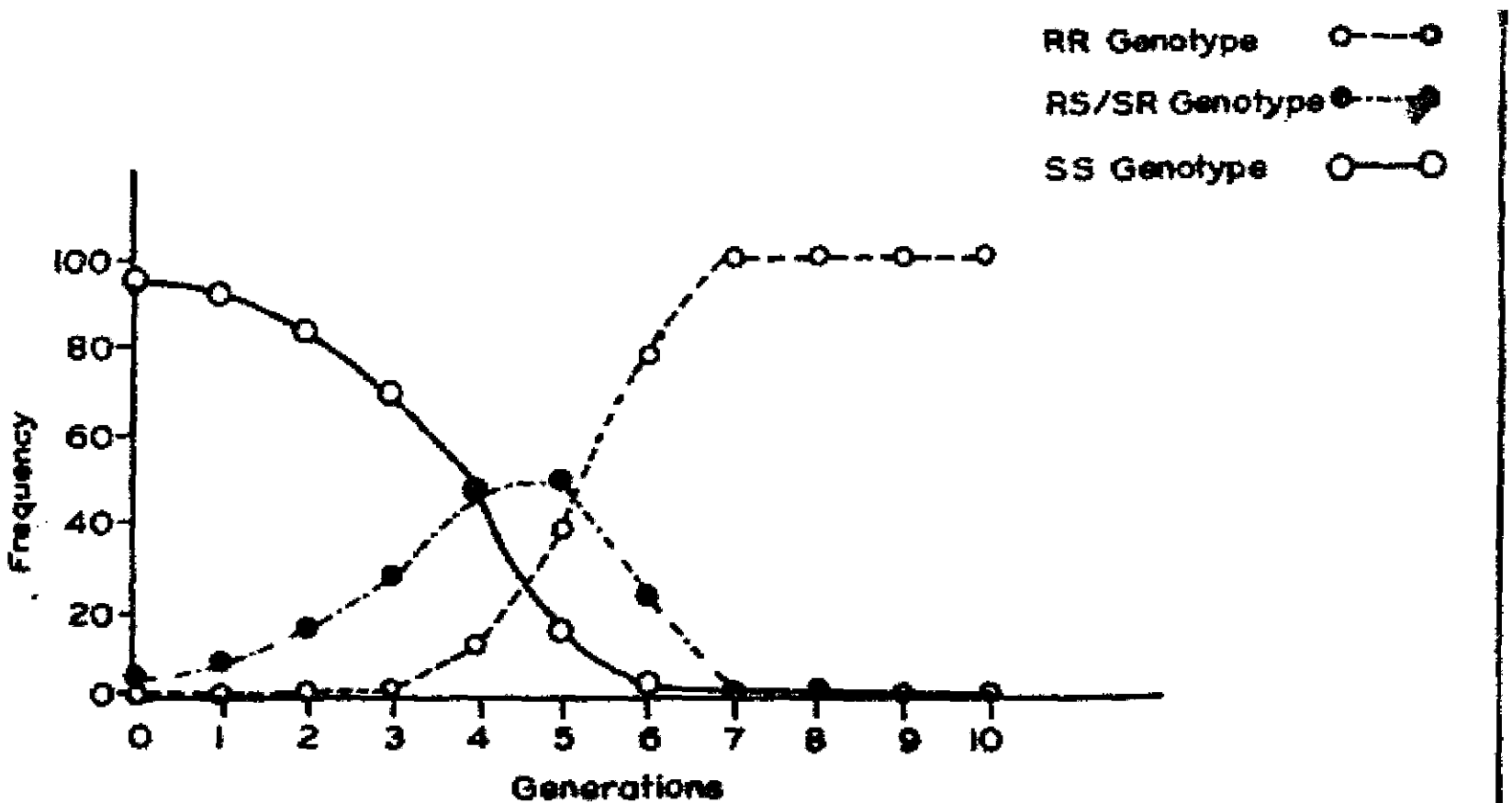

Fig.2·3 EXPECTED FREQUENCIES OF GENOTYPES IN DIFFERENT GENERATIONS UNDER DDT SELECTION AT A PRESSURE AT LD_{50} AND ABOVE

become steep due to homogeneity of the populations resulting from the selections of homozygous resistant class (Hoskins and Gordon 1956). The curves may show plateau when the population is heterogenous containing different genotype classes in high frequencies; the factor for resistance being a single one with pronounced effect.

2.3.15 Comparision of emperical data with that obtained theoratically

The frequency curve of R-alleles for different generations under selection also is like a growth curve (Fig. 2.4).

The increasing LD_{50} and hence the resistance index, RI, is the function of the frequency of 'R' alleles. Therefore, the growth type of curve, based on the RI values, is not sufficient to conclude that resistance selected was due to multigenes. The most powerful parameter is the time (in generations) taken for selecting a population homozygous for resistance since given other circumstances being equal, the fewer the factors involved, more quickly they will become homozygous and hence the convergence to near limit of resistance (Hoskins and Gordon 1956). Therefore, the estimate of the parameter from the emperical observation is compared with the theoratical one based on selection of single gene for resistance. It may be seen from the Table 2.9 that after three generations of selection the frequency of susceptible class (SS-genotype) was reduced to less than 50 percent of the total population and by the seventh generation of selection all the SS genotypes and (RS/SR) genotypes were eleminated from the population. The strains 'A' and 'L' thus expected to be homozygous resistant, containing only the 'RR' genotypes.

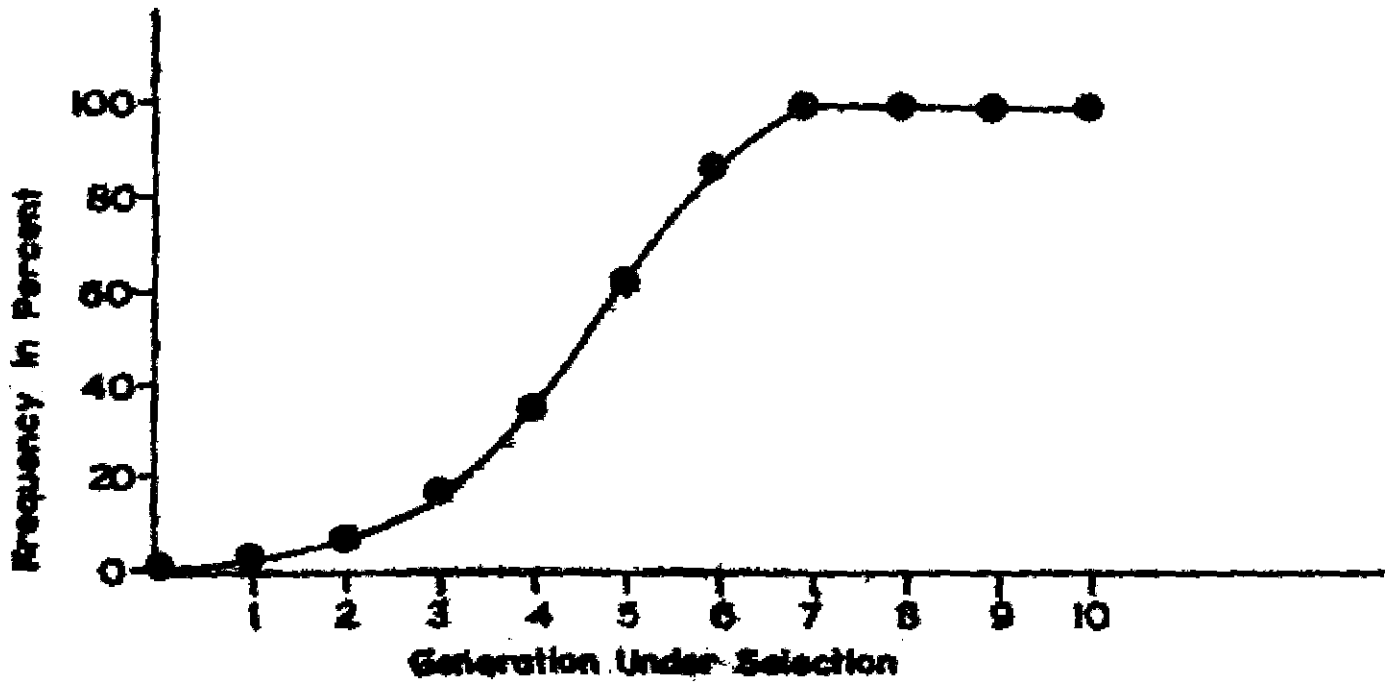

Fig. 2·4 EXPECTED FREQUENCY OF RESISTANT ALLELE IN DIFFERENT GENERATIONS UNDER DDT SELECTION AT A PRESSURE LD_{50}

after seven generationsof selection (Fig. 2.1 and 2.2) when the evolution of DDT resistance in A.stephensi is due to directional selection in favour of a single resistant gene with major effect. But the emperical results showed that the populations did not become homozygous resistant even after 57 and 58 generations of selection at LD_{50} and above level. Therefore, the hypothesis of selection of pre-existing single gene of major effect is considered not tenable. The alternative hypothesis of integration, organisation of multigenes and their selection as developed in section 3.2.2, 2.3.5 is accepted.

2.4. Discussion

The progenies of A.stephensi M-strain were almost as susceptible to DDT as their parents. Similarly the progenies of A.stephensi S-strain were as resistant to DDT as their parents. Susceptibility and resistance to DDT are thus stable characteristics of the species passed on from parents to off-spring and hence their controls are genetical. Change in the character from susceptible state to a resistant one under the DDT pressure over several generations was cumulative and it (the change) resulted in better survival of the species population under the insecticidal contaminated environment. Therefore the experimental results on the selection of A.stephensi by DDT and the inferences drawn there-on (c.f.2.3.1 - 2.3.11) are discussed in this section, in the light of existing genetical theories to systematise and comprehend the phenomenon of insecticide resistance in insects.

2.4.1 Stabilising Effect of Natural Selection

A.stephensi M-strain was collected from Madras City long before the introductions of DDT and other synthetic insecticides. The strain was maintained in the laboratory without exposure to any toxic chemical. LD_{50} of DDT for this strain was found to vary between 0.02 ppm and 0.06 ppm. The regression coefficient varied between 2.5 and 3.3 (c.f.2.3.1). Therefore the LD_{50} and the regression coefficient, b, value within such a small limits of variations could be considered as the estimates of the optimum level of expression of DDT susceptibility favoured by the insecticide free environment. Higher or lower susceptibility than the optimum ones are, hence, notadvantageous to the species for its survival under the insecticide free environment. This shows highly susceptible and resistant individuals are selected out from the population. The genetic contribution of highly susceptible and resistant individuals to the next generation is, therefore, as good as zero. The natural selection i.e. the effect of insecticide free environment could, therefore, be considered to be a stabilising one.

2.4.2 Directional Selection by DDT and evolution of Resistance

A.stephensi E-strain showed higher value of LD_{50} and greater variance of response to DDT (c.f.2.3.9). The A,L and LA strains of A.stephensi, under DDT pressure for large number of generations, also showed higher value of LD_{50} and greater variance of response to DDT (c.f. 2.3.2 ~ 2.3.5). Higher LD_{50} than the base line one indicated evolution of the resistant strain. Resistance, thus, is a character

advantageous to the species for its survival under the DDT contaminated environment. The LD_{50} of the homozygous resistant strain is, therefore, a new optimum level of average susceptibility to which the population moved under the continued pressure of DDT. The susceptible (within a limit) individuals favoured by the natural selection were weeded out by the DDT selection. The pressure of DDT, therefore, had the effect of directional selection on the populations of A.stephensi.

The results of laboratory studies on the selection of A.stephensi by DDT is in agreement with the phenomenon experienced in field. I Insecticide when used for the first time to control insect vectors/ pests was found to be highly toxic. The insect populations, exposed to it, declined by 'crisis' to almost eradication point. But sooner than expected the declining trend of the population was reversed to a rising one. The populations maintained an upword trend of growth inspite of the continued applications of insecticide. Susceptibility tests revealed that the rise in the population was due to replacement of initially susceptible strain by a resistant one (see the reference books by Brown 1958, Brown and Pal 1971). The appearance of the insecticide resistance in the field populations, at a high cost in term of mortality, is thus a clear demonstration in a short span of time, of the theory of evolution. Selection of resistant strain in the laboratory (c.f.2.3.2 - 2.3.4), is an experimental confirmation of the theory.

2.4.3 Genetic Variance of Resistance and Selection Pressures

According to Fisher's fundamental theorem of natural selection the rate of increase in the fitness is equal to the additive variance of

of fitness at that time. Resistance is a genetical character; the genes are mainly located in the chromosomes (Brown 1967). The evolution of the resistant strain, therefore, is due to work of DDT pressure on the genetical variability. Very high mortality experienced by an insect species at the initial stages of its exposure to insecticide may be due to genetical invariance since stabilizing selection will best be met by, and will therefore favour, genetical invariance (Mather 1973).

Genetic variation is the basis of adaptive and evolutionary change for no other variation is so widespread in the population (Mather 1973). Laboratory studies showed (c.f.2.3.2-2.3.4) that under DDT pressure the DDT-specific resistant strain of A.stephensi had evolved though the probit/log dose regression lines of the stock colony and earlier generations of the selected strains did not give evidence of high genetic variance. The genetic variance on which the DDT pressure worked successfully, must have, therefore, existed in the potential state wherein genes and alleles were linked together in such a fashion that their contributions towards resistance were neutralized.

Resistance is a metrical character, it was measured precisely by the ratio of LD_{50} values of the reference strain and the candidate strain of a species. The probit/log dose regression lines of the earlier generations were usually continuous (c.f.2.3.2-2.3.4) and hence following Mather (1973) resistance could be expected to be polygenic. The inheritance studies reported in Chapters III and IV gave also experimental evidence for polygenic basis of DDT resistance in

A.stephensi. It is an essential property of polygenic system that number of genotypes can give closely similar, if not identical, range of phenotypes (Mather 1973). Therefore the DDT susceptible M-strain of A.stephensi could be considered to be composed of different geneotypes and hence contained much genetical variance in potential state. This potential variance under bi-sexual mating and closed breeding in the laboratory colony slowly transformed to free variability through crossing over in heterozygous chromosomes and segregation of genes and alleles. It is this free variability that was utilized by the directional pressure of DDT.

2.4.4 Different classes of resistant genes and their economic values for species

Surveillance on the progress of selection of A.stephensi by DDT showed that the first response to selection was the evolution of resistance of vigour tolerance (VT) type; specific resistance of high intensity evolved much later (c.f.2.3.2-2.3.4). The VT type resistance was expressed both in adult and larval stages but the specific resistance had its manifestation only in the larval stage (c.f.2.3.2 - 2.3.4). Development of specific resistance in adult stage, however, was indicated long after the appearance of larval resistance under prolonged high pressure of DDT (2.3.6). The VT-type resistance was extended to BHC and dieldrin also (c.f.2.3.7). From these experimental information it is found that the resistance to DDT as detected by toxicological method is a complex one; VT-type of resistance, specific high larval resistance and specific high adult resistance are seperate and

hence they must have distinct genetical basis. They cannot be considered to be pleiotropic effect of single class of genes. Cases of co-existance of VT type resistance and specific high resistance both in adult and larval stages, usually observed with field population of insect species, could be due to linkage of different classes of genes.

The VT-type resistance was considered to factor from improved nutrition, extra weight, or any other factor associated with extra vigour. Such a resistant strain is also developed by breeding only from survivors from diverse stresses such as extremes of temperature, lack of moisture, abnormal food or injurious chemical. This type of resistance is extended to unrelated insecticide and often notably facilitate the process of selection with second chemical (Hoskins and Gordon 1956). The VT-type resistance could, therefore be considered to have significant economic value to a species under inimical ecological situations. In an insecticide contaminated environment VT-gives sufficient time to the species to reorganise its genetical resources for developing high degree of specific resistance which gives maximum fitness to species for its survival. The results of the selection experiments are evidences to it. Vigour, adjustment and fitness are the property of genotypes which have passed the test of natural selection and have survived because they possess these properties (Mather 1973). Therefore such genotypes could exist in higher frequency in 'free state' than the genotypes of specific resistance in the natural populations. It is because of this pre-existing high gene frequency that the VT-type resistance was observed to develope earlier (on or before 10th generationd)than the

specific resistance (developed on or after 30th generation) when the species population of A.stephensi was exposed to DDT (c.f.2.3.6).

2.4.5 Evolution of Gene Cluster for Specific Resistance - Its Stability and Behaviour.

One of the characteristic features of the VT-type resistance is that the slope of the dose-response metametric line is steeper that the similar line for natural (reference) populations. Such a line for VT-resistance suggested normal distribution of tolerance over dosages with smaller variance than the variance of unselected natural population. This indicated involvement of polygenic system controlling the VT, which was confirmed by experiments (see Chapter IV). A comparison of the estimates or response to selection, R, for different generations under selection by DDT on one hand and R for DDT, BHC and dieldrin on the other of A.stephensi. A-strain, L-strain, AL-strain and E-strain (c.f.2.3.2, 2.3.3, 2.3.4, 2.3.5 and 2.3.7) showed that there was no increase in magnitude of VT even after continued pressure for large number of generation and this was so even in case of field strain (c.f.2.3.9) where selections was against large and out-breeding populations. This suggested a limit of the expression of VT-resistance (around R=4.0) controlled by polygenic system of non-specific effect. The limit as measured by response to DDT selection was considered to be 4.0(c.f 2.3.5).

Specific DDT-resistance in the earlier generations was characterised by higher LD_{50} and variance of tolerance distribution The probit/ log dose regression lines were straight with low slope. This was due

due to continuous variations in tolerance distributions which indicated involvement of polygenic system. But with the continuation of DDT pressure the probit/log dose regression line was found to have plateau (c.f.2.3.2,2.3.3,2.3.4) which is indicative of discontinuous distribution of tolerance attributable to major factor(s). The major factor could result from close clustering of polygenes due to linkage bond. In the newly formed cluster the strength of linkage was weak and hence during its transmission the cluster could disintegrate giving rise to continuous variation in response. This was suggested by presence and absence of platean on probit/log dose lines.In some generations it was observed and in the following it was not observed (Table 2.2 and 2.4). With the continuance of DDT pressure the cluster of polygenes ultimately became stable (c.f.2.3.5) and behaved like a single major factor even in inheritance (see Chapter III). Hence it could be stated that the polygenes for specific DDT resistance undergoes a process of evolution culminating in a major stable cluster (factor) of pronounced resistance. This statement based on the results of experiments on selections of DDT resistance, an indirectly estimatable quantal response character, is in agreement with Mather's statement (Mather 1949,p.150), based on the experiment of directly measurable biometric character, that through recombination linked group of polygenes is built up which appear more or less as one large major factor. In case of DDT resistance such a single factor gives maximum fitness and survival value to the species exposed to DDT contaminated environment. Suitably organized and closely linked genes forming a cluster of high-specific resistance are, therefore, of great economic value to

species for its survival under insecticidal pressure than their (genes) existance in an unorganized and/or unlinked state. Whether the process of organization and increasing strength of linkage of genes are mechanical random phenomenon or they are achieved through effort of the species directed by its biological property, the urge for survival under the given insecticide contaminated environment, are difficult questions to answer from the results of the selection experiments. But in view of the fact that almost all the insect species of medical importance have, sooner or later, developed resistance to all types of insecticides brought to use against them (Brown and Pal 1971), it appeares that the drive for survival have also a significant role in the organization and stability of the resistant cluster. At individual level the success of the drive for survival depends on its magnitude and the genetical resources possessed by the individual. The adults of A.stephensi when subjected to DDT pressure did not immediately show resistance in adult stage but it did make effort to reorganise its available resources which resulted in organizations of the genes for larval resistance. Continued effort could be attributed in finally freeing the genetic variance which when organized had given adult resistance as well.

In view of the emerging fact that development of resistance to insecticide is rather a rule than exception it will be too much a mechanical and simplified explanations if appearance of resistant strain is attributed to selection of spontaneously occuring macro-mutation in a populations, by insecticide. Further it also ignores an important

biological property-the drive for survival. On the otherhand micro-mutations are enumerable and then in a given system of their organization preserve high potentiality for survival which when needed could be utilized by the 'survival drive' of a species to meet a given situation successfully. Hence this theory looks more plausible.

2.4.6 Chromosomal Inertia Visa-vis Speed of Selection and Reversion of Resistance

Genes are linearly arranged on the chromosone; the order in which they are found is the optimum state of their organization which gives optimum survival value under the given environment. Hence genic combination found are those survive the test of selection and therefore a given set of gene in a given organizational state are good under a given situation. The order of arrangement of different genes and systems of polygenes are maintained at more or less a stable state by the linkage bond. The strength of linkage is the functions of their (genes) spatial distance, it is more with closely located genes than with those which are widely seperated linkage endows the genetical system with the property of inertia. The inertia will show down the response to new selective force (Mather 1973). In the selection studies the length of the lower asymtotic phase representing the generation lag in response to DDT selection was attributed to the slow transformation of the potential polygenic variance to free state (c.f. 2.3.5). Such a slow rate could be due the chromosomal inertia resulting from the effect of long stabilizing selection of DDT free environment. The inertia was broken due to crossing over of the genes in heterozygous chromosomes

resulting from bisexual mating of different genotypes. Crossing over is a gene control phenomenon and the genes controlling it could be primed for action under a given intensity of stimulus originating from the enviornment. Once the inertia is broken the genes could be reorganized at a faster rate to meet the demand of envornment. It is probably due to this fact that resistance to unrelated group of insecticide developed at a faster rate in newly selected/evolved resistant strain than in the unselected natural population. Similarly when the chromosomal inertia is weak resistance will quickly revert back to susceptible state, when the insecticide selection pressure withdrawn.

2.4.7 Multi-resistance and Correlated Response to a given type of Selection

Generally specific high resistance to DDT does not extend over to gamma BHC and dieldrin. But in the selection study with A.stephensi the response to DDT selection of L-strain as measured by BHC and dieldrin susceptibility was of a magnitude higher than that expected for VT-type of resistance (c.f.2.3.7 and Table VI). Such a phenomenon of multi-resistance was also observed with culex fatigans (Mohan 1960) and in few other species (WHO/VBC/67.26). The genetical basis for such a typical phenomenon could be attributed to correlated response to selection resulting from weak linkage of some of the genes or the genes of BHC and dieldrin resistance with the gene(s) for DDT resistance. It is due to such allelic linkage that selection by one insecticide may, at times, select resistance or hyper susceptible strains to other unrelated groups of insecticide and other morphological and physiological characters for which selection was not practical.

2.4.8 Gene-Dose, Intensity and Limit of expression of resistance

The theory of polygenic/multigenic basis of a heritable character assumes that each gene of the system has small and equal contribution towards the intensity of expression of the character; the intensity of the character expressed is the cumulative effect of the number of genes present (Mather 1949). Hence intensity of expression of a character is a functions of gene dose. A comparision of the results of susceptibility tests obtained with the laboratory selected strain and field selected strain showed that in both the strains specific DDT resistance was developed in larval stage first which was followed by development of resistance in adult stage. In addition larvae and adults of both the laboratory and field selected strains showed nonspecific resistance of VT-type. In the field strain the intensity of expression of specific resistance to DDT was much higher than that in laboratory strain but the magnitude of expression of VT was similar in both the strains (c.f.2.3.9). These information suggested that the number of genes involved in specific larval resistance developed in field strain was much larger than the number of genes involved in the laboratory population. This is obvious for polygenic basis of resistance because the size of field population was much larger than that of laboratory population and the out breeding in field was more than that in the laboratory. In a larger population with random mating more genes will be available for selection than in smaller population, with some restricted mating.

The number of genes of a given character, however, cannot be unlimited. This is suggested by equal magnitude of VT-type resistance in both the field and laboratory strains. Failure to increase the intensity of larval resistance in A.stephensi B-strain by high selection pressure for 29 generations (c.f.2.3.10) could also be considered another observation suggesting existance of limit in the number of genes.

2.5.0 Summary

Laboratory studies were carried out to obtain information needed for comprehension of the genetic basis of insecticide resistance in insects and for development of efficient methodology of vector control with minimum cost and risk of hazards.

On the considerations of its vectorial importance, amenability to colonization in laboratory, and readily available colony with its history-sheet, A.stephensi type, was selected as an experimental animal. Chlorinated hydrocarbon insecticides were commonly used in control/ eradication of malaria and other insect borne diseases and hence DDT was chosen as selecting agent of resistance. Response to selection was assessed against DDT, HHC and dieldrin.

The studies on the speed of selection and intensity of expression of resistance due to different modes of DDT pressure involved in selection of adults, larvae and both adults and larvae of A.stephensi at LD_{50} and above levels of DDT for 57-59 generations were carried out. Survivors of each generations were used to raise the next generation. For the studies on the effect of withdrawal of selections the strains after

57-59 generations of selections pressure were cultured for eight generations without DDT pressure and then assessed for resistance. A.stephensi Erode strain was highly resistant to DDT. This strain was colonized in the laboratory and subjected to very high (60.00 parts for million) pressure of DDT for 29 generations and then assessed for resistance to see the effect of increased pressure on highly resistant strain. For evaluation of the effect of DDT selections the adults and larvae of 'stock colony' of A.stephensi were also tested for susceptibility to DDT, BHC and dieldrin and used for comparision with the test results of experimental strains.

The status of susceptibility/resistance to DDT, BHC and dieldrin of the adults and larvae of A.stephensi stock colony and the different experimental strains were assessed by susceptibility tests following standard methods. The tests with DDT were carried out to estimate the specific response to selection by DDT, and the test with BHC and dieldrin were under taken to study the phenomenon of non-specific vigour tolerance, pleiotropy, multiple resistance due to allelic correlation.

The dose-response data obtained from the susceptibility tests were transformed to log dose and probit metameters and then used to estimate the LD_{50} and regression coefficient values. LD_{50} was an estimate of the mean of the tolerance (resistance) distributions; the regressions coefficient being inversely proportional to the variance of tolerance distributions was used as a statistic to see the variance of resistance. The probit/log dose regression lines was also examined for existance of plateau which when found was considered as due to

major genetic factor for resistance. The LD_{50} of DDT for different generations were also used to see the trend of change in mean susceptibility status of different experimental strains over generations. Existence of increasing trend of ^{R} , was taken as a confirmation of the interpretations of the LD_{50} and regression coefficient values for the progress of selection . The progress of selections followed a growth curve; genetical activities under the lower asymptatic phase, exponential phase and upper asymptatic phase were stated. Trends of regression coefficient values were also studied and its behaviour was interpretated for genetic variance resulting from and required by stabilizing and 'directional' selections, respectively. On the hypothesis of pre-existing single major factor for resistance a simple model for the progress of DDT selections, at LD_{50}, of resistance, under bi-sexual random mating, was worked out. The model was used to fit the experimental data on selections to infer the number of gene(s) involved in specific resistance.

The LD_{50} and regression coefficient, b, values for larvae of A.stephensi stock colony was found to vary between 0.02 to 0.06 part per million of DDT and 2.5 to 3.3 respectively. These values for adult varied between 0.5 percent to 1.0 percent of DDT and 4.2 to 5.5 respectively Therefore to minimise the errors in accepting a case of resistance when it is not or in over estimating the magnitude of response to selection, the highest LD_{50}, 0.06 ppm and 1.0 percent, and the lowest value of b, 2.5 and 4.2 for larvae and adults respectively were considered as the base line statistics for assessment of the progress of DDT selection.

The first response to selection was evolution of resistance of vigour tolerance type (VT-type); specific resistance in larvae developed much later. Adult resistance evolved last in the sequence. The magnitude of expression of VT was less than or equal to 4.0 times higher than base line value and was due to polygenes which expressed in both the stages. Its early selections was attributed to pre-existance of VT-genes in high frequency. VT was considered to be of significant economic value to the species as it provides sufficient time for development of specific resistance and protect the specific genes from ecological hazard other than specific insecticide. The specific resistance developed due to slow concentration and organisation of large number of genes into a cluster which gets selected. The cluster under, DDT pressure evolved to stable state which gives maximum fitness to the species. A stable cluster would behave like a single major factor of resistance during its transmission. In view of sequential development of VT, larval resistance and adult resistance they were not considered as expression of the same gene, - each type was considered to be due to a set of 'seperate genes. All the three types of resistance could arise due to close linkage of the different sets of genes. The longer time for development of specific resistance was attributed to chromosomal inertia - weaker the inertia quicker the development and reversion of resistance. Low level of BHC and dieldrin resistance was due to correlated response to DDT selection. The speed of development of specific resistance was related with the intensity of selection pressure and the larval stage under selection. Natural selection was found to have stabilizing effect; it worked against highly

resistants and hyper-susceptibles. The DDT selection had directional effect - it worked against susceptible genotypes and favoured the specific resistant ones. Intensity of expression of resistance was the function of the 'gene dose' and 'gene organization'.

2.6 References

1. Brown, A.W.A. (1958) : Insecticide Resistance in arthropods, Geneva (World Health Organization: Monograph Series, No.38).

2. Brown, A.W.A. and Pal, R (1971) : Insecticide resistance in arthropods, Geneva (World Health Organization: Monograph Series, No.38).

3. Brown, A.W.A. (1967) : Genetics of insecticide resistance in insect vectors, In: Wright J.W. & Pal, R., ed. Genetics of insect vector of disease, Amsterdam, Elsevier, pp 505-552.

4. Busvine, J.R. & Nash, R (1953) : Bull. Ento. Res. 44, 371-376

5. Crow, J.F. (1957) : Ann. Rev. Ent. 2, 227-246.

6. Docker, G.C. and Bruce, W.N. (1952) : Amer. J. Trop Med. Hyg. 1, 395-403.

7. Finney, D.J. (1952) : Probit Analysis (2nd Ed.) Cambridge University Press, Cambridge.

8. Hoskins, W.M. and Gordon, H.T. (1956) : Ann. Rev. Ent. 1, 89-122.

9. Mather, K. (1949) : Biometrical Genetics, Methuen Co. Ltd. London.

10. Mather, K. (1973) : Genetic Structure of Population, Chapman and Hall. Ltd. London.

11. Mather, W.B. (1963) : Principles of Quantitative Genetics. Burgess, Minneapolis.

12. Miland, R. (1956) : Riv. Parassit., 17, 233-246.

13. Miland, R. (1959) : Genet. agr., 10, 288-308.

14. Singh, N.N. (1961) : Biometric study of insecticide resistance phenomenon in insects with spacial reference to mosquitoes - An approved dissertation for partial fulfilment of statisticians Diploma of the Indian Statistical Institute, Calcutta.

15. WHO (1960) : Expert Committee on Insecticides: Tenth Report, Geneva (Wld. Hlth. Org. Techn. Rep. Ser. No.191).

16. Wright, J.W. (1958) : Ind. J. Malar. 12, 269-277.

CHAPTER III

INHERITANCE OF SPECIFIC RESISTANCE TO DDT IN ANOPHELES STEPHENSI

3.1. Introduction

One normally expects a resistant species of mosquito to exhibit the character in both adult and larval stages. This expectation was realised in Culex fatigans. In this species both the adults and larvae were found resistant to DDT . (Pal and Singh 1958). In case of A.stephensi, however, such expectation was not fulfilled. In this species high degree of DDT resistance was first observed in larvae alone (Rajagopalan, Vedamanikkam and Ramoo, 1956; Mammen as quoted by Pal 1958). High degree of DDT resistance in adults, however, had subsequently developed in the field population. (Bhombore, Roy and Samson 1963).

Laboratory study, presented in Chapter II, on the effect of DDT pressure on A.stephensi confirmed the field observations. From the results of the laboratory study it was considered that the high degree of larval resistance was due to slow concentration and suitable organizations of large number of resistant alleles, each with small contribution towards resistance, into a cluster. In its earlier stage of development the cluster was considered to be un-stable one in its transmission but slowly it developed to a stable state under the DDT pressure. It was suggested that a stable cluster could behave like a single Mendelian factor in its expression and transmission. To test the validity or otherwise of these considerations and suggestions suitable experiments were carried out in the laboratory to study the mode of inheritance of resistance to DDT. The results of these experiments are presented in this chapter.

3.2 Material and Methods

3.2.1 Material

To study the mode of inheritance of larval resistance in A.stephensi crossing experiments were carried out with A.stephensi Madras strain (Strain M) & A.stephensi Erode strain (strain E). The strain-M was susceptible to DDT but the larvae of strain E was highly resistant, which developed in field. Prior to its use in crossing experiments, the strain E was maintained in the laboratory for 29 generations under DDT pressure at larval stage. Detailed history of these strains and the methods of their culturing were the same as given in Chapter II. The strain-M and the strain-E are hereafter called parent 1 (P_1) and parent 2 (P_2) respectively.

3.2.2 Breeding Plan

Crosses between P_1 and P_2 were made in both directions viz female P_1 X male P_2 and female P_2 X male P_1. The progeny of these two crosses are referred to as ME_{F_1} and EM_{F_1} respectively. The second generation obtained by in-breeding ME_{F_1} and EM_{F_1} are called as ME_{F_2} and EM_{F_2} respectively. In addition three back-cross populations, out of eight types of back-crossings, from the crosses like female P_1 X male EM_{F_1}, female EM_{F_1} X male P_1 and female ME_{F_1} X male P_1 were also obtained. The remaining five possible back crossings could not be carried out due to shortage of mosquitoes of appropriate genotypes. In the crossing experiments the females and males were mated en masse. Due care was taken to use only the virgin females for different crossings. To ensure virginity, Pupae were hatched singly in specimen tubes and the adults emerging from them were kept in separate cages, sex wise.

3.2.3 Testing Method

To detect and estimate resistance in larvae of P_1, P_2, F_1, F_2 and the three back crosses, late third or early fourth instar larvae were tested against DDT by WHO method using WHO susceptibility test kit The DDT doses higher than 5.0 ppm were not supplied with the WHO kit and hence they were, locally prepared with p,p' DDT in alcohol.

3.2.4 Statistical Method

The dose response data obtained from the susceptibility tests were transformed to log-probit metameters which were used to estimate the LD_{50} and regression coefficient, b, values by eye estimation method. The LD_{50} and b values for heterogenous populations are of low precision and so of little value. These statistics were, therefore, not estimated for F_2 and back-cross generations. The probit/log dose curves for these populations were, however, used for interpretations of the data following Hoskins and Gordon (1956), Coker (1958), Pal and Singh (1958) and Singh (1961).

3.3 Results

3.3.1 Level of resistance in parents and location of the genes

The results of the larval susceptibility tests of the parent strains and of the progenies of different crossings are given in Table 3.1. It could be seen from the table that the LD_{50} values of DDT for the susceptible parent (P_1) and resistant parent (P_2) were 0.02 ppm and $>$ 60.0 ppm respectively. This suggested that at the time of crossing experiment the P_2 was more than 3,000 times as resistant as the P_1. The LD_{50} of DDT for ME_{F_1} and EM_{F_1} were 1.14 ppm and 0.28 ppm which when compared with the similar value of P_1 showed that ME_{F_1} and EM_{F_1} were 57 times and 14 times, respectively, as resistant as the P_1.

of susceptible and resistant strains of *A. stephensi* and of the progenies of different crossings to varying doses of DDT

Dose of DDT in parts per million(ppn)	P_1		P_2		ME_{F_1}		EM_{F_1}		ME_{F_2}		EM_{F_2}		B_1		B_2	
	λ	p (n)	λ	p (n)	λ	p (n)	λ	p (n)	λ	p (n)	λ	p (n)	λ	p (n)	λ	p (n)
60.000			4	46.00 (87)	2	100.00 (40)	2	100.00 (40)								
30.000			4	42.20 (83)	2	100.00 (40)	2	100.00 (40)								
15.000			4	20.90 (86)	4	100.00 (90)	2	100.00 (40)	3	63.20 (66)	4	81.30 (75)				
7.150			4	25.80 (89)	4	90.00 (90)	2	100.00 (40)								
5.000			2	0.00 (36)					5	59.40 (101)	6	76.00 (125)				
3.750					4	81.10 (90)	2	97.50 (40)								
2.500	2	100.00 (40)			4	76.40 (89)	6	98.60 (140)	6	57.50 (120)	6	73.60 (125)	6	97.30 (150)	6	92.40 (118)
0.500	4	100.00 (90)			4	26.10 (88)	6	68.60 (140)	4	39.80 (83)	4	56.00 (75)	4	78.70 (75)	6	68.60 (121)
0.250									2	40.00 (50)	6	38.40 (125)	2	52.00 (50)	2	66.00 (50)
0.100	4	96.70 (90)			4	0.00 (89)	6	20.00 (140)	4	7.60 (77)	6	11.20 (125)	6	48.30 (147)	2	58.00 (50)
0.020	4	68.90 (90)					5	1.00 (100)	2	0.00 (50)	6	0.00 (125)	5	20.00 (95)	6	16.90 (118)
0.004	4	2.20 (90)											5	0.00 (100)	3	0.00 (65)
Control	4	0.00 (80)	4	0.00 (83)	4	0.00 (90)	4	0.00 (90)	2	0.00 (50)	5	0.00 (100)	5	0.00 (100)	4	0.00 (82)
LD_{50}		0.02		>60.00		1.14		0.28	Population were heterogenous and so the LD_{50} and b values were not estimated from the dose-response data of these generations.							
b		2.50				1.70		2.7								

Foot Note (Table 3.1)

P_1 : Madras (Susceptible) strain, [redacted]

P_2 : Erode (resistant) strain

ME_{F_1} : Generation 1 of the Cross female P_1 x male P_2

EM_{F_1} : Generation 1 of the Cross female P_2 x male P_1

ME_{F_2} : Progeny of female ME_{F_1} x male ME_{F_1}

EM_{F_2} : Progeny of female EM_{F_1} x male EM_{F_1}

B_1 : Progeny of female P_1 x male EM_{F_1} and female EM_{F_1} x male P

B_2 : Progeny of female ME_{F_1} x male P_1

where x stands for cross

λ : Number of replicate tests

p : Mortality rate (%)

(n) : Number of larvae exposed

Hence it could be concluded that DDT resistance in larvae was transmitted to the offspring through both male and female parents. Such a phenomenon is possible only when the gene - allele(s) for resistance is located in the chromosomes rather than in the cytoplasm. Cyto-genes for resistance would have expression only in the progeny of resistant female alone since they are not present in the sperm.

3.3.2 Dominance of Alleles

The log LD_{50} values of ME_{F_1} and EM_{F_1} were slightly less than half the difference between the log LD_{50} values of P_1 and P_2 (Fig. 3.1). This suggested slight dominance of susceptible allele(s) over the resistant ones.

3.3.3 Influence of other chromosomal and extra-chromosomal factors on the expression of resistant alleles.

The intensity of resistance was not the same in ME_{F_1} and EM_{F_1} since their LD_{50} were 1.14 ppm and 0.28 ppm. respectively (Table 3.1). Based on these LD_{50} values ME_{F_1} was considered four times as resistant as the EM_{F_1}. Further, the variance of the distribution of resistance over log-doses was higher with ME_{F_1} than with EM_{F_1} as indicated by the b values, 1.7 and 2.7, respectively. Higher mean and variance of resistance in the offspring of male resistant parent than those for the offspring of female resistant parent could be due to the influence of genes located on the male-sex determining chromosome and/or the cytoplasmic factors of the female susceptible parent with which the chromosomal gene(s) for DDT resistance could interact. Thus the expression of the chromosomal

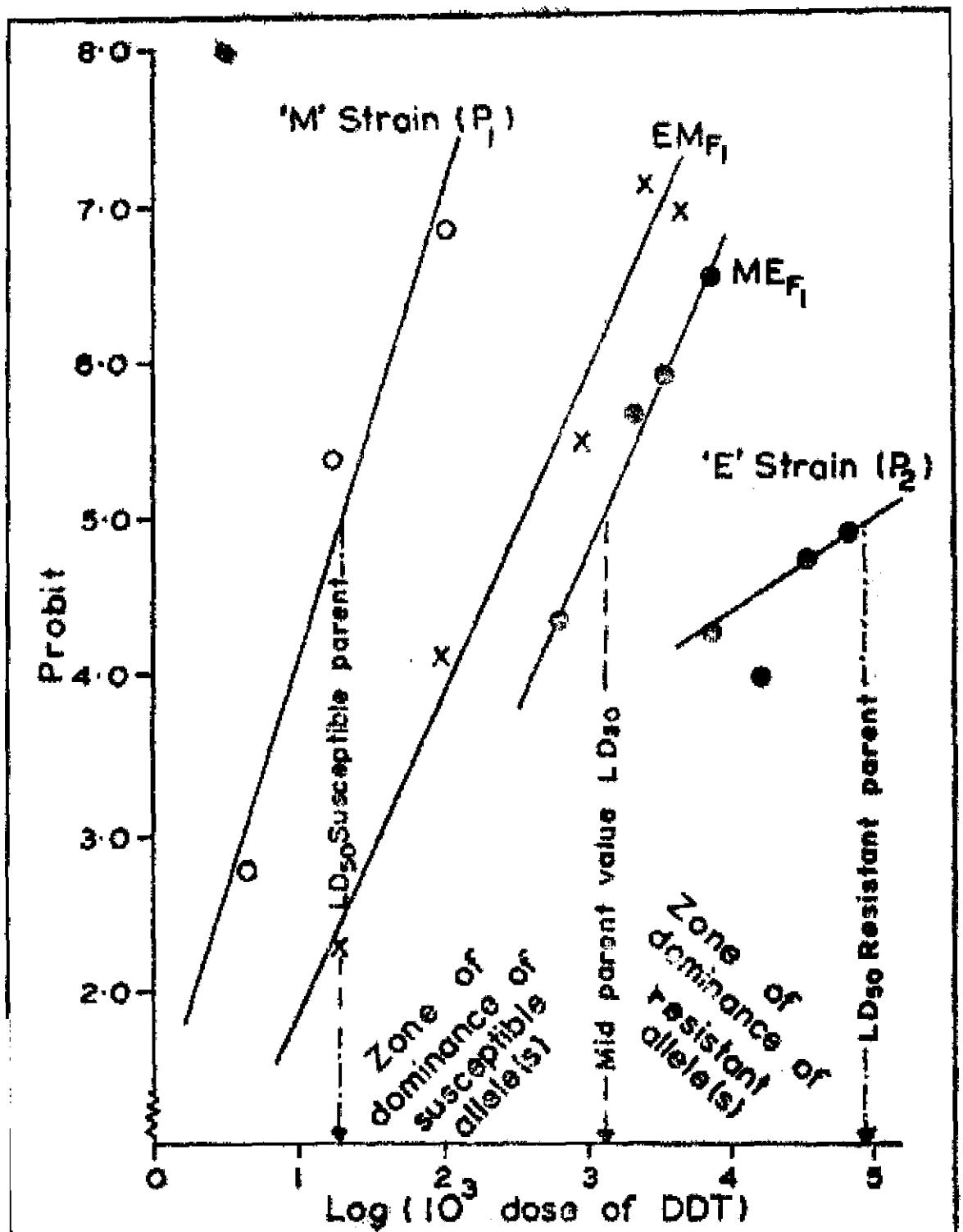

Fig. 3·1 PROBIT/LOG DOSE REGRESSION LINES OF THE PARENTS, F_1 GENERATIONS & THE LOG 10^3 LD_{50} VALUES OF PARENTS AS WELL AS THE MID PARENT VALUES

gene(s) for resistance is influenced by the other genes located on the male-sex determining chromosome and/or the cytoplasmic factors of P_1 females which constitute the immediate environment of the chromosomes.

3.3.4 Mode of inheritance

To study the mode of inheritance of resistance the probit/log dose curves of F_2 and back-cross generations were considered. The probit/log dose line of ME_{F_2} was not linear; it had two small plateaus one over the doses 0.25 ppm to 0.5 ppm and the other over 2.5 ppm to 5.0 ppm (Fig.3.2). This first plateau was over LD_{100} of P_1 and the second plateau was over LD_{85-90} of ME_{F_1}. Mortality rate of ME_{F_1} at the doses under the first plateau was 26.1 percent and the mortality rate of P_2 at the doses under the second plateau was zero percent. At the LD_{100} of ME_{F_1} (15 ppm) 20-25 percent of P_2 were also killed (Table 3.1). This information suggested that the population of ME_{F_2} was a mixed one; consisting of three classes like P_1, ME_{F_1} and P_2, but none of the three classes of populations could be clearly discriminated by discriminating doses. Presence of three classes of populations in the F_2 generation suggested that a single gene with two alleles, each having large effect, are involved in determining the character, resistance vs susceptibility.

The probit/log dose curve of the larvae of EM_{F_2} was also not linear; it flattened at the top, over the doses 2.5 ppm to 15 ppm (Fig.3.3). This suggested the presence of two phenotype classes which could be discriminated by the dose 5.0 ppm of DDT since at this dose all the P_1 and EM_{F_1} type of populations would be killed but none of the P_2 type populations would be affected (Table 3.1). The survivals at 5.0 ppm will,

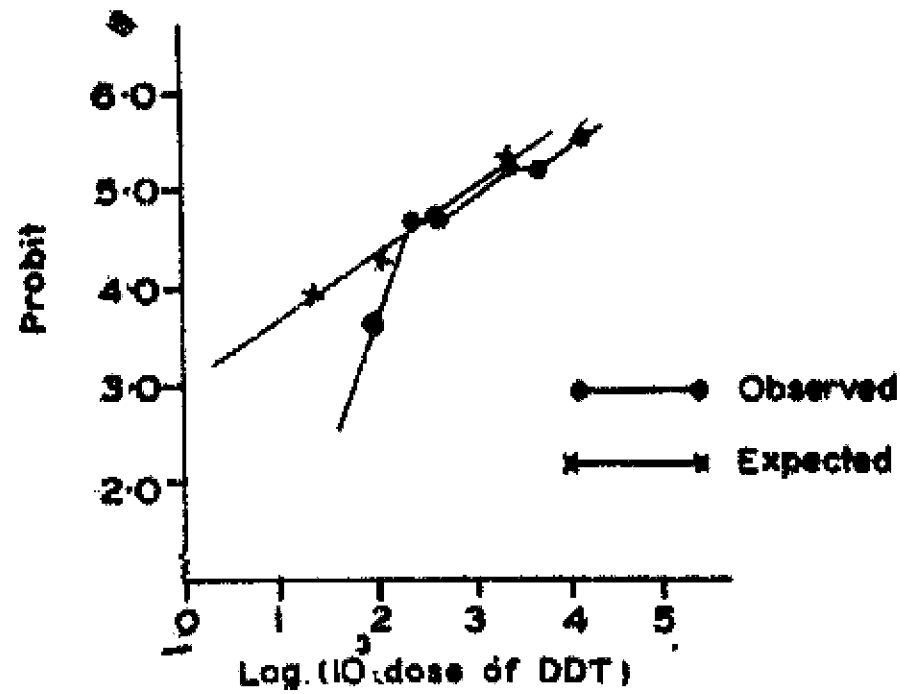

Fig.3·2 PROBIT LOG (10^3 DOSE) CURVE FOR ME_{F_2}

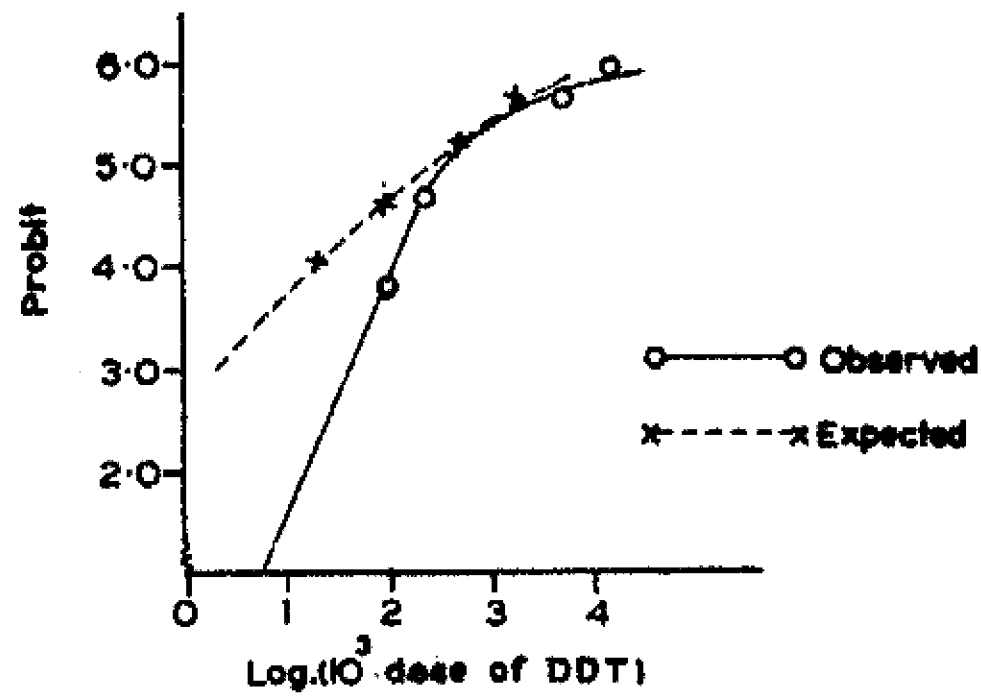

Fig.3·3 PROBIT LOG (10^3 DOSE) CURVES FOR EM_{F_2}

therefore, be only of P_2 type. The result of the susceptibility tests showed that the survival rate at 5.0 ppm was 23.2 percent which was close to 25 percent expected on the di-allelic single gene (monofactorial) basis of resistance. Thus the EM_{F_2} also showed that a single chromosomal gene with two alleles determined the DDT susceptibility/resistance in larvae of A.stephensi. Absence of plateau at LD_{100} of P_1 in EM_{F_2} could be due to almost same status of susceptibility of P_1 and FM_{F_1} at higher doses; mortality rates of 100 percent and 98.6 percent was observed for P_1 and EM_{F_1}, respectively, at 2.5 ppm of DDT (Table 3.1).

The probit/log dose lines of the back-cross generations, B_1 and B_2 also suggested plateaus over the doses 0.1 ppm to 0.25 ppm and 0.25 ppm to 0.5 ppm, respectively (Fig.3.3 & 3.4). At these doses of DDT almost complete kill of P_1 population and 20 and 26 percent kill of EM_{F_1} and ME_{F_1} populations, respectively, were observed (Table 3.1). Single plateau in each case on the probit/log dose line of the B_1 and B_2 generations suggested that each population was a mixed one, consisting of two classes, viz P_1 and EM_{F_1} in B_1 and P_1 and ME_{F_1} in B_2. Such a mixed populations with non-linear probit/log dose line could be possible when resistance is due to a di-allelic gene with pronounced effect. Thus the information obtained from the F_2 and back-cross generations gave the same conclusion that DDT resistance in larvae was controlled by a di-allelic gene having pronounced effect. The alleles were in heterozygous combination in F_1 and they segregated and recombined randomly giving three genotype classes in F_2 and two genotype classes in the back-cross generations.

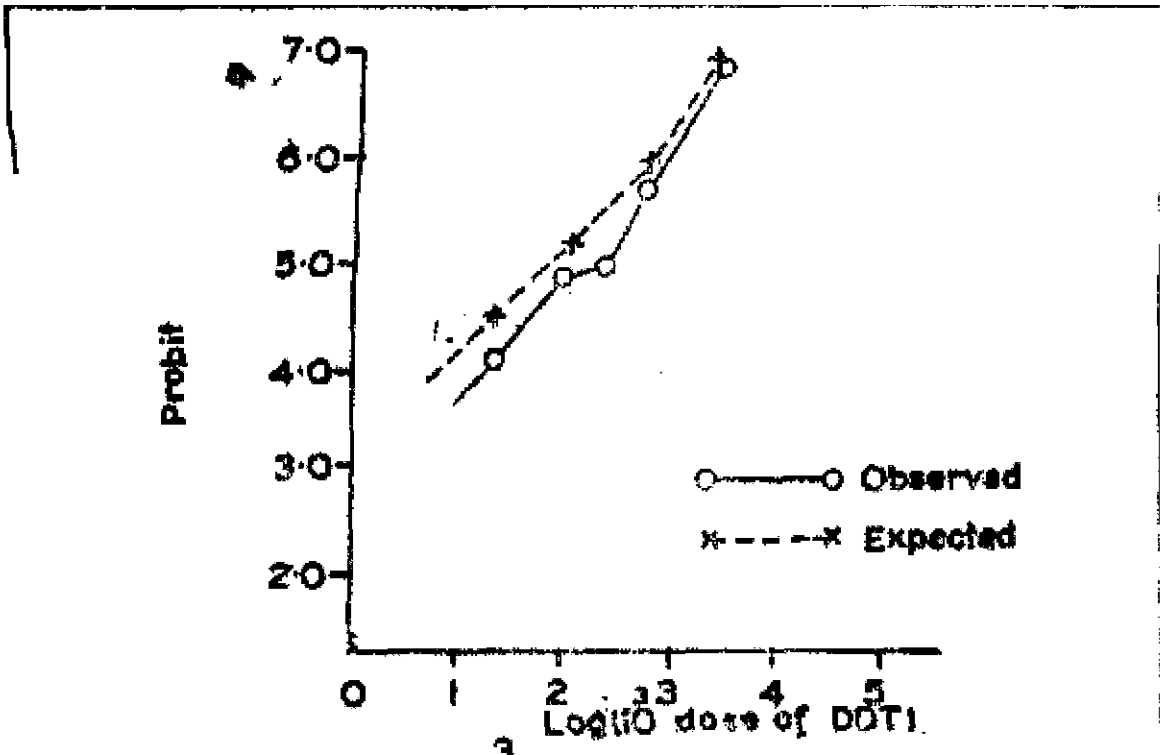

Fig.3·4 PROBIT LOG (10^3 DOSE) CURVES FOR B_1 GENERATIONS

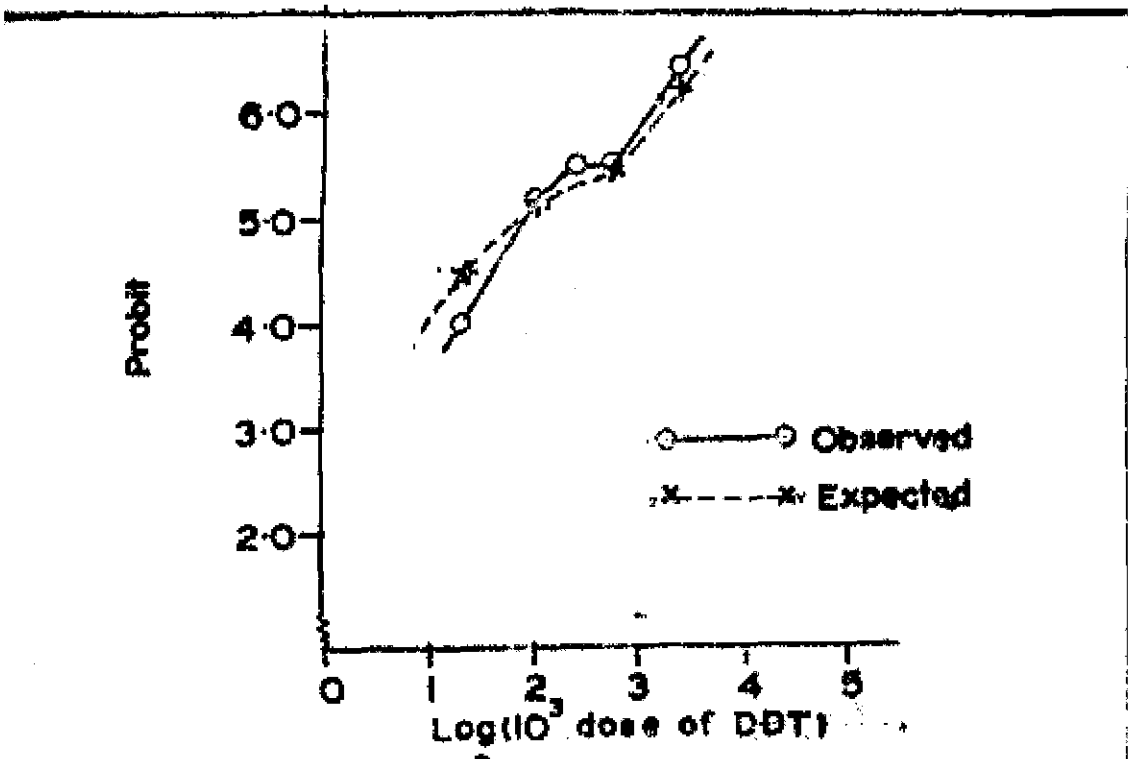

Fig.3·5 PROBIT LOG (10^3 DOSE) CURVES FOR B_2 GENERATIONS

3.3.5 Additional Polygenic System

The inference on genetic basis of DDT resistance in larvae of A.stephensi drawn in the preceeding section was based on the existance of plateau(s) on the probit/log dose curves of the segregating generations. Plateau(s) on the probit/log dose curves is a necessary condition for monofactorial basis (Single gene with pronounced expression giving rise to frequency data rather than measurements) of resistance but it is not a sufficient one since the shape of the probit/log dose curve due to multifactorial basis of resistance could be under certain condition, similar to the one, for a monofactorial basis of resistance Tsukamoto (1963). Further, the use of discriminating dose to estimate the frequency of different classes may miss the the small contribution towards resistance of the minor genes due to the masking effect of high doses. Therefore, to improve upon the inference, the hypothesis of monofactorial basis of resistance was tested by χ^2 test at a number of doses. For this purpose expected numbers of dead larvae for each dose, x_1 were estimated as

$$r_x = n_1 P_{P_1} + n_2 P_{F_1} + n_3 P_{P_2}$$

where r_x = number of insects killed by a dose x

$n_1 : n_2 : n_3$ is as 1:2:1 and

$n_1 + n_2 + n_3 = n$ the number of insects to the dose x

P_{P_1}, P_{F_1} and P_{P_2} are the probability of kill at dose x for the Parent populations (P_1 and P_2) and F_1 generation of their reciprocal crosses. The probability of kill is estimated from the Probit/log dose regression lines of each population.

It could be seen from the Table 3.2 that the sum of the χ^2 values for each dose was 27.4615, 59.5040, 15.8967 and 18.8293 for ME_{F_2}, EM_{F_2}, B_1 and B_2 respectively. These values with 4 degree of freedom (df) were highly significant. This suggested that the hypothesis of single gene for resistance was not tenable.

A perusal of [redacted] χ^2 values for each dose seperately, however, showed that the values with df=1, for the lower doses (0.02 ppm and 0.10 ppm) were highly significant. These high values contributed to significantly high sum of χ^2 values for all doses. In this connection it may be seen from Table 3.2 and Figs. 3.2 to 3.5 that the observed kill was consistently lower than the expected kill in lower doses for almost all the segregating generations. None of the χ^2 values, with df=1, for the doses 0.5 ppm and 2.5 ppm for ME_{F_2}, EM_{F_2}, B_1 and B_2 was significant at P=0.01.

From the above it is inferred that the larval resistance was controlled by a major gene in associations with large number of small genes, poly-genes. The intensity of resistance due to major gene was high but it was of low magnitude for the poly-genes and hence was detected only at lower doses of DDT.

3.4 Discussion

DDT resistance in both adults and larvae of A.stephensi has been reported to be governed monofactorially, the expression of the resistant gene (factor) being modified by genetic background (Davidson and Jackson 1961). These conclusions were arrived at from genetical studies carried out with A.stephensi of Iraq and India. The degree of resistance in both adults and larvae of the Iraq strain was of a lower order.

Table 3.2

Statistical Test of the Hypothesis of Single Gene Basis of DDT Resistence in Larvae of A.stephensi

Generation	Source	Dose - Response Data												Total Chi^2 d.f.(4)
		0.02 ppm			0.1 ppm			0.5 ppm			2.5 ppm			
		Alive	Dead	Total	Alive	Dead	Total	Alive	Dead	Total	Alive	Dead	Total	
ME_{F_2}	Observed	50.00	0.00	50.00	71.00	6.00	77.00	53.00	35.00	88.00	51.00	69.00	120.00	
	Expected	41.98	8.02	50.00	55.97	21.03	77.00	54.12	33.88	88.00	40.08	79.92	120.00	
	Chi Square value			8.3982			14.7187			0.0601			4.2845	27.4615
EM_{F_2}	Observed	125.00	0.00	125.00	111.00	14.00	125.00	32.00	43.00	75.00	33.00	92.00	125.00	
	Expected	104.09	20.91	125.00	80.41	44.59	125.00	28.13	46.87	75.00	26.38	98.62	125.00	
	Chi. Square value			23.9238			32.6223			0.8519			2.1055	59.5040
B_1	Observed	76.00	19.00	95.00	76.00	71.00	147.00	16.00	59.00	75.00	4.00	146.00	150.00	
	Expected	65.13	29.87	95.00	60.64	86.36	147.00	11.55	63.45	75.00	2.10	147.90	150.00	
	Chi Square value			5.5575			6.6225			1.9033			1.7434	15.8267
B_2	Observed	98.00	20.00	118.00	21.00	29.00	50.00	38.00	83.00	121.00	9.00	109.00	118.00	
	Expected	81.54	36.46	118.00	24.80	25.20	50.00	47.67	73.33	121.00	16.17	101.83	118.00	
	Chi Square value			10.7535			1.1552			3.2366			3.6840	18.8293

Chi Square value for each dose has 1 degree of Freedom.

For drawing more valid conclusions on the genetic basis of resistance, Davidson and Jackson (1961) suggested the need for a study based on susceptibl and resistant strains originating from the same part of the world. The susceptible and resistant strains of A.stephensi used in the present study originated from the same geographical region, Tamilnadu state of India. Furthermore the degree of resistance in one of the strains (Strain-E) was of a very high order (>3,000 times) in larval stage only. The susceptible strain (Strain-M) also possessed some specific and non-specific polygenes for resistance. The polygenes under DDT pressure at LD_{50} and above levels and under closed breeding were found to give rise to DDT resistance in larval stage (Chapter I).

The results obtained in the inheritance study with these strains showed that F_1 generation was more resistant to DDT than the susceptible M-strain and less resistant than the resistant E-strain. This finding is in conformity with that reported by Davidson and Jackson (1961) for A.stephensi. The high resistance of F_1 than the susceptible parent should not be taken as an indication of dominance of alleles for resistance. Resistance is a quantitative character and hence to decide the dominance of either types of alleles the LD_{50} of F_1 generation was compared with the mid-parent LD_{50} values (Fig.3.1). The result showed that the susceptible alleles were slightly dominant over the resistant alleles.

The F_1 generations of the reciprocal crosses of strain-M and Strain-E were not equally susceptible to DDT. Larvae of the line obtained by crossing the female susceptible strain with male resistant strain were four times as tolerant as the line obtained by reciprocal

crossing. Further the variance of distribution of resistance over log doses was also higher in the former case than the latter. Similar differences in resistance levels in F_1 generation of reciprocal crosses were also observed by Davidson and Jackson (1961). These findings suggested the presence of minor genes in the male-sex determining chromosome of the resistant strain, which (minor genes) also contributed towards the total resistance expressed in A.stephensi. This conclusion was considered valid in view of the results of the classical experiments of Crow (1957). Using 'mutant markers' Crow (1957) showed that the DDT resistant genes in Drosophila melanogester were present, at varying levels, on all chromosome pairs.

The results of susceptibility tests with F_2 and back-cross generations of A.stephensi showed that DDT resistance was due to one major factor (a di-allelic gene with pronounced expression) in association with minor factors (polygenic system). This information obtained from the inheritance studies showed that the resistant cluster developed under DDT-pressure in field was fairly stable intransmission.

A comparision of the results of inheritance study with A.stephensi of M-strain and E-strain with those reported by Davidson and Jackson (1961) showed that the mode of inheritance of DDT resistance of Iraq and Erode strains were similar. Resistance in the Iraq strain, however, was expressed at both adults and larval stages while in Erode strain it was expressed only in the larval stage. Susceptibility tests before the commencement of the inheritance study indicated a beginning of the

development of DDT resistance in adult stage under DDT pressure. These information suggested that the cluster of genes for larval resistance was a stable one but not a matured one; it was in the process of maturity and evolution through integration and organizations of genes which would result in better fitness of the species for survival at both larval and adult stages of its lifecycle under the DDT contaminated environments. Resistance in A.stephensi E-strain was developed in field, at least six years before the inheritance study was carried out, due to the use of DDT under the Malaria Control Programme. Hence it could be considered that during the period of at least six years the DDT specific resistance cluster was evolved and became stable enough to be inherited like a single gene as observed in this study. Whether the resistance cluster had developed to the state of absolute stability can be decided only by repeated backcrossing with susceptible strain and selection.

It was evinced from the results of reciprocal crosses that the DDT specific resistance cluster was located on the chromosome which is also the site of other genes including the genes controlling VT-type of resistance (Chapter IV). Following Mather (1973) it could be considered that the system of polygenes controlling VT-type of resistance and stable cluster of genes for specific DDT resistance are likely to be intermingled along the length of the same chromosome and hence the genes of the two systems could be partially linked to one another. The lower kill in the segregating generations (F_2 and back-crosses) than expected on single gene hypothesis, at the lower doses of DDT could be attributed to such linkage phenomenon.

3.5 Summary

Laboratory studies were carried out on the inheritance of DDT resistance in larvae of A.stephensi. The resistant strain was highly resistant to DDT (more than 3,000 times) at larval stage alone. Both the susceptible and resistant strains originated from Tamilnadu state of India.

The allele for susceptibility to DDT was found to be slightly dominant over the allele for resistance. Auxiliary factors, located on the male-sex determining chromosome were found to contribute towards the DDT resistance.

DDT resistance was, however, controlled mainly by a stable cluster of genes in association with a system of polygenes. The mode of inheritance and the dominance of DDT-resistance cluster were found to be similar to that reported for A.stephensi of Iraq. A comparision of the result suggested that the specific DDT resistance cluster in A.stephensi Erode-strain was a stable one but not a matured one; it was in the process of evolution through intergration and suitable organization of poly genes to give resistance in adult stage as well.

3.6 References

1. Bhombore, S.R. Roy, R.G. and Samson, F. (1963) : Bull.nat.Soc.India Malar. 11, 35-38

2. Coker, W.Z. (1938) : Ann.trop.Med. Parasit., 52, 443-445.

3. Crow, J.F. (1957) : Ann. Rev.Ent. 2, 227-246.

4. Davidson, G. and Jackson C.E. (1961) : Bull.Wld.Hlth.Org.25, 209-217.

5. Hoskins, W.M. and Gordon, H.T. (1956) : Ann.Rev. Ent. 1, 89-122.

6. Mammen quated by Pal, R. (1958) : Indian J.Malar 12, 383-399.

7. Mather, K. (1973) : Genetic Structure of Population. Chapman and Hall; Ltd., London.

8. Pal, R and Singh, N.N. (1958) : Indian J. Malar 12, 499-516.

9. Rajagopalan, N., Vedamanikam, J.C. and Ramoo, H. (1956) : Bull. nat.Soc.India Malar., 4, 126-128.

10. Singh, N.N. (1961) : Biometric study of insecticide resistance phenomenon in insects with special reference to mosquitoes - An approved dissertation for partial fulfilment of Statistician's Diploma of the Indian Statistical Institute, Calcutta.

11. Tsukamoto, M. (1963) : Botyu-Kagaku, 28, 91-98.

CHAPTER IV

GENETICAL BASIS OF NON-SPECIFIC DIELDRIN RESISTANCE IN ANOPHELES STEPHENSI

4.1. Introduction

Insecticide resistance/tolerance as diagnosed by quantal response of insects to toxic chemical compounds was classified into three types viz. non-heritable resistance, heritable group specific resistance and heritable group non-specific resistance (Singh 1961). Non heritable resistance was due to the effect of age, sex, nutritional status of the test insects and the environmental factors like temperature, humidity, crowding etc., during the testing/rearing periods. This type of resistance being of low magnitude is of no problem in the vector control actions. The group specific heritable resistance was usually of high order and could be obtained by the selection pressure of any one of the toxicant belonging to a given group. Thus resistance selected by the pressure of DDT was extended over to methoxychlore, DDT belonging to the DDT group and not over the toxicants of cyclodiene derivatives and gamma HCH (Busvine 1954, Davidson 1956 and Brown 1958). This type of resistance is a great problem in the vector control scheme. Studies on selection/development and inheritance of DDT specific resistance were presented in Chapter II and Chapter III. The non-specific heritable resistance was usually of low magnitude and it extended over the toxicants belonging to other groups. This type of resistance was termed by Hoskins and Gordon (1956) as "Vigour tolerance" and could be diagnosed by the nature of the log-dose probit regression line.

The studies on the selection of resistance by the pressure of DT in A.stephensi showed the precipitation of DDT specific resistance and non-specific resistance to BHC and dieldrin (DL)(Chapter II). Non-specific heritable resistance (vigour tolerance), on the basis of toxicity tests and nature of dose-response regression line, was considered to be polygenic. Experimental evidence on its genetical basis has so far not been reported. Genetical studies of the non-specific DL resistance selected by the DDT selection pressure in A.stephensi has, therefore, been carried out. The results of that study are recorded in this Chapter.

4.2 · Material and Methods

4.2.1 Biological material

The study was carried out with A.stephensi Madras strain (strain M) and A.stephensi DDT resistant Erode strain (strain E). These strains were hereinafter referred to as P_1 and P_2 respectively.

The P_1 was found to contain, in an unorganised State, determinants genes) for DDT specific resistance and non-specific resistance to dieldrin and BHC. Under the selection pressure these determinants were organised in an effective form which gave phenotypic expression of resistance to DDT, DL and BHC (Chapter II).

The larvae of P_2 strain were found to be highly resistant to DDT also showed low degree of resistance to BHC and DL. Resistance to fferent insecticides was selected in the field mainly due to the use DDT. The strain was colonized in the laboratory and maintained under

ith selection pressure of DDT at the larval stage. The detailed history f both the strains and the methods of their culturing were described in apter II (c.f. 2.2).

4.2.2 Breeding ...on

Reciprocal crossings, en masse, were carried out with P_1 and P_2. ...s F_1 generations of the reciprocal crosses were inbread and F_2 progenies ...re obtained. Biparental progenies (BIP) were also obtained from randomly ...lected 100 female mosquitoes of F_2 generation which were exposed to ...masse sibmating*. The method of collections of material for crossing experiments and the precautions taken to avoid contamination during the breeding experiments were the same as described in Chapters II and III (c.f. 2.2. and 3.2.2).

4.2.3 Method of testing

The parent strains and their progenies obtained from the different crossings were characterised on the basis of the response of larvae to different concentrations of DL. For that purpose susceptibility tests were carried out following the WHO technique and using 'WHO test Kits' for mosquito larvae (WHO 1963). In some cases intermediary and lower concentrations of DL were locally prepared by diluting the WHO 'stock solutions'. P_1, P_2 and F_1 larvae were tested simultaneously under the same condition. The larvae of F_2 and BIP were tested later. The environmental conditions were, however, almost similar during all the tests.

The proportion of female mosquitoes inseminated by more than one male is very low (Kitzmeller and Laven 1958). A male mosquito was, however, found to inseminate more than one female mosquito in one night of exposure. The inseminating efficiency of the male mosquito was affected by factors such as density of mosquitoes, male and female ratio in the population, age of the mosquitoes etc. (Krishnamurthy, Singh and Verghese 1965-66).

4.2.4 Biometrical methods and inferences

The dose response data obtained from the susceptibility tests (Table 4.1) were analysed by probit analysis method following Finney (1952). The dose metametres were obtained after multiplying the concentrations of DL, giving less than 100 percent mortalities in the tests, by 10^4 to avoid the negative log-values. The mortality rates were converted to probit. Linear regression models were fitted to the dose-response metametres. The validity of linear model, in each case, was tested by χ^2 tests. In all cases the linear regression models were found valid. The maximum likelihood estimates of the regression coefficients were different in different cases and therefore the hypothesis of parallelism of the regression lines for P_1, P_2, F_1 and F_2 were tested. The analysis of χ^2 showed the validity of this hypothesis (Table 4.2). LD_{50} in log-unit, m, and natural concentration units, parts per million (ppm), with their fiducial limits at 95 percent levels of probabilities and the regression coefficient values with their standard errors were estimated. The LD_{50} values were the estimates of the mean response rates of the different strains. The regression coefficient values being proportional to the inverse of the standard deviation of tolerance distribution of the population was used to judge the variance. Therefore, the LD_{50} values in log unit and natural unit and the regression coefficient values were used to characterise the strains and their progenies and to draw inference on the nature of the determinants, their dominance, segregation and linkage.

4.3 Results

4.3.1 Characterisation of Parent Strains

The LD_{50} values of DL for P_1 and P_2 strains were estimated as 0.00070 ppm and 0.00256 ppm, respectively (Table 4.3). The upper fiducial limit of P_1 did not overlap with the lower fiducial limit of P_2 estimated at 95 percent level of probability showing the significant difference between the mean response of the two strains. A comparision of the two LD_{50} values suggested that P_2 was 3.7 times as resistant to DL as the P_1.

The regression coefficient, b, values of P_1 and P_2 strains were estimates as 3.36 ± 0.36 and 2.54 ± 0.25 respectively. The difference between these estimates were not significant (Table 4.2). This suggested that the variance of tolerance distributions of the two populations were identical.

Both the strains were cultured and tested for their susceptibility to DL under identical conditions and hence the effect of non-heritable factors on the means and variances of tolerance distributions could be considered to be of same magnitude in both the strains. These estimates of LD_{50s} and b_s could, therefore, be considered as the biometrical characteristics of the P_1 and P_2 strains.

4.3.2 Location of Resistant determinant(s)

The results of susceptibility tests with the larvae of F_1 generation obtained from the reciprocal crosses between P_1 and P_2 strains were within the limits of normal variations (Table 4.1). This

Table 4.1: Larval mortality rate (percent) at different doses of dieldrin (DL)

Strains/Crossing	Symbol	Doses of dieldrin(DL) in parts per million (ppm)										
		2.5	0.5	0.1	0.02	0.004	0.001	0.0008	0.0004	0.0002	0.00016	Control
Susceptible Madras Strain (M)	P_1			100.0 (50)	100.0 (100)	99.0 (100)	- -	59.0 (98)	- -	- -	1.0 (100)	0.0 (100)
Resistant Erode Strain (E)	P_2	100.0 (100)	100.0 (100)	100.0 (100)	99.0 (99)	68.0 (100)	- -	10.0 (96)	- -	- -	- -	0.0 (100)
Female M x Male E	$M \times E$ F_1			100.0 (50)	100.0 (100)	92.0 (100)	- -	23.0 (150)	- -	0.- (75)	0.0 (50)	0.0 (125)
Female E x Male M	$E \times M$ F_1		100.0 (25)	100.0 (25)	100.0 (75)	94.0 (99)	40.0 (50)	32.0 (100)	- -	- -	0.0 (50)	0.0 (75)
Total of F_1 of the reciprocal crosses	F_1		100.0 (25)	100.0 (75)	100.0 (175)	93.0 (199)	40.0 (50)	27.0 (250)	- -	0.0 (75)	0.0 (100)	0.0 (200)

Figures in parenthesis are the numbers of larvae exposed.

Table 4.27 - Test of Parallism of Probit/Log dose Regression lines for different strains and generations

Strain/ Generation	Sxx	Sxy	Syy	χ^2	DF
P_1	7.8096	25.5543	86.3660	0.5060	1
P_2	16.3944	41.7031	106.1825	0.2567	1
F_1	19.2005	57.1837	170.2271	0.1271	1
F_2	14.9720	51.4255	178.0906	1.7001	2
Total	58.1765	175.6669	540.8662 531.7124	2.5899	5
	$\frac{(Sxy)^2}{Sxx}$ = 531.7124		9.1538		

Analysis of χ^2		
Source	DF	SS
Parallelism of regression	3	6.5639
Residual Heterogeneity	5	2.5899
Total	8	9.1538

$\chi^2_{[5]}$ = 2.5899 for residual heterogeneity is not significant at P=0.05. Therefore SS for parallelism was tested as χ^2 since χ^2 =6.5639 is also not significant at P=0.05 the Hot regression lines are parallel was accepted. The regression coefficient from the pooled data was estimated as be 3.023.

Table 4.3

Statistical Summary of Susceptibility Test Results:
Susceptibility to DL in Par ents and offsprings

		P_1	P_2	F_1	F_2	BIP
Probit/log dose relation:		Y=2.1643+3.36x	Y=1.4214+2.54x	Y=1.7176+2.98x	Y=0.7452+3.3.43x	Y=3.8065+1.41x
Validity chisquare: (degree of freedom)		0.50 (3)	0.26 (4)	0.13 (4)	1.70 (3)	2.18 (2)
$m \pm \varepsilon E$:		0.8440 ± 0.034	1.4089 ± 0.040	1.0148 ± 0.023	1.6750 ± 0.030	0.8465 ± 0.068
LD_{50} (ppm) :		0.00070	0.00256	0.00103	0.00473	0.00070
Fiducial limit of m (α= 0.95)	Lower:	0.7773	1.3305	0.9697	1.6162	0.7132
	Upper:	0.9107	1.4873	1.0599	1.7338	0.9798
Fiducial limits of LD_{50} (α= 0.95	Lower:	0.00060	0.00214	0.00093	0.00413	0.00052
	Upper:	0.00810	0.00307	0.00115	0.00542	0.00095
Regression coefficient ±SE		3.36 ± 0.36	2.54 ± 0.25	2.98 ± 0.23	3.43 ± 2.60	1.41 ± 0.15

Note : $x = \log (10^4 \times \text{dose of DL})$

$m = \log (10^4 \times LD_{50})$

suggested that there were no difference in the susceptibility to DL of the larvae obtained from the reciprocal crosses. It could, therefore, be inferred that the determinant(s) for DL resistance were borne on the chromosomes and cytoplasm was not involved as loci for these determinants.

4.3.3 Major gene(s) versus polygenes

The specific resistance to DDT in the P_2 strain was of the very high order in the larvae (Chapters II and III) but the non-specific resistance of the larvae to DL was only 3.7 times. Varying proportions of P_1 and P_2 populations were also found to be phenotypically indistinguishable when exposed to certain concentration of DL (Table 4.1). Hence the level of non-specific DL resistance could be considered of low magnitude which could be readily mimicked by factors contributing to non-heritable resistance. Further the linear regression models were 'good fit' to the dose response data of the segregating generations viz. F_2 and BIP - the values of $\chi^2_{[3]}$ and $\chi^2_{[2]}$ being 1.70 and 2.18 respectively (Table 4.3) which were not signficient at $P = 0.05$. This suggested continuous variation of phenotypes as diagnosed by quantal response of the larvae to the varying concentrations of DL. The dose response curve of F_2 also did not suggest plateau which was invariably found when the resistance was controlled by a single major factor having pronounced expression (See Brown 1967). Further the determinants for DL-resistance were found to be located on the chromosome. Therefore, following Mather (1949), it could be said that the non specific DL resistance in A.stephensi selected by the DDT selection pressure was polygenic.

The non-specific DL resistance could be considered distinct one and was different from the specific DL resistance as in the latter case, whenever studied, the phenotypic expression of resistance was of high magnitude and was controlled by a major factor (Davidson 1956

4.3.4 Dominance of Alleles .

The magnified ($x10^4$) LD_{50} values in logarithmic unit, m, were estimated from the pooled dose response data of F_1 larvae obtained from the reciprocal crosses were 1.0148 ± 0.023 (Table 4.3). This value was very close to the mid-parent value, 1.1, in the same unit. It could, therefore, be considered that there was almost no dominance of the non-specific DL resistant character over the susceptible and vice versa, controlled by specific sets of alleles, in the given environment.

4.3.5 Segregation and Linkage

The characteristics of nuclear borne genes are segregation and linkage. In case of polygenes the independent segregation would result in a compound population in F_2 generation consisting of populations like P_1, F_1 and P_2 in the ratio of 1:2:1. Hence the expected value of LD_{50} in log unit, m, will be $\frac{1}{4}\left(m_{P_1} + 2m_{F_1} + m_{P_2}\right)$ = 1.0706. The observed value of m for the F_2 generation was 1.6750 which was higher than the expected value. The pected value of m, 1.0706, was however, equal to the similar observed lue, 1.015 ± 0.023, of F_1. The variance of the tolerance distribution f log doses for the F_2 generation was equal to those of the P_1, P_2 and since the probit/log dose regression lines for these population were llel. According to Mather (1949), in case of polygenes, the segregation ld result in the same mean measurement of F_2 as that of F_1 but the dll generally, though not invariably, show greater variation than ler parents or F_1. The experimental data, therefore, could be sidered not to conform with the hypothesis of independent segregation. value of mean response rate of F_2 higher than the expected value

and the F_1 with similar variance could be due to;

i) the effect of non heritable factors causing higher tolerance to larvae and/or

ii) effective organization, through crossing over, of the determinants for resistance available with chromosome belonging to P_2 and supplemented by the chromosomes of P_1.

The larvae of F_2 was not tested for their susceptibility to DL alongwith the larvae of F_1. The conditions of the test during F_2 was however, kept as far as possible, similar to those tests carried out with P_1, P_2 and F_1. Even then some effect of non heritable factor on increased tolerance of the F_2 should not be totally ignored.

The F_1 strain was known to contain some determinants in an unorganised state. Under DDT selection these determinants, through suitable organization, were reported to exhibit non-specific resistance to DL-BHC (c.f. 2.3.7). Therefore, the integration of supplementary determinants of P_1 releasedfrom potential stage due to hybridization and crossing over, alongwith the determinants available with P_2, in an effective form, through crossing over, was considered more likely cause for higher mean tolerance of F_2 population.

The value of m in the BIP was 0.846 $\pm$ 0.068. This value was significantly less than the value of m, 1.675 $\pm$ 0.030 of F_2 but it was within the range of the normal variability of the mean response of P_1. The maximum likelihood estimate of the regression coefficient for the BIP was 1.41 $\pm$ 0.15 which was lower than the regression coefficient β.023, estimated from the combined estimates for P_1, P_2, F_1 and F_2 (Table 4.2). This suggested that the variance of tolerance distribution

over log doses of DL was maximum with the BIP. Lower mean with higher variance of tolerance distribution could be attributed to breakdown of the effective organization of the determinants, arrived at F_2, - through crossing over at varying rates.

4.3.6 Linkage of nonspecific DL resistant determinants with specific DDT resistance cluster

The studies reported in Chapters II and III suggested linkage of non-specific DL-BHC resistance factors (VT-genes) with the stable cluster of determinants controlling high degree of larval resistance to DDT. The specific DDT resistance cluster was inherited like a single chromosomal factor. The ideal method for detection of linkage of the non-specific polygenes with specific major factor for the DDT resistance was to classify the three genotypes, *homozygous resistant, heterozygous* and homozygous susceptible, of the F_2 individuals and then to estimate in them the *nonspecific* DL resistance. But in the case of resistance such method is not suitable as the classification is only possible by toxicological tests and the classified material cannot be retested against other group of insecticides. Hence for evidence of linkage of the nonspecific polygenes for DL resistance with the major factor for specific DDT resistance the LD_{50} of DL in log scale for three genotypes of DDT resistance, RR, RS/SR and SS as present in P_1, F_1 and F_2, respectively, were examined. The value of m for the F_1 generation, 1.015 ± 0.023, was found to be very close to the mid-parent value, 1.1, in the same scale. That suggested that mean nonspecific DL resistance in F_1 and P_2 was nearly proportional to the number of allelomorphs of the specific

DDT resistance. This indicated linkage between the Polygenic system and the DDT resistance cluster.

4.4 Discussion

Selection of A.stephensi Madras strain by DDT had resulted in the selection of DDT specific resistance in larval stage alone and DL-BHC nonspecific resistance in both adult and larval stages. (Chapter II). The correlated non-specific resistance of low magnitude was considered partly due to linkage of the alleles (c.f. 2.3.7). The present study on the genetical basis of nonspecific DL resistance showed that the phenomenon was polygenic and with almost no dominance of either of the complimentary systems of alleles for resistance and susceptibility. The polygenes were considered by Mather (1949) to be linked with one another and with the major factor (cluster) for the DDT resistance. These observations alongwith their implications are discussed in this section.

The nonspecific resistance was, ucually, encountered before the selection of specific resistance (Hoskins and Gordon 1956) if at all it is selected. In the selection studies the non-specific DL-BHC resistance was observed both in adult and larvae of A.stephensi whereas the specific resistance to DDT was found only in larvae. This clearly suggested that the non-specific DL resistance was not due to pleiotropic effect of specific DDT resistance factor, it was due to another set of genes.

The non specific polygenes were found to be linked into systems. Additional non-specific factors when available through/ breeding would get organized into effective order (+++, ---) through linkage and supplement the expression of the character. This was evinced by F_2 results.

In case of weak linkage the effective order of the polygenic system(s) is likely to get disorganised through crossing over, resulting in the lowering of the mean nonspecific resistance. But, in absence of selection the population would have a greater variance as revealed by the BIP data. Such a population when again subjected to selection pressure would quickly develop the non-specific resistance. This could be considered as a possible machanism to explain the phenomenon of reversion and reselection of non-specific resistance encountered in the field. The evidence of linkage of the non-specific polygenes with the DDT specific major factor(s) is a confirmation of the observation made on the linkage of genes for non-specific VT with the gene(s)/cluster for specific resistance.

The non-specific heritable resistance, apart from the selection by the injurious chemicals, could also be developed by breeding only from those individuals which survive exposure to such diverse stress as extremes of temperature, lack of moisture, abnormal food. This type of resistance was considered to have an altered ability to withstand many kinds of stress including chemicals (Hoskins and Gordon 1956). The polygenes for non-specific resistance could, therefore, be considered, following Mather(1949), as genes of fine adjustment. These genes are, usually, selected quickly under insecticide selection and give the selected population an opportunity, due to their improved fitness to survive under the inimical environment, for the development of the specific major factor through the integration and effective organization of the minor factors into a cluster as observed with A.stephensi (Chapter II). The linkage of major

factor for specific resistance, with the non-specific polygenic system, will also result in better survival value to the resistant population under the dynamic ecological set up. Thus the polygenes could be considered to play an important role in the evolution and economy of strains of mosquito species.

The non-specific DL resistance could be considered distinct of and different from the specific DL resistance which invariably showed resistance of high magnitude controlled by major nuclear gene (Davidson 1956, Reviews by Brown 1967, Brown and Pal 1971).

4.5 Summary

Laboratory studies were carried out to collect the information on the genetical aspect of non-specific dieldrin (DL) resistance selected in the larvae of Anopheles stephensi by DDT selection pressure. The non-specific DL resistance was of low magnitude and it was distinct and different from the specific DL resistance. The non-specific DL resistance was not due to the pleiotropic effect of specific DDT resistance factor. It was found to be controlled by polygenes located on the chromosomes. There was no dominance of non-specific DL resistant alleles over the susceptible alleles and vice versa in the given environment. The non-specific polygenes for DL resistance showed linkage with one another as well as with the major DDT - specific factor which was reported to have evolved through slow concentration and suitable organization of determinants into a closely linked cluster. The polygenes for non specific resistance were considered to play an important role in the evolution and economy of strains of a mosquito species.

References

Brown, A.W.A. (1958) : Insecticide resistance in Arthropods, Geneva (World Health Organization: Monograph Series, 38)

Brown, A.W.A. and Pal, R. (1971) : Insecticide Resistance in Arthropods, Geneva (World Health Organization: Monograph Series, No.38).

Brown, A.W.A. (1967) : In: Wright, J.W. & Pal, R., ed. Genetics of insect vectors of disease, Amsterdam, Elsevier, pp 505-552

Busvine, J.R. (1954.) : Nature (Lond.) 174, 783-785.

Davidson, G. (1956) : Nature (Lond.) 178, 705-706

Finney, D.J. (1952) : Probit Analysis (2nd Edition) University Press, Cambridge.

Hoskins, W.M. and Gordon, H.T. (1956) : Ann.Rev. Ent. 1, 89-122.

Kitzmiller, J.B. and Laven, H. (1958) : Amer. J. Hyg. 67, 207-213.

Krishnamurthy, B.S., Singh, N.N. and Verghese, A.K. (1965-66) : Annual Report, Natl. Institute of Comm. Disease, 137

Mather, K. (1949) : Biometrical Genetics, Methuan & Co. Ltd. London.

Singh, N.N. (1961) : Biometric study of Insecticide Resistance phenomenon in insects with special reference to mosquitoes. An approved dissertation for partial fulfilment of statistician's Diploma Examination of the Indian Statistical Institute, Calcutta-35

WHO (1963) : Wld Hlth Org. techn.Rep. Ser., No.265.

CHAPTER V

INHERITANCE OF DDT RESISTANCE IN CULEX PIPIENS FATIGANS

5.1. Introduction

Inheritance of specific resistance to DDT and non-specific resistance to dieldrin were studied in A.stephensi, an important malaria vector (Chaptor III and IV).. DDT resistance in A.stephensi was an atypical case since resistance was expressed in larval stage alone. Usually resistance was expected and was encountered in both the adult and larval stages of a species. Therefore, before generalization of the genetic information obtained from the studies on A.stephensi, it was considered necessary to confirm them with the experimental results on another species, in which DDT resistance had its expression in both adults and larvae, using a different method of susceptibility test. The study carried out with C.p.fatigans are, therefore, considered in this chapter.

5.2 Material and Methods

5.2.1 Considerations for choice of C.p.fatigans as experimental animal

The motives of the genetical study of resistance were to collect information which could be utilized to comprehend the phenomenon of insecticide resistance in insects and which inturn could also be used to develop and/or modify the methodology and strategy of vector control and thereby disease control. C.p.fatigans is a widely distributed tropical house mosquito. It is an important vector of bancroftian filariasis. Further, in India, it was the first species of mosquito which became resistance to DDT (Newman et al 1949 and Pal et al 1952). Resistance in this species developed to so high a level and it was

o widespread in space that filariasis control by use of powerful
ynthetic residual hydrocarbon insecticides had to be given up. In view
of its medical importance, close association with man and domestic animals
ide range of geographical distributions, urgent need for development
of methods and strategy of its control and amenability to colonization in
aboratory C.p.fatigans was selected as an experimental animal for genetic
tudy.

2.2 Source of Material

DDT resistant C.p.fatigans was collected from the village Khuraji
f Delhi state and colonized, following the method similar to that described
n section 2.2.3, in the insectary of the then Malaria Institute of India,
elhi, for one generation. The progeny of this strain and that of the
usceptible strain, maintained at that Institute over a large number years,
ere used for the breeding experiment. The susceptible strain(S-strain)
nd the resistant strain (R-strain) were referred to as P_1 and P_2
respectively in this study.

2.3 Breeding Experiments

Adults of both P_1 and P_2 strains were obtained by hatching single
pupa, seperately, in specimen tubes. This assured virginity of the
females of both the strains. When sufficient number of adults were
obtained, reciprocal crossings, en mass, were carried out; female P_1 x
male P_2 and female P_2 x male P_1. The hybrids, SR_{F_1} and RS_{F_1}, respectively,
obtained from the reciprocal crossings were inbred to obtain SR_{F_2} and RS_{F_2}
respectively. One backcross generation RRS_{F_1} was obtained by crossing
female R_2 with male RS_{F_1}.

5.2.4 Heterogenous X-population

A heterogenous populations, called X-population, was simulated. It consisted of female mosquitoes of F_1, SR_{F_1} and F_2 in the Mendelian ratio of 1:2:1. The females of different populations used were reared under similar conditions and they were of the same age groups.

5.2.5 Methods of susceptibility tests

To detect and estimate the level of tolerance/resistance in larvae and adults of F_1, F_2 and their F_1 and F_2 generations, obtained from reciprocal crosses, and RRS_{F_1}, susceptibility tests were carried out with both adults and larvae. Adults of X-population was also subjected to parallel tests with SR_{F_2} populations for drawing valid inference.

Larval susceptibility tests were carried out in enamel bowls (of 1850 ml capacity and 26 cm. surface diameter) containing 1000 ml. tap water. The temperature of water was found to vary between 20^0_C - 30^0_C. DDT (technical) alcoholic solutions of varying concentrations was applied to each bowl at a rate of 1 ml. of solutions per bowl. The doses of DDT were expressed as parts per million (ppm). 30 larvae of late 3rd or early 4th instar were exposed to each concentration. Mortality counts were made after 24 hours of continuous exposure. Larvae found 'dead beyond doubt' were recorded as dead. Pupae, if formed during exposure, were excluded from the number of larvae exposed for purpose of calculations of larval mortality rates. Controls, using 1 ml. of absolute alcohal, were set up with each batch of experiment.

In adult-tests 4-8 days old, glucose fed female mosquitoes were treated with varying doses of DDT by topical application method using micro-metre syringe. For this purpose mosquitoes were anaesthetised with carbon dioxide, and DDT (technical) alcoholic solutions of varying concentrations were applied to the mosquitoes on dorsal surface of the thorax at the rate of 0.001 ml of solution per mosquito. Doses of DDT applied were expressed as μg/mosquito. Control was set up with each experiment where in 0.001 ml of alcohol alone was applied to a mosquito. The treated mosquitoes were kept under observation for 24 hours in glass chimneys and provided with glucose solution. At the end of observations period mosquitoes found 'dead beyond doubt' were treated as dead. Dosewise record of number of mosquitoes treated (n) and number found dead(r) were maintained. In each test the level of doses were more than two.

The mortalities in control, both in the larvae and adults, were used for correcting mortality rates observed at different concentrations.

5.2.6 Design of the Experiment and Method of Estimation of Parameters

The method of detection and measurement of insecticide resistance is almost similar to that of the 'bio-assay' method. In bio-assay one insect population is used in tests against varying doses of standard and 'candidate' insecticides. But in the resistance study two insect populations, standard (susceptible) and candidate, are used in tests against varying doses of an insecticide. The basic principles of selection of levels of doses etc. are the same for both bio-assay and resistant tests. Hence to estimate the mean and variance of response of both

the larval and adult populations to DDT, tests were, as far as possible, designed and planned following the suggestions of Finney (1952a, 1952b) given for bio-assay. In the susceptibility tests with RS_{F_2}, SR_{F_2} and L-populations the doses of DDT used included also the LD_{50} of DDT for P_1 and P_2 strains.

5.2.7 Method of estimation

Mortality data obtained from the susceptibility tests were analysed by 'Probit Analysis Method'. The LD_{50} and the regression coefficients(b), for different strains/populations were estimated by the maximum likelihood estimation (MLE) method. For this purpose the computational plan of Finney (1952a) was followed. The validity of the linear probit/log dose regression model, in each population, was tested by χ^2 test. The χ^2 value in no case was found significant. This suggested the validity of the linear regression model in each case and hence estimates of LD_{50} and b were used to characterise a population and estimate the level of resistance. For drawing inference the standard errors (SE) of LD_{50} (in log scale) and their fiducial limits (in dose scale) at 95 percent level of confidence were calculated. SE of b was also estimated to compare the variance of tolerance of different populations since b is inversely proportional to variance of tolerance of a population. The degree of resistance, R, was estimated as

$$R = m_{p_2} - m_{p_1}$$

where m_{p_2} and m_{p_1} are the MLE$_s$ (log units) of p_2 and p_1 strains respectively.

In order to test the segregation ratio in F_2 and back-cross generations sample of mosquitoes of these generations were exposed to

Table 5.1

Basic data on susceptibility of different strains of C.p.fatigans (Adults) to DDT (Topical application method)

Strains/ Generations	DOSAGE OF D.D.T µg/MOSQUITO																				
	10.0			5.0			2.5			1.25			0.625			0.3125			0.15625		
	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p
P_1	-	-	-	-	-	-	72.1	65.1	90.3	31.8	59.8	73.1	96.0	52.0	54.2	107.0	25.0	23.4	39.9	2.9	8.8
P_2	166.6	114.6	68.8	199.0	101.0	50.7	193.1	55.1	28.5	125.4	21.4	17.1	-	-	-	-	-	-	-	-	-
SR_{F_1}	-	-	-	-	-	-	68.6	56.6	82.5	64.6	41.6	64.4	130.8	62.8	48.0	65.2	15.2	23.3	86.1	12.1	14.0
RS_{F_1}	-	-	-	-	-	-	42.9	31.9	74.3	30.2	14.2	47.0	67.0	17.0	25.4	19.1	1.1	5.7	-	-	-
SR_{F_2}				78.7	71.7	91.1	73.6	61.6	83.7	-	-	-	73.2	32.2	44.0	82.8	17.8	21.5	-	-	-
RS_{F_2}				149.7	115.8	77.3	99.3	70.3	70.8	-	-	-	101.2	21.2	20.9	102 2	22.2	21.9	-	-	-
Bx(F3R) (Back cross)	-	-	-	54.4	40.4	74.3	30.5	19.5	63.9	49.5	21.5	43.4	20.0	6.0	30.0	-	-	-	-	-	-
X	18	17	93.9	31	27	85.8	-	-	-	-	-	-	34	13	32.2	36	6	8.7	-	-	-
Z	77	58	75.3	78.6	49.2	62.6	75.0	26.0	34.7	73.6	18.5	25.1	-	-	-	-	-	-	-	-	-

n = number of mosquitoes treated (after correction).
r = number of mosquitoes dead (after correction).
$p = \frac{100r}{n}$

NOTE:- The control mortality rates in different replicate tests were found to vary between 0-26 per cent. Since in differ replicates the same dosages were not always tried there was a danger of either over-estimating or under-estimating the true mortality rates in different dosages if Abbott's formula is applied to summed up results. Therefore, n an were corrected for the control mortality rates of each test and summed up. These values were then used to estimate the mortality rates in different dosages. In such cases, weighting coefficient value for further calculations were read from Table 5.2 (Finney, 1952) for C=0.

best available discriminating doses to estimate the individuals belonging to P_1, P_2 and F_1 populations.

The probit/log dose regression lines of different populations showed that no dose of DDT existed for clear discrimination of any two or more populations due to over-lapping of the probit/log dose regression lines of P_1, P_2 SR_{F_1} and SR_{F_2} (Fig. 5.1). This point was given due considerations in calculating the expected mortalities for segregating populations based on Mendelian Law. In order to calculate expected mortalities some simple formulae were developed and applied (Appendix 5.1).

5.3 Results

The basic data on the susceptibility tests with adults and larvae of different strains/generations are given in Table 5.1 and 5.2 respectively. The statistical summaries of basic data are presented in Table 5.3 and 5.4.

5.3.1 Characterisation of the parent populations [redacted].

The P_1 strain was subjected to selection pressure of DDT for large number of generations without any success in selection of specific resistance to DDT. The failure to develop resistance could be due to homogenity and homozygosity of the strain. LD_{50} and b values of P_1 adults were estimated as 0.63 µg/mosquito and 2.2 ± 0.2, respectively (Table 5.3), as maximum likelihood estimates of the two parameters. The LD_{50} and b values estimated from the results of larval susceptibility tests were 0.078 ppm and 1.3 ± 0.05 respectively (Table 5.4). These values of LD_{50} and b were used as characteristics of the adult and larval populations.

Table 5.2

Basic data on susceptibility of different strains of C.fatigans larvae to DDT

Strains of C.fatigans	Dosages of D.D.T. in parts per million (p.p.m).																															
	20.0			10.0			5.0			2.5			1.25			0.625			0.3125			0.078125			0.0390625			Cont		
	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r
P_1													1271	1196	93.9				1751	1396	79.1	1620	824	50.2				300	0	
P_2	641	579	89.5	1130	1001	85.9				1126	771	66.9				563	272	45.7													273	13
SR_{F_1}													148	146	98.6				144	120	88.3	141	79	55.3	139	10	7.2	26	-			
RS_{F_1}																			141	117	83.0	143	68	47.5	136	20	14.7	28	-			
SR_{F_2}													517	499	96.1	542	444	79.9				365	189	46.4	309	112	29.1	30	8			
RS_{F_2}										267	255	95.2	466	431	92.1	96	81	83.5				422	112	22.2	285	41	9.4	73	4			
S				67	60	89.5	65	34	52.3							30	5	16.7										28				

n = number of larvae exposed.
r = number of larvae dead.
p = corrected mortality rate.

Table 5.3

Equation of the fitted probit/log dose regression lines and the parameters with their standard errors estimated from the susceptibility test data of different strains of C.p.fatigans adults to DDT

Strains of C.fatigans	Equation of the fitted ld-pr line (X=log 100 Z)	χ^2(d.f)*	n (Log 100/µg/ mosquito)	Variance of n	LD_{50} µg/ mosquito	Fiducial limits of LD_{50} (µg/ mosquito)	Regressio Coefficient (b)
P_1	Y=2.1734X + 1.0926	0.86 (13)	1.793 ± 0.032	0.001064	0.63	0.73 - 0.54	2.2 ± 0.2
F_2	Y=1.5957X + 0.6746	3.57 (19)	2.711 ± 0.012	0.000148	5.14	5.42 - 4.87	1.6 ± 0.2
SR_{F_1}	Y=1.7016X + 1.3212	0.27 (14)	1.8691± 0.039	0.001590	0.74	0.88 - 0.62	1.7 ± 0.2
RS_{F_1}	Y=2.2495X + 0.2061	0.23 (8)	2.131 ± 0.05	0.002815	1.35	1.72 - 1.06	2.2 ± 0.4
SR_{F_2}	Y=2.0727X + 0.9514	6.46 (12)	1.953 ± 0.039	0.001513	0.90	1.07 - 0.75	2.1 ± 0.1
RS_{F_2}	Y=1.4141X + 1.9793	9.11 (18)	2.14 ± 0.04	0.002030	1.38	1.66 - 1.15	1.4 ± 0.1
$R(RS_{F_1})$	Y=1.2844X + 2.1468	0.18 (4)	2.221 ± 0.085	0.007281	1.66	2.44 - 1.13	1.28 ± 0.3
X	Y=1.3404X + 1.1007	0.43 (2)	2.119 ± 0.092	0.008426	1.31	1.99 - 0.87	1.8 ± 0.3
Z	Y=1.6484X + 0.7699	1.24 (10)	2.566 ± 0.046	0.002129	3.68	4.5 - 3.0	1.6 ± 0.2

* Degree of freedom (d.f.) = SB - 2
where SB = Total number of batches of mosquitoes for all dosages in the experiment.

Table 5.4

Equation of the fitted probit/logdose regression lines and the parameters with their standard errors estimated from the susceptibility test data of different strains of C.p.fatigans larvae to DDT

Strains of C.fatigans	Equation of the fitted ld-pr line (X = log 100 Z)	χ^2(d.f)*	m in log 100 dose 1 (p.p.m.)	Variance of m	LD_{50} (p.p.m.)	Fiducial limits of LD_{50} (in p.p.m.)	Regression coefficient b
P_1	Y=1.2689X + 3.0693	9.14 (160)	0.891 ± 0.024	0.000576	0.079	0.087 - 0.0698	1.3±0.05
P_2	Y=0.9536X + 3.1553	6.25 (122)	1.934 ± 0.042	0.001776	0.859	1.037 - 0.711	0.9±0.05
SR_{F_1}	Y=2.3756X + 2.5345	8.36 (22)	1.016 ± 0.052	0.002703	0.104	0.131 - 0.082	2.4±0.13
RS_{F_1}	Y=1.9625X + 2.979	0.50 (13)	1.030 ± 0.045	0.001191	0.107	0.131 - 0.087	1.96±0.20
SR_{F_2}	Y=1.3706X + 3.5655	17.44 (62)	1.041 ± 0.035	0.001255	0.110	0.129 - 0.094	1.4±0.09
RS_{F_2}	Y=1.7100X + 2.6775	2.3 (56)	1.358 ± 0.028	0.000900	0.228	0.259 - 0.201	1.7±0.07
Z	Y=1.4428X + 1.6922	4.1 (6)	2.293 ± 0.070	0.004931	1.963	2.67 - 1.43	1.4±0.17

* Degree of freedom (d.f.) = SB - 2
where SB = Total number of batches of larvae for all dosages in the experiment.

The LD_{50} of the P_2 population was reported as 5.0 µg/mosquito (Bami et al 1957) and the b value was observed as 1.7 (Kalra, 1958). These estimates were based on the results obtained from topical application method of susceptibility tests. After nine months' interval, the populations was again tested and the LD_{50} and b values were found to be 5.14 µg/mosquito and 1.6 ± 0.2, respectively (Table 5.3). There had been no change in the mean susceptibility and homogeneity of P_2 populations during all these months; hence P_2 could be considered homogeneous. The estimates of different parameters were, therefore, considered stable over time. The LD_{50} and b values of larvae of P_2 were 0.859 ppm and 0.9 ± 0.05, respectively (Table 5.4).

A comparison of the LD_{50} values of P_1 and P_2 populations showed that adults of P_2 were 8.2 time as resistant to DDT as P_1. Similarly the larvae of P_2 were found 11 times resistant (Tables 5.3 and 5.4). The b values of P_1 and P_2 adults were 2.2 ± 0.2 and 1.6 ± 0.2, respectively, and they were not significantly different (Table 5.3). Hence the variances of tolerance over doses of DDT for P_1 and P_2 adults were considered similar. In the larval tests the variance of tolerance distribution over log doses of P_2 population was greater than that of P_1 population since the values of b were 0.9 ± 0.05 and 1.3 ± 0.05 respectively (Table 5.4).

5.3.2 Choice of a Valid Scale

Resistance is detected by comparision of the tolerance of the two populations; the candidate population and the standard (susceptible) population. Tolerance is indirectly estimated from the response of an individual to a given dose of insecticide (stimulus). Individuals of a homogenous population show variations in their tolerance levels to a given dose and hence LD_{50} value is taken as an estimate of mean of the population's tolerance distribution. The LD_{50} being stable statistic is used to measure the degree of resistance as R=LD_{50} of C÷LD_{50} of S; where C & S denote the candidate and standard populations. Resistance, therefore, is a metrical character. In the study of resistance the biometrical constants like LD_{50} and b had to be used since the individuals could not be classified in to mutually exclusive phenotype classes.

The quantitative description of a biometrical character can be valid only interms of scale. In an empirically adequate scale the quantitative effects of the gene(s) on an average become simply additive which conform with the assumptions of biometrical genetics (Mather 1949). The scaling test following Mather, for the F_2 generation, is

$$\bar{F}_2 = \frac{1}{4}\left(\bar{P}_1 + \bar{P}_2 + 2\bar{F}_1\right)$$

which in case of resistance could be stated as

$$LD_{50} \text{ of } F_2 = \frac{1}{4}\left(LD_{50} \text{ of } P_1 + LD_{50} \text{ of } P_2 + 2\ LD_{50} \text{ of } F_1\right).$$

The right side of the above equation is the arithmatic mean of the LD_{50} values of the segregating classes which is the expected LD_{50} of F_2. The value of the left hand side of the equality sign is the observed one; empirically estimated from dose-response data of F_2.

Table 5.5

Results of the Scaling Tests

Stage of life cycle	Generation	Mean of the response in dose scale (LD_{50})		mean of the response in log dose scale	
		observed (LD_{50})	Expected* (LD_{50})	observed (m_o)	Expected* (m_E)
Adult	SR_{F_2}	0.90	1.81	1.953	2.061
	RS_{F_2}	1.38	2.12	2.140	2.193
Larvae	SR_{F_2}	0.110	.286	1.041	1.214
	RS_{F_2}	0.228	.238	1.358	1.221

* Weighted mean of the means of P_1, P_2 and appropriate F_1; weights being proportional to Mendelia ratio of segregation ($P_1:F_1:P_2$ as 1:2:1).

The LD_{50} values were first estimated in log-scale on which the response were normally distributed. This was validated by the non-significant values of χ^2 (Tables 5.3 & 5.4). Subsequently for physical relevance it was transformed to actual dose scale. The scaling tests were, therefore, tried with both the log scale and dose scale of the estimates of LD_{50} values of adults and larvae (Table 5.5). It would be seen from the table that the log LD_{50}(m) was a better description of biometric character of the adults than LD_{50} in actual dose scale since deviation between the empirical and expected values were smaller in log scale than actual dose scale. In the larval tests also the log scale was considered a good choice.

5.3.3 Dominance of Alleles

Information on the dominance or otherwise of a biometric character controlled by gene-alleles are greatly dependent on the scale used for the measurement of the character (Mather 1949). In the present study log LD_{50} values were considered the best unit for study of dominance since tolerance was normally distributed over log dose and since it was found a better unit in the scaling test (c.f. 5.3.2). The midparent log LD_{50} was 2.254 for adult mosquitoes. This value was higher than the log LD_{50} of RS_{F_1} (=2.131) and SR_{F_1} (=1.868) adults (Table 5.3). This suggested that susceptibility was slightly dominant over the resistance in adults. In the larval tests the mid-parent LD_{50} in log scale, m, was 1.412 which

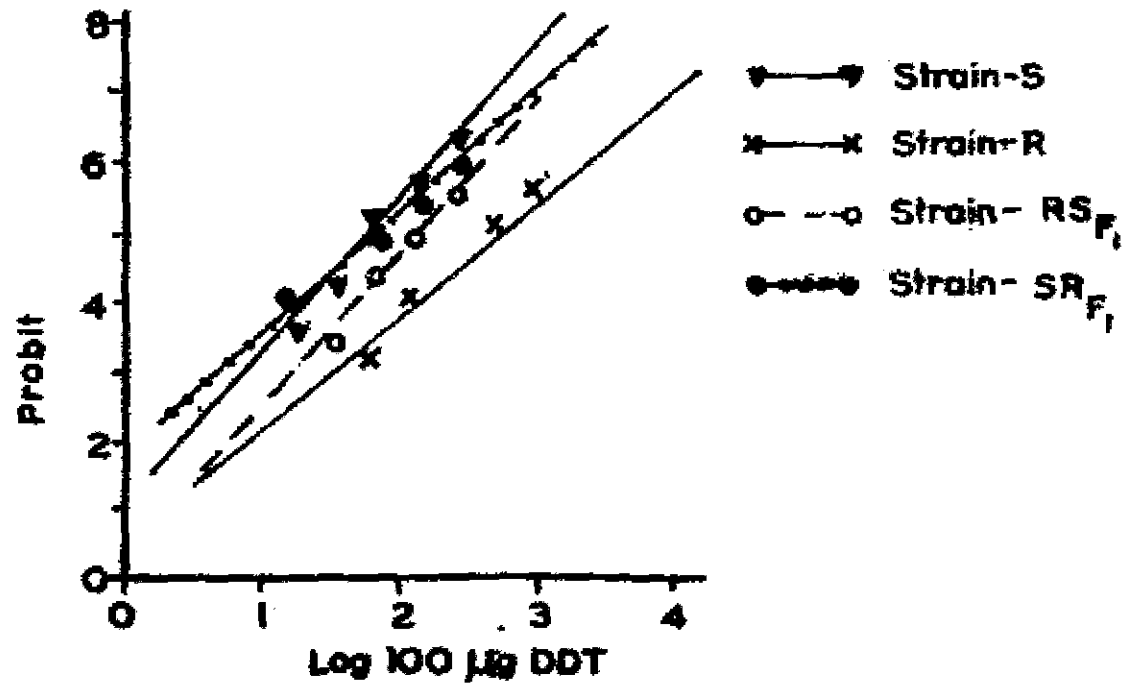

Fig. 5.1 PROBIT/LOG DOSE REGRESSION LINES OF P_1, P_2 STRAINS AND THE F_1 PROGENY OF THEIR RECIPROCAL CROSSES

was also greater than m values of RS_{F_1} (=1.030) and SR_{F_1} (=1.016) vide Table 5.4. Hence it is concluded that the susceptible alleles controlling DDT susceptibility in both adults and larvae were slightly dominant over the resistance alleles controlling DDT resistance in both the stages of C.p.fatigans.

5.3.4 Influence of extra-chromosomal factors on level of resistance

Resistance when determined by only the chromosomal factors would show equal intensity of its expression in the F_1 and F_2 generations of the reciprocal crosses between P_1 and P_2. But the experiments showed that the LD_{50} values of the RS_{F_1} and RS_{F_2}, 1.35 µg/mosquito and 1.38 µg/mosquito, were significantly higher than the corresponding values, 0.74 µg/mosquito and 0.90 µg/mosquito of SR_{F_1} and SR_{F_2} populations, respectively. The suggested influence of cytoplasmic factors of resistance females on the expression of DDT resistance in C.p.fatigans adults. Such influence in larvae was not significantly expressed in F_1 generation. The RS_{F_2} was more tolerant than SR_{F_2} which showed some influence of cytoplasmic factor of F_2 on the level of expression of resistance. Considering the results of adult and larval test it could be said that contribution of cytoplasmic factor resulted in 1.5 to 2.0 times increase in resistance. The contributions of cytoplasmic factors in the expression of resistance has been reported in Blattella garmanica. (Cochran et al 1952) in Musca domestica (Johnston et al 1954) and in Drosophila (Tattersfield and Kerridge 1953). A critical examinations of the susceptibility test results reported by Davidson (1958) also suggested the influence of cytoplasmic or maternal factor on the inheritance of DDT resistance in A.sundaicus.

5.3.5 Distributions of tolerance to DDT in the Populations of segregating generations - their genetic implications

Resistance if determined by nuclear gene(s), would give rise to different phenotype classes of varying degree of resistance in the F_2 and back-cross generations due to segregation of gene-alleles. The F_2 and back-cross generations would, therefore, be heterogenous which would be reflected on the probit/log dose regression lines. Hence the probit/log dose regression lines of adults and larvae for F_2 and back-cross generations were examined.

The probit/log dose regression lines were used to represent the dose-response data of adult female C.p.fatigans. The χ^2 tests showed that the linear regression model was valid as none of the χ^2 values for RS_{F_2}, SR_{F_2} and the backcross population (RRS_{F_1}), with appropriate degree of freedom, was significant (Table 5.3). A perusal of the results of statistical analysis of dose-response data given in Table 5.2 showed the linear regression model, in probit and log dose metametre, was also valid for the RS_{F_2} and SR_{F_2} populations of C.p.fatigans larvae (Table 5.4). Hence it is inferred that the DDT tolerance of adult and larvae of the segregating generations were normally distributed over the log dose. Normal distributions of tolerance of the adults and larvae of the segregating generations suggested that DDT resistance in C.p.fatigans was polygenic/multi-factorial. Had the DDT resistance been due to a single stable factor, the linear probit/log dose regression model would have been poor to respresent the data since the distribution

of resistance in segregating generation would have been a compound one consisting of two or more normal distributions.

5.3.6 Genetic Inference based on LD_{50} and regression coefficient

LD_{50} is the mean of the distribution of resistance over doses and the regression coefficient is inversely proportional to the variance of the distribution of resistance. According to Mather (1949), the mean of F_2 generation is equal to the mean of F_1 generation if the character is controlled by polygenes. The variance of a polygenically controlled character in F_2 will generally, though not invariably, be greater than either F_1 or parents even when the parents are not nearly true breeding. The LD_{50} and b values of the parents, F_1 generation and F_2 generation of the reciprocal crosses are, therefore, examined in this section to draw inference on the genetic basis of resistance.

LD_{50} of RS_{F_1} and RS_{F_2} adults were estimated by maximum likelihood method as 1.35 µg/mosquito and 1.36 µg/mosquito, respectively. The values were close to each other and they did not differ significantly since their fiducial limits at 95 percent level of probability overlapped. Similarly the LD_{50}s of SR_{F_1} and SR_{F_2} were estimated as 0.74 µg/mosquito and 0.90 µg/mosquito, respectively, and they were also found not to differ significantly (Table 5.3).

The maximum likelihood estimates of b for P_1, RS_{F_1} and RS_{F_2} were 2.2 ± 0.2, 2.2 ± 0.4 and 1.4 ± 0.1, respectively for the female adults. The value was lowest for RS_{F_2} and it was significantly different from P_1 (Table 5.3). Hence the variance of resistance was significantly

more than that of the P_1. The regression coefficient value of SR_{F_1} and SR_{F_2} were 1.7 ± 0.2 and 2.1 ± 0.1, respectively and they didnot differ significantly and so was the case when the b values of SR_{F_2} and P_1 were compared. Hence the variance of SR_{F_2} could be considered of the same magnitude as those of P_1 and SR_{F_1}.

In the larval tests the LD_{50} of RS_{F_1} and RS_{F_2} were estimated by maximum likelihood method as 0.107 ppm and 0.228 ppm, respectively. These values also did not differ significantly as the fiducial limits at 95 percent level of probability overlapped each other. Hence the mean levels of resistance was the same for both RS_{F_1} and RS_{F_2} generations. The LD_{50} of SR_{F_1} and SR_{F_2} were 0.104 ppm and 0.110 ppm respectively. They also did not differ significantly (Table 5.4). Therefore, it could be concluded that mean resistance of the larvae of SR_{F_1} and SR_{F_2} were similar.

The regression coefficients for P_1, SR_{F_1} and SR_{F_2} were estimated as 1.3 ± 0.05, 2.4 ± 0.18 and 1.4 ± 0.09, respectively. The values of SR_{F_1} and SR_{F_2} showed that the variance of resistance in SR_{F_2} was higher than SR_{F_1}. When the b values of P_1, RS_{F_1} and RS_{F_2} were considered they were found as 1.3 ± 0.05, 1.96 ± 0.20 and 1.7 ± 0.07, respectively. Though the values of RS_{F_1} and RS_{F_2} did not differ significantly but when compared with the P_1, the variance of RS_{F_2} was found to be significantly less (Table 5.4). This could be due to linkage or extra chromosomal factors.

From the above studies of LD_{50} and regression coefficients for adults and larvae of P_1 and F_1 and F_2 progenies of the reciprocal

crosses it could be concluded that DDT resistance of C.p.fatigans was due to polygenes. This conclusion is an expected one for numerical character and is in agreement with the one drawn from the normal distribution of resistance, in the segregating generations (c.f.5.3.5). The variance of back-cross population ($RoRS_{F_1}$) was also higher than P_1 (Table 5.3 which also supported the conclusion arrived at .

5.3.7 Test of Segregation of Resistance

Inference drawn on the polygenic basis of resistance was based on the comparision of the LD_{50} and regression coefficient values of P_1, F_1 and F_2 populations (c.f.5.3.6). The model of the relative behaviour of mean and variance of a quantitative character was developed on the assumptions of segregations of genes and characters in F_2 generation. But the assumed behaviour of the mean and variance could also result from the influence of enviornment with or without the involvement of gene-segregations. Therefore, it was considered necessary to test the segregation of resistance using the means of the expected classes of phenotypes in F_2 population. The result of the test is presented in this section.

It is assumed that in F_2 generations, due to segregation of the gene-alleles, P_1, F_1 and P_2 types of population would be present. If this be so the expected LD_{50} of given F_2 generation would be the weighted mean of the LD_{50} of the segregating genotypes. The expected means were worked out on log scale since in the present case it was seen to be the better choice. (c.f. 5.3.2). The expected values, M_E, when compared with the observed one, M_O, were found to be well in agreement

(Table 5.5). This showed that value of mean, m, found from the adult and larval tests of RS_{F_2} and SR_{F_2} were mainly due to the presence of three expected phenotype populations in Mendelian ratio rather than due to enviornmental factors. Hence the hypothesis of segregation is considered tanable.

5.3.8 Validity of the test of Segregation of Resistance

The test of segregation of resistance was based on the comparision of expected mean, m_E, with observed mean, m_O, of F_2 generations (c.f.5.3.7). The expected mean was the weighted average of the values of m for P_1, F_1 and P_2 populations which were tested simultaneously for their susceptibility to DDT under identical conditions. The weight were 1,2, and 1 respectively based on Mendelian ratio of segregation of P_1, F_1 and P_2, respectively. F_2 generations, were, however tested for susceptibility after a lapse of time. Hence there is a need to establish the validity of the test of segregation of resistance and the conclusions drawn there on.

To establish the validity, a heterogeneous population (X-population) consisting of mosquitoes of P_1, SR_{F_1} and P_2 classes in 1:2:1 was made and subjected to parallel susceptibility tests with SR_{F_2}. The estimates of log LD_{50} and regression coefficient values of X-populations and SR_{F_2} populations were compared. Further these values of X-population were also compared with the expected mean, m_E used for segregation test.

The m values of X-population and SR_{F_2} were 2.119 ± 0.092 and 1.953 ± 0.039, respectively. The difference between the two values were not significant. The regression coefficient values of X-population

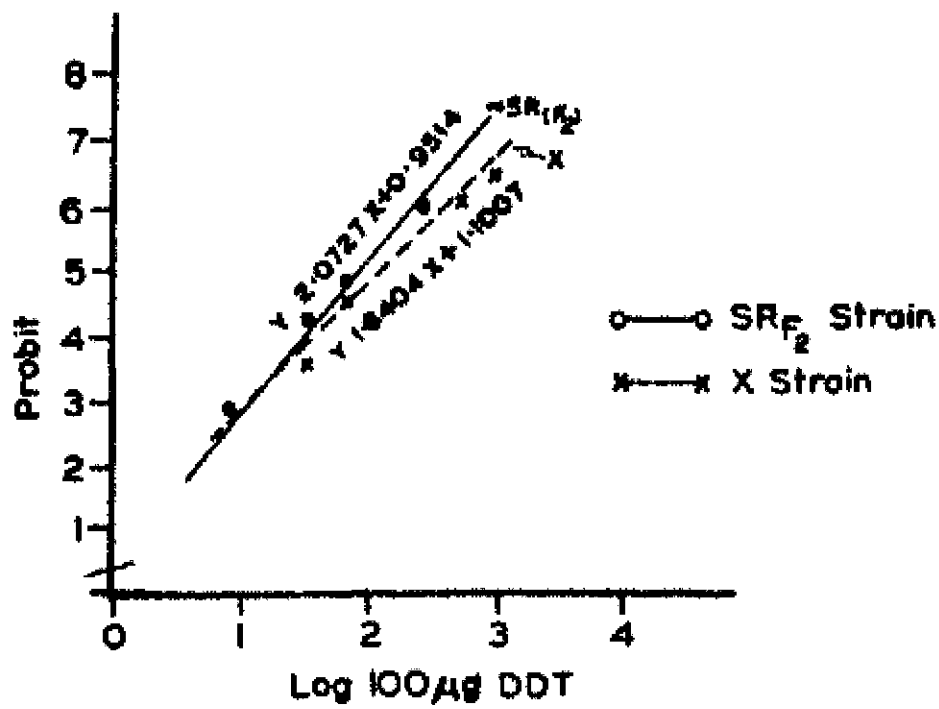

Fig. 5·2 PROBIT/LOG DOSE REGRESSION LINES OF THE SR$_{F_2}$ AND X-POPULATIONS

nd SR_{F_2} were 1.8 ± 0.3 and 2.1 ± 0.1 respectively (Table 5.3). The two stimates of regression coefficient were within the limit of sampling ariations (Fig. 5.2). Therefore the SR_{F_2} could be considered to ontain P_1, SR_{F_1} and P_2 genotypes in the Mendelian ratio. The expected alue used for test of segregation of resistance in SR_{F_2} was 2.061. This alue when compared with the emperical value, 2.119 ± 0.092, of X-population, t was found that they belonged to the same population. This validated he test of segregation of resistance.

5.3.9 Method of discrimination and its validity

The inference and conclusions drawn on the genetic factors and on their segregations were mainly indirect, based on the biometric constants estimated from dose-response data (c.f. 5.3.5 to 5.3.8). Direct count of different genotypes/phenotypes in the segregating generations in desirable to test the Mendelian hypothesis - number of genes involved, in resistance nd mode of inheritance. Due to the vary nature of the character it as not possible to score the frequencies of different genotypes/ henotypes. Estimation of the genotype/phenotype frequencies by oxicological method, using 'discriminating dose', was made difficult since he tolerance distributions of P_1, P_2 and F_1 populations of C.p.fatigans are overlapping. With the help of statistical methods and using oxicological tests an attempt was, however, made to estimate the enotype/phenotype frequencies in the populations of the segregating enerations. The resistant phenotypes, thus scored, were bred and the rogeny was tested to establish the validity and power of discrimination. he results of this study are recorded in this section.

Number of adults of RS_{F_2}, SR_{F_2}, RRS_{F_1} and X-populations and the number of larvae of RS_{F_2} and SR_{F_2} populations found dead at the LD_{50}s of P_1, RS_{F_1} and P_2 populations were compared with the expected number dead at these doses. (Table 5.6). LD_{50} values were used because it is most precise of all the dose levels. The expected mortalities at those doses were calculated on the hypothesis of Mendelian ratio of segregations of di-allelic single factor for resistance (Appendix 5.1).

It may be seen from Table 5.6 that the difference between observed and expected mortalities were significant at p=0.05 for RS_{F_2} larvae alone. For all other populations the differences between the observed and expected mortalities at three discriminating doses were well with in the limit of sampling fluctuations. A good agreement of the results of discrimination of phenotypes of X-population with the known actual numbers of different phenotype populations showed that the discriminating method used was a valid one. Hence the conclusion that the segregating generation contained different phenotype populations in Mendelian ratio could be considered valid. The validity was further confirmed by estimating the LD_{50} of the progeny of those females called Z-population which had survived the LD_{50} of P_2 population. The LD_{50} for adults of Z-population was 3.68 µg/mosquito was close to that of the P_2 population. Both the P_2 and Z-populations had the same variance of distribution of resistance as the regression coefficient value, 1.6 ± 0.2, was same for both (Table 5.3). The resistance level of Z-population was 5.8 times as high as the P_1 population. The larvae of Z-population was also 25 times as resistant as P_1 (Table 5.4). This showed that the survivors

Table 5.6

Results of testing of hypothesis of segregation of characters

ing	Hypothesis of segregation (phenotypic ratio of characters in the population)	DISCRIMINATING DOSAGES											
		LD_{50} of P_1				LD_{50} of RS(F_1)				LD_{50} of P_2			
		d	n	s	c	d	n	s	c	d	n	s	c
(A)	(SS+SR):RR=3:1	3.4	73.2	4.2	0.81	..	..	..	..	4.0	78.7	3.1	1.3
$(A)_2$	SS:RS:RR= 1:2:1	4.3	101.2	4.4	1.1	..	..	..	..	7.1	149.7	4.7	1.5
$(A)_{F_1}$	RS:RR=1:1	..	..	..	..	4.7	49.5	3.3	1.4	2.2	54.4	3.4	0.65
	SS+SR:RR=3:1	0.8	31	2.7	0.03	..	..	..	..	2.2	34.9	2.5	1.1
(L)	SS+SR:RR=3:1	77	398.8	9.3	6.2*	..	..	..	..	..	..	..	..
$(L)_2$	-do-	16	328.5	8.9	1.81	..	..	..	..	..	..	..	..

gnificant at P.-0.05

deviation of observed mortalities from the expected mortalities.

total number under observation.

$\sqrt{pqn}$, where p is probability of dying of an individual, q is 1-p.

d

s

Adults

Larvae

of the LD_{50} of P_2 were mainly the resistant phenotypes since their offsprings were also resistant to DDT. These experiments thus, clearly, showed that the discriminating dose method was valid and reasonably powerful.

The test for goodness of fit of the segregations of genes and alleles in Mendelian ratio further confirmed the inheritance of resistance was governed by the Mendelian principles. Its study, however, involved special biometric methods.

5.3.10 Evolution of a major factor for Resistance

DDT resistance in C.p.fatigans was considered to be polygenic in view of the good fit of linear probit/log dose regression model to the dose response data of the segregating generations (c.f. 5.3.5). In the study of selection of DDT resistance in A.stephensi it was concluded that a major factor for resistance, under DDT pressure, could evolve through concentration of poly/multi genes. The intensity of expression of resistance would depend on the number of genes in the cluster and their organizations. A stable cluster would behave like a single factor (Chapter II). To study the evolutions of resistance cluster in C.p.fatigans, the probit/log dose regression line for adults of RS_{F_2} generations was examined. It could be seen from fig 5.2 that the regression line contained two plateaus which could be taken as an evidence of clustering of genes into major factor(s) for resistance. The goodness of fit of the linear regression line suggested that the cluster was in the initial stage of formations and contained few genes.

5.4 Discussion

There are five reports on the inheritance of DDT resistance in C.p.fatigans. The first publication on the inheritance of DDT resistance was by Pal and Singh (1958) which was based on the experimental results of the present Chapter. The second report was by Rozeboom and Hobbé(1960) based on the cross Philippine DDT resistant strain with a Texas Laboratory susceptible strain. The resistance strain was 13 times as resistant, in larval stage, as the susceptible one. Based on the response of larvae to discriminating dose genetic interpretations were made. Using the discriminating dose method against adults Davidson (1964) studied the mode of inheritance of laboratory selected DDT resistance in this species. The susceptible strain used for crossing was originated from same geographic area. Thomas (1966) studied the inheritance of DDT resistance in C.p.fatigans using the Klang resistant strain and Lamir susceptible strain, both these strains originated in Malaya. Using highly resistant (250 fold) strain and susceptible strain of Rangoon Tadano and Brown (1967) studied the inheritance of DDT resistance in larvae. The results of these studies are summarised in Table 5.7. It could be seen from the table that the conclusions drawn by the authors were not consistant.

The inconsistancy of the informations on the dominance could be attributed, among other factors, to the method of the study.(testing design) and the scale. Using different scales different types of information on the dominance of gene(s)/character could be inferred from the same experimental results.(Mather 1949). In the present chapter the inference on the slight dominance of susceptible allele(s)

Summary of the published information on the inheritance of DDT resistance in Cx.p.fatigans

Item of Information	SOURCE OF INFORMATION				
	Pal & Singh (1958)	Rozeboom & Hobbs (1960)	Davidson (1964)	Thomas (1966)	Tadano and Brown (1967)
Source of resistant Parent	Khurajee village Delhi State	Philippine	Laboratory selected Conoor Strain	Klang-Malaya	Rangoon Strain
Method of selections	Selected due to use of DDT under Malaria control		Laboratory selection at LD_{50}	-	late selection
Source of the susceptible Parent	Susceptible Colony strain	Texas laboratory colony	Susceptible Conoor strain	Lamir - Malaya	Rangoon strain selected for susceptibility
Stage of lifecycle studied	Adult & Larvae	Larvae	adult	-	Larvae
Method of susceptibility test	Topical applications of DDT on adult & larval test	Discriminating dose following larvae testing method	Discriminating dose(4.0% X 4 hrs) through contect	-	Scalar dose larval susceptibility test
Method of drawing conclusion	Scalar & Discriminating dose	Comparing the observed mortality rate with expected	Comparing observed mortality rate with expected one	Scalar dose	Scalar dose-platean & comparison of rates
Information on dominance	Slight dominance of susceptibility	Dominance of resistance	Dominance of resistance	Resistance dominant	Resistance
Information on maternal/ paternal effect	Matroclinous effect	Matroclinous	Matroclinous	Matroclinous	Leek of matroclinous evidence
Conclusion on mode of inheritance	Single factor	Single factor	Single factor	Polyfactorial	Single factor

was based on log LD_{50} which was found to be an adequate scale in the scaling test (c.f.5.3.2). Therefore, with the biomaterial and testing methods used, the conclusion could be considered a valid one. Chromosome determining the male sex or containing the factor for male sex was found to contribute towards DDT resistance in A.stephensi (c.f. 3.3.3). But four out of five reports recorded maternal effect on DDT resistance in C.p.fatigans. This showed that cytoplasmic factor(s) had also significant contribution towards phenotypic resistance. The contribution of cytoplasmic factors towards resistance had also been reported in Blattella germanica (Cochran et al, 1952) in Musca domestica (Johnston et al, 1954) and in Drosophila (Tattersfield and Kerridge, 1953). A critical examinations of the susceptibility test results reported by Davidson (1954) also indicated the influence of matroclinous effect DDT resistance in A.sundaicus. According to Perry there is an increasing number of examples demonstrating the microsomal enzyme system in insects as a system responsible for large variety of oxidasive mechanisms resulting both in intoxication and detoxication processes. Hydroxylating and hydrolytic mechanisms responsible for detoxications of organophosphorous, carbamate and chlorinated hydrocarbon insecticides also reside in the microsomal system. Thus it is found that the genetic system of DDT resistance is a complex one; involving at times the nuclear factors as well as cytoplasmic factors which could act and interact with each other and one another.

The reports of Rozeboom and Hobbs (1960), Davidson (1964) and Tadano and Brown (1967) showed that the DDT resistance in

C.p.fatigans was due to single Mendelian factor. The clear demonstration of monofactorial basis of DDT resistance by Tadano and Brown (loc cit 196 was based on a long plateau in the dose-mortality regression line of the backcross (F_1 ♂S) generation which segregated in a 1:1 ratio. This monofactorial basis of DDT resistance was confirmed by using (Yellow or Orange larvae) marker gene which was located on the chromosome 2 (Laven 1967). Through a suitable crossing experiment Tadano and Brown (loc cit) showed that the DDT resistant gene was linked with Y gene at a cross-over distance of 15-20 units. But the genetical studies by Thomas (1966) proved that the DDT resistance in Malayan strain of C.p.fatigans was polygenic since the regression lines for F_1 and F_2 generations were identical. Following Milani (1957) the inconsistant information on this aspect could be attributed to material and method of study. The inconsistant information could also be due to criteria used for drawing inference. Pal and Singh concluded that DDT resistance in C.p.fatigans of Khurejee was due to single factor since segregation followed Mendelian ratio. Such a ratio could be found with members of polygenes since they are located on the chromosome which segregate and so the genes on it. A critical examination of the data showed that resistence was due to many genes. (c.f. 5.3.5 - 5.3.9). The valid criterion for polygenic basis of resistance is the continuous normal distribution of resistance over log doses in segregating generations. Continuous distribution could be possible only when each member of polygenic system had small contribution which is additive in a suitable

. In case of monofactorial basis of resistance the dose mortality
ssion lines of segregating generations must contain a plateau over
luating doses due to compound nature of segregating population.
the linear regression model will be a poor fit to the emperical data.

Based on the studies with A.stephensi (Chapter II) it could be
ated that specific polygenes undergo a dynamic process of evolution
their reorganisation and linkage. This model is helpful to
end the dynamic changes in evolution of reistance genes into a
e cluster. The cluster when matured would behave like a single factor
ts transmission. This hypothesis is helpful to comprahend the apparent
sistant information viz. monofactorial vs polyfactorial/polygenic basis
T resistance in C.p.fatigans. The monofactor is the limiting stage
lution of resistance factors through integration and organisation
polygenes/multifactors as found in Chapter II.

SUMMARY

Genetic basis of DDT resistance, expressed in both adult and
e of C.p.fatigans, were studied. The method of testing involved
cal application' of DDT on the drosal surface of female adults
ntinuous exposure of larvae to DDT. The results of susceptibility
were rigor ously analysed and argued too. Matroclinous effect
T resistance was observed. Susceptibility to DDT was considered
tly dominant over resistent. DDT resistance was considered
polygenic which was evolving to a single Mendelian factor.

5.6 Appendix 5.1

Method of estimation of expected mortality at discriminating dosages and test of the Mendelian hypothesis of monofactorial basis of resistance

When inheritance of one character determined by a single gene is r study, according to 'Mendelian Law', the expected genotype ratio in population of F_2 generation is 1:2:1. If the character is completely sive, the phenotype ratio of the individuals possessing the cters is 3:1, but when it is completely dominant, the ratio is 1:3. case of intermediate expression, the phenotype ratio is 1:2:1.

In the resistance study, characters are estimated by the mean se rates, of the populations. As such, the LD_{50} values for the ts and hybrid populations are used as discriminating dosages to the segregation of characters in F_2 generations.

If a random sample of size n is drawn from the population of F_2 ration, according to the Mendelian Law, the sample is likely to sist of 0.25_n susceptible, 0.5_n hybrids and 0.25_n resistant individuals.

When n number of mosquitoes are exposed to LD_{50} of susceptible s) the expected mortality in the susceptible class is

$$\frac{0.25_n}{2} = 0.125_n \quad \ldots\ldots\ldots\ldots\ldots\ldots (a)$$

Since this dose, $LD_{50}(s)$, also causes some mortality to the squitoes of hybrid and R-classes (vide Fig.5.1), it is essential estimate these mortalities also.

The expected mortality of hybrid class at $LD_{50}(s)$ is

$$0.5_{np}{}^{hs} \quad \ldots\ldots\ldots\ldots \text{(b)}$$

where p^{hs}, is the probability of dying of the hybrid individuals at $LD_{50(s)}$.

Similarly, the expected mortality of resistant class at $LD_{50}(s)$ is

$$0.25_{np}{}^{rS} \quad \ldots\ldots\ldots\ldots\ldots\text{(c)}$$

where p^{rS} is the probability of dying of the resistant individuals at $LD_{50}(s)$. Therefore, the sum of the mortalities of different strains, expected number dead, i.e., $EM.(s)$, at $LD_{50}(s)$ is

$$E.M.(s) = 0.125_{n} + 0.5_{np}{}^{hS} + 0.25_{np}{}^{rS} \ldots\ldots\text{(sum of a, b and c).}$$

$$\text{or } E.M.(s) = n(0.125+0.5_{p}{}^{hS} + 0.25_{p}{}^{rS})\ldots\ldots\ldots\ldots(1)$$

In order to estimate the values of p^{hS} and p^{rS}, first probit values are worked out solving the ld-pr line equations of the hybrid and resistant populations respectively by substituting the log LD_{50} value of the susceptible population for x. Probit values thus worked out are referred to Table 1 (Finney, 1952) and the probabilities of dying of different populations are estimated from the percentage mortalities read in this table.

Similarly the equations for the expected number dead at LD_{50}s of hybrid and resistant populations, EM(H) and EM(R), are worked out. Those are

$$EM(H) = n(0.25\ p^{sH} + 0.25 + 0.25\ p^{rH})\ldots\ldots\ldots(2)$$

and $$EM(R) = n(0.25\ p^{sR} + 0.5_{p}{}^{hR} + 0.125) \ldots\ldots\ldots(3)$$

where p^{sH} and p^{rH} are probabilities of dying of susceptible and resistant individuals respectively at $LD_{50}$of hybrid strain and p^{sR} and p^{hR} are probabilities of dying of susceptible and hybrid strains respectively at LD_{50} of resistant strain.

On the above rationale the following equations for estimating the expected mortalities are worked out when the character is (a) recessive and (b) dominant.

(a) When the character (resistant) is recessive:

$$EM(S) = n(0.375 + 0.25_{p}rS) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$$EM(R) = n(0.75_{p}sR + 0.125) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

(b) When the character (resistant) is dominant:

$$EM(S) = n(0.125 + 0.75_{p}rS) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$EM(R) = n(0.25_{p}sR + 0.375) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

On the similar line, the formulae for estimation of expected numbers dead, for the population obtained by the back cross of F_1 hybrid and either of the parent stock, are worked out and given below. In these cases, the LD_{50} for hybrid and the parent (used for crossing) are used as discriminating dosage. According to theory 50:50 ratio of hybrid and parent (used for crossing) are expected and they can only be discriminated if they are pehnotypically found different.

$$EM(H) = n(0.25 + 0.5_{p}pH) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

$$EM(P) = n(0.5_{p}hP + 0.25) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

Where P^{pH} and P^{hP} are the probabilities of dying of parent class used for crossing and hybrid (F_1) individuals at LD_{50} of hybrid F_1 and parent strains respectively.

EXAMPLE

The RS(F_2) strain showed 20.9 per cent and 77.3 per cent mortality rates at LD_{50} for S strain and R strain respectively (c.f. Table 5.1). In this case resistance was observed to be slightly dominant. It is intended here to test the hypothesis that resistance is determined by a single gene.

If resistance is controlled by a single gene, according to Mendelian Law, the susceptible, hybrid and resistant genotypes are expected to be present in Rs(F_2) in the ratio of 1:2:1.

Owing to overlapping phenotypes, the expected mortalities at LD_{50}(s) and LD_{50}(R), are estimated as

$$EM(S) = n(0.125 + 0.5_p h + 0.25P^{rS}) \ldots\ldots\ldots \text{(by equation 1)}$$

To solve this equation the values of p^{hS} and p^{rS} are required, estimation of which are as follows:

Estimation of the probability of dying of hybrid class at LD_{50} of S strain, (p^{hS})

$Y = 2.2495_x + 0.2061$ .....equation of the fitted ld-pr, line for RS(F_1) strain (vide Table 5.3)

$Y = 2.2495 \times 1.798 + 0.2061$ (substituting the log Id_{50}(s) value for x).

or $Y = 4.2507$* (in probit).

From Table 1 (Finney, 1952), 22.7 per cent response rate is read for the probit value 4.2507.

---

* The probit values can also be read approximately from the graphs of ld-pr lines of RS(F_1) and R strains for LD_{50} of strain.

The probability of dying of the RS(F_1) population at LD_{50}(s) is 0.227.

Estimation of the probability of dying of R strain at LD_{50} for S strain, (p)rS.

$Y = 1.5957x + 0.6746$.....(equation of the fitted ld-pr line for R strain).

$Y = 1.5957 \times 1.798 + 0.6746$...(substituting the log LD_{50}(s) for x).

or $Y = 3.5437$ (in probit).

From Table 1 (Finney, 1952), 7.3 per cent response rate is read for the probit value 3.5437.

The probability of dying of R strain at LD_{50}(s) is 0.073.

Putting the estimated values of p^{hS} and p^{rS} and n in equation (1),

$$EM(S) = 101.2\ (0.125 + 0.5 \times 0.227 + 0.25 \times 0.0730) = 26$$

Likewise, the expected mortality at LD_{50} of R strain, EM(R), is estimated as 122.9.

The number of mosquitoes observed dead when exposed to LD_{50}(s) for S and R strains were 21.2 and 115.8 respectively. The standard error, s, of the mortalities at LD_{50}(S) and LD_{50}(R) were

$4.4 (= \sqrt{pqn} = \sqrt{0.257 \times 0.743 \times 101.2})$ and

$4.7 (= \sqrt{0.821 \times 0.179 \times 149.7})$ respectively.

The normal deviate, c, is the ratio of d (deviation of observation from expectation) to s (Mather, 1951), and so in the present case at LD_{50}(s) is $\frac{4.8}{4.4} = 1.1$. Similarly the value of c for the data obtained at LD_{50}(R) was calculated to be 1.5.

Both the values of c, i.e., 1.1 and 1.5 are less than 1.96 and therefore the differences between the observed and expected values are not considered significant at conventional level of probability ($P = 0.05$). The hypothesis of 1:2:1 ratio of susceptible, hybrid, RS(F_1) and resistant mosquitoes in RS(F_2) strain was therefore considered tenable.

5.7 References

1. Bami, H.L., Sharma, M.I.D. and Kalra, R.L. (1957) : Bull.nat.Soc.India Malar., 5, 246-247

2. Cochran, D.G. Grayson, J.M. and Lovitan, M. (1952) : J.econ.Ent. 45, 997-1001.

3. Davidson, G. (1958) : Bull.WHO. Hlth.Org. 18, 579-621.

4. Davidson, G. (1964) : Ann.trop.Med.Parasit. 58, 180-188

5. Finney, D.J. (1952a): Probit analysis, 2nd edit. Cambridge University Press.

6. Finney, D.J. (1952b): Biological Standardization, 2nd edition by J.H. Burn, D.J. Finney & L.G. Goodwin, Oxford University Press.

7. Johnston, E.F., Bogart, R and Lindquist A.W. (1954) : J.Hered. 45, 177-182.

8. Kalra, R.L. (1958) : Personal Communication.

9. Laven, H. (1967) : Formal Genetics of Culex pipiens. In: Wright, J.W. and Pal R. ed. Genetics of Insect Vectors of disease Amsterdam, Elsevier, pp 17-65.

10. Millard, R. (1957) : WHO.Hlth. Org. Information Circular on resistance problem - Supplement -C, Oct. 1957.

11. Mather, K. (1949) : Biometrical Genetics, Methuen & Co., Ltd. London.

12. Mather, K. (1951) : Statistical analysis in Biology 4th edition, Methuen & Co., Ltd. London.

13. Newman, J.F., Aziz, M.A. and Koshi, T. (1949) : Proc. Indian Acad.Sci., B30, 61-68.

14. Pal, R., Sharma, M.I.D. and Krishnamurthy, B.S. (1952) : Indian J.Malar. 6, 303-316.

15. Pal, R & Singh, N.N. (1958) : Ibid 12, 499-516.

16. Rozeboom, L.E. & Hobbs, J. (1960) : Bull.Wld.Hlth.Org. 22, 587-590.

17. Tadano, T & Brown, A.W.A. (1967) : Ibid 36, 101-111

18. Tattersfield, F & Kerridge, J.H. (1953) : Ann.Appl.Biol. 40, 630-644.

19. Thomas, V. (1966) : J. econ. Ent. 59, 779-786.

CHAPTER VI

SYSTEMATISATION OF INFORMATION FOR COMPREHENSION OF INSECTICIDE RESISTANCE PHENOMENON

6.1 Introduction

Facts collected through observations and experimentation are likely to become sterile if they are not meaningfully organised in system(s) and logically argued to fruitful conclusions. In this chapter, therefore, the experimental information on selection and inheritance of different types of insecticide resistance (c.f. Chapters II-V) are meaningfully organised in and logically argued to a set of six statements on response to insecticide selection - course of development of different types of resistance and evolution of resistance gene cluster (R-cluster)/resistance factor (R-factor) of major effect from polygenic system; probable physiological/biochemical roles of the constituent members of R-cluster/system(s); chromosomal mechanism of evolution of R-cluster; position effect of the R-cluster and constituent genes within the cluster; speed of selection and reversion of resistance and theory of evolution of resistant strain. Using the published informations on genetic of resistance and on physiological and biochemical basis of resistance, which are at times divergent, an attempt has been made to demonstrate the usefulness of the statements in comprehensions of the resistance phenomenon. In view of its objective, emphasis in this chapter. is given on the atypical information rather than on an exhaustive review of all published information. An exhaustive and excellent review of information on the arthropod resistance to insecticide has already been made by Brown and Pal (1971) in the classical WHO monograph series No.38.

6.2 Divergent Information - Their Sources and Control

Ever since the detection of DDT-resistance in houseflies, large number of studies on genetics of insecticide resistance, mainly in arthropods of medical importance, have been published (vide reviews by Perry (1966), Hoskins (1967) Brown (1967) and Brown and Pal (1971) and others quoted by these reviewers). These reviews showed that the information are divergent on several aspects. Divergent information on specific aspects of genetics of insecticidal resistance are indicated in Table 6.1.

Sources likely to contribute towards the inconsistancies of the information on different aspects, as indicated in Table 6.1, could be visualised as

(i) lack of precise identification of the type of resistance for valid comparision;

(ii) purity of the strain for a given type of resistance;

(iii) technique and methodology used in the study;

(iv) unit (scale) of measurement of resistance and

(v) inadequacy of the genetical concepts used for comprehension of the resistance phenomenon.

6.2.1 Types of Resistance and Need for their Identification

All cases of insecticide resistance, detected by the dose-response data, were not identical with respect to phenotypic expression, genetical determinants and physiological and bio-chemical defence mechanisms. Hoskins and Gordon (1956), therefore, suggested that whenever possible the different types of resistance should be identified. Singh (1961) suggested that insecticide resistance should be classified into three

Table 6.1

(Inconsistent information on different aspects of the Genetics of Insecticide Resistance*)

Genetical Aspects	Species Studied	Insecticide used in the study	Information	Reference	Remarks
(1)	(2)	(3)	(4)	(5)	(6)
Response to selection	A.stephensi Delhi strain	DDT	6.6 times resistance in larvae after 17 generations of selection by DDT.	Davidson(1958)	Selection resulted in vigour tolerance (v.t.). Studies were carried out in the Ross Institute of Hygiene, London.
	"	"	1.9 times resistance in larvae after 5 generations of selection by DDT	Weyer (1957)	Selection resulted in v.t. Studies were carried out in the Tropen Institute, Hamburg.
	"	"	5 times resistance after 12 generations of selection	Mosna (1956)	Selection resulted in v.t. Studies were carried out in the Institute Superiore di-Sanita.
	A.stephensi Madras strain	"	34-55 and 1.8-2.4 times resistance to DDT and DL respectively in larvae after 57 generations of selection. The adults were 2 & 3 times resistant to DL - DDT respectively.	Mohan & Singh (1965)	Selection resulted in v.t. and specific DDT resistence in larvae and v.t. in adults. Delayed appearance of larval resistance was considered due to slow concentration and suitable organisation of minor determinants into a cluster. Studies were carried out in the South India Branch of the N.I.C.D., Mettupalayam.
	C.P.fatigans Khurajee strain	"	5 times resistance after 39 generations of selections.	M.I.I. Delhi(1957)	Selection resulted in v.t. studies were carried out in the Malaria Instt. of India, Delhi.
	"	DDT & BHC	1.6 and 1.7 times resistance to DDT and HBC respectively after 40 generations of selection by a	"	"

Contd...

(1)	(2)	(3)	(4)	(5)	(6)
Response to Selection	C.p.fatigans Kalar strain	DDT	More than 150 times resistance to DDT, 9 times resistance to EIC & high resistance to DL after 65, 76, and 76 generation of selection by DDT at the larval/ adult stages.	Mohan(1960)	The Laboratory Colony was Established with a single female. Dose-response curves were very steep indicating highly homogenous population. Studies were carried out in the South India Branch of the N.I.C.D - Delhi.
	C.p.fatigans (Rangoon strain)	p,p'-DDT	High resistance to p,p'-DDT	Kalra(1970)	No significant resistance to o,p'-DDT
	C.p.fatigans Delhi. Normal strain	p,p'-DDT	High resistance to p,p'-DDT and also to o,p'-DDT.	Kalra(1970)	Studies were carried out in the N.I.C.D. - Delhi.
	Spider-mites	Chlorinated acaricides	Selection by chlorinated acaricides caused resistance also to the O.P. compounds but selection by the O.P. compounds did not cause resistance to the chlorinated acaricides	See Review by Brown & Pal(1971)	
	Mosquitoes	O.P. compounds	Selection by OP compounds caused a concomitant resistance to OP and CH compounds but selection by CH compounds did not result in resistance to the OP compounds.		
Expression of resistance	A.stephensi	DDT	Transient resistance to DDT in the larval stage alone.	Mammen as quoted by Pal(1958)	Observed with wild population. Selection was against the larvae.
			Transient resistance to DDT in the larval stage alone. Adults showed w.t. continuation of selection caused resistance in the adults in the latter generations.	Mohan and Singh(1965)	Observed in the Laboratory studies carried out in the South India Branch of the N.I.C.D.
	A.subpictus		Transient resistance to DDT in the adult stage only.	Sharma & Kalara (1958)	Observed in the wild population in Delhi and nearby area. Selection was directed against adults.

contd.

(1)	(2)	(3)	(4)	(5)	(6)
	C.p.fatigans	DDT	Resistance in both adult and larval stages	Pal & Singh(1958)	Observed in the wild population in Delhi and nearby areas. Selection was directed against adults.
	C.p.fatigans Kaiar strains	DDT	Resistance in both adults and larval stages against DDT & BHC	Mohan(1960)	Laboratory studies were carried out in the South India Branch of N.I.C.D.
Expression of M.domestica resistance		DDT	Resistance was limited to the male housefliés	Kerr(1960, 1961) Hiroyoshi (1961)	It's a lone observation. Resistance in insects were, usually, found in both sexes.
Transmission of Resistance	A.stephensi	DL	The Non-specific DL Resistance (i.e v.t) was polygenic.	Singh(1976)**	
"		DDT	The specific DDT resistance was due to a stable cluster transmitted like a single Mendelian factor in association with other determinants.	Singh & Mohan (1965)	
"	"	DDT	Monofactorial inheritance	Davidson Jackson (1961a & 1961b)	The R-gene was dependent on genetical background for its expression.
	C.p.fatigans	DL	Monofactorial inheritance of specific DL - resistance	Davidson(1964)	
"	"	DDT	Monofactorial inheritance of the pecific DDT-resistance	Pal & Singh(1958) Rozeboom and Hobbs(1960) Davidson (1964) Tadano Brown(1967)	
"	"	DDT	Polyfactorial inheritance of the specific DDT resistance	Thomas(1966)	

Contd...

(1)	(2)	(3)	(4)	(5)	(6)
Extra-chromosomal effect	C. fatigans	DDT	Matroclinous effect observed. The tolerance to DDT in F_1 of the cross using resistant female was higher than the F_1 of the cross using susceptible female.	Pal & Singh(1958) Rozeboom & Hobbs (1960) Davidson (1964) Thomas(1966)	1.
	C. p. fatigans	DDT	Matroclinous effect was not observed	Iadano and Brown (1967)	
	A. stephensi	DDT	Matroclinous effect was not observed	Singh & Mohan (1965)	
Dominance of the character	C. fatigans	DDT	Resistance dominant	Rozeboom & Hobbs (1960) Davidson (1964) Thomas (1966) Iadano & Brown(1967)	
"	"	DDT	Resistance recessive	Pal & Singh(1958)	
"	"	o,p'-DDT	partially recessive	Kalra (1970)	
	"	p,p'-DDT	dominant	- do -	
	A. stephensi	DDT	Partially recessive	Davidson and Jackson(1961a, 1961b)	Dominant in association with DL-H gene (Davidson, 1966)***
	"		Intermidiary resistance without any dominance of either types of alleles.	Singh and Mohan(1965)	
Gene action	Insects spp	CH, OP etc. compounds	No consistent mode of action	Review by Perry (1966)	

Contd...

Reversion of resistance	*A.stephensi*	DDT	No reversion	Mohan and Singh(1965)	The highly DDT resistant strain was cultured in the Laboratory for 28 generation without selection. Susceptibility tests did not show any significant loss of resistance.
	A.culicifacies	DL	Reversion to susceptibility within a years time when DL spraying was replaced by DDT spraying. Re-selection with DL resulted in higher degree of resistance than that observed before	Bhatia and Deobhanker (1963) Patel et al (1961)	

* The object of this table is just to indicate the inconsistency of the information on different aspects of the genetics of resistance and hence exhaustive coverage of information was not attempted. For the latter Brown and Pal (1971) may be referred.

** See Chapter IV.

*** W.H.O. Inf. Circ. Insect. Resist. No.58-59, 32.

types viz. non-heritable resistance, heritable non-specific resistance and heritable chemical group specific resistance. The first two types of resistance were usually of low magnitude and the last type was of high order with varying magnitudes. The genetical basis of non-specific and specific types of resistance were also different (Chapters III,IV and V). Toxicological, genetical and biochemical studies further indicated that the specific resistance to different analogues/isomers of a given toxicant was not the same. Wieb (1968) observed that out of 50 analogues only 8 reacted similarly to DDT in barley. The molecular structure was considered of greater importance than the chemical composition of the toxicant. Kalra and Joshi (1962) working with mosquitoes reported that the joint actions of p, p' and o, p' isomers of DDT were simple similar, complex similar and independent against C.p.fatigans, Ae aegypti and A.subpictus, respectively. Independent action was considered due to specific site of loss rather than site of action. These p,p' and o,p' isomers were found to show synergistic action against house-flies and body lice (Yasutomi 1956). In the genetical studies with Delhi - R strain of C.p.fatigans the resistance to o,p' isomer of DDT was found to be partially recessive as against dominance for the p,p' isomer. Defence mechanism(s) only for p,p' DDT and nonspecific defence mechanism(s) for both p,p' and o,p' isomers were suggested to be involved. (Kalra 1970). The bio-chemical studies showed that the disintegrating enzyme of p,p' DDT had no effect on the o,p' isomer (Perry 1966). These observations suggested the existence of specific gene control defence mechanism(s) even against the isomers of an insecticide. Therefore the heritable

specific resistance should also be sub-classified, whenever possible, with respect to the molecular structure of a chemical. Unless the resistance under study is precisely classified and pure toxicant, with respect to isomer, is used for toxicity test the chances of having converging information is rather small.

A precisely classified resistant population should be homogeneous and homozygous. The population should not contain determinants of the other types of resistance. Genetical and bio-chemical studies with such population may be expected to increase the chance for obtaining consistent results on gene transmission and gene action.

6.2.2 Bias Due to Technique and Need for Its Control

The limitation and bias of technique(s) and methodology used for the genetical studies were also found to contribute towards the inconsistency of experimental information. Genetical studies carried out with the 'discriminating dose' and with the 'scalar dose' yielded mutually contradictory information even with the same material. Resistance was found to be mono-factorial when 'discriminating doses' were used but it was found to be multifactorial when 'scalar doses' were used (Milani 1956). Similarly studies based on toxicity cum statistical methods also likely to give biased information on the number of resistance factors as revealed from the model dose-response curves given by Tsukamoto (1963). A combination of the 'discriminating doses' and 'scalar doses' methods, when 'mutant markers' are not available, could minimise the bias from these sources.

6.2.3 Bias due to Criteria and Scale

Variability of information on the dominance of resistance could be attributed to criteria of judgement and scale used to measure the character. Any higher value of LD_{50} of the F_1 generation than the susceptible parent should not be considered as a case of dominance of resistance. For this purpose the criteria suggested by Mather (1949), Singh (1961) and Singh and Mohan(1965) for deciding the dominance of an allele of bio-metric character may minimise the variability of information on this point.

The scale, unit of measurement of the quantitative character, also contribute towards the variability of information on dominance (Mather 1949). Reports on the validity of a given scale, in the genetical studies of resistance, are rare. The studies reported earlier (Chapter V) showed that the dosages of insecticides in log units were better since the log dose scale gave normal distributions of tolerance, additive effect of the alleles and null effect to gene interaction. Genetical factors like the modifier genes and/or dose of the resistance determinants could also contribute towards the variability of information on dominance of resistance.

These sources of variability of the information when controlled may still leave significantly inconsistent information due to inadequacy of the prevailing genetical concepts. In the following sections, therefore a set of concepts is suggested to comprehend the genetical basis of resistance phenomenon in its totality.

6.3. The Course of Progress of Insecticidal Selection- Evolution of the System/Cluster of Determinants

A.stephensi when subjected to DDT selection pressure at the aquatic and/or imaginal stages, in the laboratory, showed concomitant

resistance to dieldrin, BHC and DDT (Chapter II). Resistance to insecticides other than those belonging to the chemical group of the selecting one was often found to be prominent early in the development of a resistant strain. Its nature and significance were considered by Grayson and Cochran (1968) as subject of debate. The genetical experiments presented in Chapter IV had elucidated this problem. The concomitant resistance to DL in A.stephensi was of low magnitude and was determined by polygenes organised into an effective order. Chromosomal activities like crossing-over, was attributed as probable mechanism for re-organisation of the polygenes in an effective system. The effective organisation could be maintained by chromosomal inversion or linkage.

D'Alessandro et al (1957,1958 and 1962) and Mosna et al (1958,1959) reported that strains of A.albimanus, in which DDT tolerance had been induced by laboratory selection, were found to contain significantly more inversions than the parental stock. The individuals with inverted chromosomes were more DDT tolerant than the individuals without chromosomal inversions. Selection by DL also resulted in the increase of frequency of chromosomal inversion. Further, chromosomal inversion could also be induced by enviornmental stress resulting in increased vigour. It could, therefore, be concluded that chemical and other enviornmental pressure/ stress could, in addition to selection, induce the re-arrangement of chromosomal material pre-existing and/or newly introduced ones through mutation/migration. The re-organised chromosomal material would, usually, be expressed in improved vigour and would be of economic value to the species. The reorganised genes/alleles could be maintained through strong linkage bond/chromosomal inversion (heterozygous).

High chromosomal inversion rates were not found to be correlated with the varying degree of insecticide resistance. It could, therefore, be considered that the chromosomal inversion was not the cause of resistance but it was a mechanism of preservation of newly organised system of genes for resistance.

It was observed earlier that the polygenes for non-specific - resistance were the genes of fine adjustment and they play an important role in the subsequent evolution of specific resistant strain of a species.

From the above considerations it may be concluded that insecticides can play, simultaneously two roles viz. inducing and selecting insecticide resistance.

The specific resistance to the selecting insecticide was reported to evolve after the non-specific resistance. Laboratory studies on the selection of resistance in A.stephensi by DDT showed that DDT - specific resistance was not due to selection of a preexisting single factor. It was suggested that the DDT resistance in larvae was, most probably, due to slow concentration and suitable organisation of the specific minor factors into a cluster (Chapter II). The resistance cluster when adjusted to a stable state, under the specific selection, was found to be transmitted like a single Mendelian factor (Chapter III).

The progress of development of resistance, estimated by the degree of resistance in different generations, was found to approximate the growth curve (c.f.2.3.5). This observation was in conformity with the result of laboratory selection of resistance in house flies by DDT, Methoxychlore, DL, chlordane and gamma BHC (Bruce and Decker 1950).

The growth curve could, therefore, be considered as a reliable representation of the course of progress of the development of specific resistance.

The growth curve, representing the progress of development of resistance, was seen to have distinct three segments viz. lower asymptotic like segment, steep-gradient segment and upper asymptotic segment. The lower asymptotic-like segment was attributed to absence or very slow rate of increase of resistance during the earlier generations under selection. The slow rate in the build up of resistance was considered due to the inertia of chromosomes(2.4.6). The chromosomal inertia resists the re-organisation of the existing genetical determinants into a suitable cluster. The magnitude of chromosomal inertia depends on the degree of stability of the efficiently organised state of the genes under the demand of a given ecological situation. A highly stable state of organisation of the chromosomal material would, on account of its resistance to reorganisation, be eliminated under the altered ecological situation resulting from the contamination with insecticide. The individual surviving in the insecticidal contaminated enviornment, due to vigour factors, would reorganise the chromosomal material at a given locus of chromosome(s) for better fitness if the chromosomal inertia is below the critical level of resistance to reorganisation. The perceptible degree of specific resistance would be expressed only when an optimum size and level of organisation of resistance cluster(s) is built up. The success in the evolution of the resistance cluster, however, would depend on the availability of the required 'building blocks' in the

chromosomes of the species population. In absence of these 'building blocks' for resistance the development of insecticidal resistance would not occur.

The segment of the growth curve with steep gradient suggested that the rate of increase of resistance per generation was higher than the previous phase. This phase of higher rate of increase of resistance would, therefore, be considered to be the highly active phase for development of the specific resistance cluster. The higher activity could be attributed to the momentum generated by mutually accelerating process of the given State of cluster formation and its proportionally increased power of assimilation of the hitherto available elements needed for further growth of the cluster. The other factor could be the loss of chromosomal resistance to change due to continued chromosomal activities induced by insecticide specific selection pressure. The increase in the level of efficiency of the resistance cluster would result from the increase in effective 'gene dose'. This would be expressed in the rapid increase of the insecticide resistance. The observations recorded in Chapter II and that of Chovnick (1966) would be cited to support this assumption. Chovnick reported sharp change in the enzyme activity with $3ry^+ > 2ry^+ > ry^+$ and $0ry^+$ was completely inactive. The ry^+ was the rosy locus of D.melanogaster and the coefficients were the gene dosages. Mohan and Singh (1965) reported DDT resistance in the Erode Strain of A.stephensi was greater than that of the laboratory selected Madras strain. The availability of large number of

determinants in the wild population of Erode Strain than that of laboratory cultured Madras strain was attributed to this phenomenon.

The upper asymptotic segment of the growth curve representing the progress of selection was considered as the phase of maturity and stabilisation of the resistance cluster. More or less unchanging level of resistance in this phase could be attributed to non availability of specific determinants for further incorporation in the resistance cluster and/or the large size of the cluster. It is considered that there exist a size of resistance cluster with optimum organisation. Any growth beyond this size may not exhibit increase in the physiological expression of resistance. The failure to increase the level of resistance in a highly resistant strain of A.stephensi (Strain E) with high DDT selection pressure for 27 generations in the laboratory (Chapter II) could be considered as an experimental support to this hypothesis.

The upper asymptotic phase over the generation axis could in its turn be divided into three segments representing unstable, moderately stable and highly stable states of the resistant cluster. The unstable state could be at the begining of this phase and would be followed, sequentially, by the moderately and highly stable states. A highly stable cluster would behave like a monofactor in transmission. The unstable cluster would give evidence of polygenic/multifactorial inheritance of resistance. The moderately stable cluster could be expected to behave like single mendelian factor probably with some disturbance but if subjected to rigorous test like repeated back-crossing and selection

then it might give evidence of polygenic/polymeric resistance. The monofactorial evidence of DDT resistance in A.stephensi (Chapter III) and C.P.fatigans (Chapter V) could be due to stable/semi-stable states of the specific DDT resistance cluster. The experimental evidence of polygenic basis of resistance in C.P. fatigans (Thomas 1966) could be due to unstable state of the resistant cluster. The stage of development of the resistance cluster and its state of varying level of stability would, thus, yield inconsistent information on the mode of inheritance of specific resistance.

In a given wild population of an insect species stable specific/non specific resistance cluster/system could be found at any stage of development and stability due to random phenomenon or due to positive correlation between the directions of the natural selection and that of a given insecticidal selection. In such a situation the progress of selection of the resistant strain would be faster and the mode of inheritance of resistance would be found to be monofactorial. The evidence of existence of resistant individuals, in significant numbers, in anopheline populations from the unsprayed areas (see review by Spielman and Kitzmiller, 1967) and of the monofactorial evidence of resistance with field selected species of insects could be considered as evidence towards the validity of the hypothesis.

In the light of these discussions the following statement on the course of development of insecticide resistant is made.

Statement 1

Exposure of insects to a given insecticide could result in the development of three types of insecticide resistance viz non-specific resistance(R-I), group-specific resistance (R-II) and structure specific resistance (R-III). The R-I type is usually of low magnitude and could often be found prominent early in the development of resistance. It is polygenic and is developed by re-organisation of minor chromosomal determinants, if available, into an effective system through the chromosomal activity induced by the insecticide. The non-specific determinants are genes of fine adjustment and they play an important role in the development of specific type of resistance.

The specific types of resistance, R-II and R-III. are developed through slow concentration and suitable re-organization of specific chromosomal factors, if available, into a cluster. The course of development could be represented by the 'growth curve'. The slow rate of 'built up' of resistance in the earlier generation could be attributed to chromosomal inertia resisting reorganisation of the available required determinants into resistance cluster (R-Cluster). Perceptible degree of specific resistance would be expressed only when the optimum size and level of organisation of the R-cluster is built up. The phase of higher rate of development of resistance is attributed to the loss of chromosomal resistance to reorganization and increasing power of assimilation and organization of the developing cluster. The growth of the R-cluster tends to a maximum size and hence a maximum level of resistance. This phenomenon is represented by the upper asymtotic phase of the 'growth curve'. Continued exposure to insecticide, when the maxima is attained, would result in stability of the R-cluster. The unstable, moderately stable and highly stable R-cluster would give the evidence of polygenic, polymeric and monofactorial basis of specific resistence.

6.4 Composition and Function of the System/Cluster of Resistance Genes

Direct method of viewing the structure and function of the system(s)/cluster(s) of gene(s) of insecticide resistance is not available. Therefore indirect method, study through observable behaviour, is used for this purpose.

The resistant individuals of a species of insect scape the lethal action of an insecticide through a set of protective mechanisms. The likely protective mechanisms of insecticide resistance were visualised by Hoskins and Gordon (1956) and Perry (1966) as

(i) lesser penetration of insecticides through insect integument;

(ii) slower rate of transportation to the site of action;

(iii) lesser sensitivity of the nerve cells;

(iv) higher rate of enzymic conversion of the toxicant to innocuous substance(s);

(v) faster rate of detoxication of the toxin (s), if produced;

(vi) slower rate of enzymic conversion of chemicals to more toxic compounds;

(vii) higher rate of excretion of the toxins and the toxicants and

(viii) more storage of insecticides in the fats and non-sensitive tissues.

the resistant individuals than the sensitive ones.

These protective mechanisms and others, if they exist, could, rly, be classified as of anatomical, physiological and bio-mical types. The three types of protective mechanisms could be ected to differ in the two metamorphic stages, larval and adult, of quitoes, because of the differences of their enviornment and the e of biological developments. Protective mechanisms(s) orginating m the anatomical modifications could be considered to be of a manent nature - once developed, it would persist throught the life of individual. The physological and the bio-chemical types of

protective mechanisms could, however, be in the state of readiness with the high potential to operate with increased efficiency when stimulated by the specific toxicant.

Histogenesis, morphogenesis and mural development occur in an organism through the sequential and/or independent operations of specific genetical switches. All these developments could not be envisaged as a product of a single gene (Waddinton 1966). Similarly the nature of enzymes and their primary structure of different polypeptides were determined by separate genes (Garrod, 1909; Haldane, 1942; Harris, 1966). The determinants of insecticide resistance could, therefore, be considered to modify the actions of the developmental, physiological and biochemical genes at the appropriate times. Hence it could be considered that the composition of a resistance system/cluster would be of varying degree of complexities due to its complex functions.

The non-specific resistance, R-1, is due to increased vigour controlled by polygenic system(s) evolved through the induction of enviornmental stress and injurious chemicals. From the physiological and bio-chemical points the increased vigour could result, among others, from the higher rates of degradation of the injurious chemicals. Bio-chemical studies showed that the prominent among the drug disposal mechanisms are the microsomal enzymes - a class of enzymes requiring $NADPH_2$ and oxygen but having no specific substrates of their own. Such enzymes were found in several insect species to hydroxylate DDT, to act on parathion, BHC to oxidise carbaryl, pyrothrins, rotenone, and to epoxidize aldrin (review by Perry 1966). As a working

pothesis a non-specific polygenic system or a sub-system of the system(s)
uld, therefore, be considered to be induced by the enviornmental factors inclu-
g insecticide which would induce & regulate in their turn the production of
e drug disposing non-specific enzymes. Each of the other polygenic
stems or the sub-systems of a system could be attributed to modify
h of the anatomical and physiological protective mechanisms, in a
ll way, in favour of increased vigour. Likewise a cluster of specific
istance R-II or R-III, could be considered complex in its composition.
could be considered as a set of heritable units controlling the different
tective mechanisms contributing towards a given type of specific resistance.
there are n exhaustive perfectly organised heritable units each
ifying a sub-character of any type of specific resistance then 2^n
es of resistance cluster could be found on the basis of its composition.
would include a null R-cluster which is the normal (susceptible)
e of the R-cluster. The remaining (2^n-1) resistance clusters are
ly to cause variability with respect to the operation of the protective
nisms and hence expression of the magnitude of resistance within and
een the resistant strains of a given species. The magnitude of
ession of a given type of specific resistance would also depend on;

(i) type of jointaction between the elements of a R-cluster and

(ii) number of elements present in a given cluster.

intaction between the elements of a R-cluster could be;

(i) additive and/or

(ii) multiplicative.

The higher degree of specific resistance than non-specific one could be due to complex interaction of the elements of a given R-cluster.

The number of elements and their state of organisation would also determine the degree of dominance of resistance.

This hypothesis could be supported by some of the published experimental observations. Wiesman (1947) reported modification in permeability of cuticle to insecticides through the evolution of thicker and more pigmented tarsii, pulvilli etc. in a resistant strain of houseflies. Abedi and Brown (1961) observed hypersecretion of peritrophic membrane, considered to be a protective mechanism, in response to DDT by the DDT resistant Ae.aegypti of Caribbean origin. These protective mechanism were not observed with every case of resistance. In his exhaustive review of the experimental results on resistance Perry (1966) concluded that the structural and bio-chemical variability among species and strains was rather a rule than exception. Each species or strain possesses a combination of attributes for resistance which may be different from that found in others.

The variability in the distribution of the sub-units of the resistant cluster might result in the presence or absence of certain element modifying the protective mechanism at a particular morphic stage. Once this would result in the variation of the expression of resistance at different stages of metamorphosis of insects. High degree of transient DDT resistance in larval stage alone of A.stephensi (Review by Pal 1958 and Mohan and Singh (1965) and in adult stage alone in C.pipiens fatigans (Sharma and Kalra 1958) could be due to absence of factor

in the resistance cluster to protect or to activate the protective element(s) for its function at adult and larval stages respectively. The elements of a given resistant cluster could act sequentially or otherwise. In case of sequential operation of the elements of a resistance cluster, an element A is set off to produce a substance α which activate another element B of the cluster to produce another substance β to act upon the third element C and so on. But when the elements do not act sequentially, each of them is primed by a common substance or different substances produced by the element not belonging to the cluster. This model of actions of elements could show variation of a kind in which an element of resistance cluster could be activated by a substance γ produced by some element X. X may or may not be a member of the given resistance cluster. Further, certain elements could be activated by alternative substances produced by different elements belonging to same or different cluster(s).

This scheme on the mode of actions of the elements of the resistance cluster could be used to comprehend the unusual phenomenon of supervened DDT resistance observed in malathion selection of *Ae.aegypti* Brown and Abedi, 1960 ; Mat.umera and Brown, 1963 and Pillai and Brown, (1965) Malathion resistance due to DDT selection was not so far, reported in insects. The factors for DDT and malathion resistance in *Ae.aegypti* were reported to be on the chromosome II. DDT resistance supervened in malathion selection could be due to priming/activating of some elements of an incomplete DDT cluster, evolved by chance, by activating/priming product of an element of malathion resistance cluster selected under

specific pressure. The failure of selection of malathion resistance by DDT pressure could be due to;

(i) non susceptibility of the element(s) of malathion resistance cluster to any product of the element belonging to DDT resistance cluster or/and

(ii) absence of even an incomplete malathion resistance cluster.

From these considerations the following statements on the components and functions of the non-specific polygenic system(s) and specific resistance cluster is made:

Statement No.2

Each polygenic system or each sub-system of a given polygenic system could be assigned a specific role in the development of R-I type of resistance. The environmental factor and toxic chemicals are likely to induce and regulate, among other things, the production of drug disposing non-specific enzymes. The specific resistance cluster(s), R-II and R-III, could be composed of a number of specific sub-units each interacting to modify the developmental, physiological and biochemical processes in favour of a given type of resistance. If there exists n number of exhaustive sub units for a resistance cluster then there could exist 2^n types of cluster. One of the 2^n clusters would be a 'null' cluster which could be considered the susceptible allele of the resistance factor(s). The remaining (2^n-1) types of resistant cluster will differ with respect to their composition and protective actions. The variability in the composition of R-II and R-III clusters could be considered to effect variability with respect to expression of resistance within and between the stages of mosquito's developments, dominance of the character (due to variations in the critical level of gene dose), physiological and biochemical defence mechanisms. The mode of actions of the elements of the R-cluster could be attributed to the necessary but not sufficient cases of double resistance.

6.5 Chromosome Mechanism and Resistance

The determinents of non-specific and specific types of insecticide resistance in A.stephensi and group specific type of resistance in C.fatigans were found to be chromosomal (Chapters III,IV and V). These observations are in agreement with the reported information on the genetics of insecticide resistance in vector species of insects (Review by Brown 1967). Chromosome could be viewed as an integrated organisation of genes with complex interaction and control in replication, transcription and segregation. Chromosomal activities like inversion, linkage - recombination, translocation etc. could, therefore, play important role in the evolution, transmission, action and expression of the system/cluster of resistance genes.

Inversions, homozygous and heterozygous, cause alteration in the order of genes in a given linkage group. The change in the order of genetical determinants could affect the expression of resistance in varying magnitude and direction. In the case of heterozygous inversion each block of chromatin would tend to become allelically different from the comparable blocks because of random Mutations that might accumulate in absence of recombination. Selection would, in such situation, operate not on single gene but on total genotype and be more effective than in caseof gene sequence constantly being reshuffled through crossing over (Swanson, Merz and Young; 1967). In A.stephensi (M-strain) inversion in the chromosome R-III was observed (Rishikesh, 1959). High rate of heterozygous inversion, without any correlation

with the degree of resistance, under insecticide pressure was reported by D'Alessandro et al (1957 and 1958) and Mosna et al (1959). Inversion was, therefore, suggested as a likely mechanism of evolution of resistance system/cluster.

Linkage is regular co-transmission of adjacent loci. It provides selective value through increased mechanical economy during cell division. It could be detected by the presence of recombinant phenotype classes in proportions not commensurable with the hypothesis of independent assortment of non-homologous chromosomes. In a given ecological situation crossing over of a given type of resistance determinants could result in a new linkage arrangement that may lead to;

(i) evolution of the R-system/cluster;

(ii) increase in the efficiency of the resistance system or the cluster or

(iii) disintegration of the R-system/cluster.

Linkage and crossing over would also contribute towards the variability of information on effective factors and their transmission and action.

In the case of insecticide resistance linkage could be between

(i) elements of a given system/cluster of resistance;

(ii) non-specific system(s) and specific cluster(s) of resistance;

(iii) clusters of different types of specific resistance;

(iv) a given type of R-cluster and some elements of another type of R-cluster and

(v) R-system/cluster and determinants of unrelated character(s).

Non specific resistance to DL and BHC was developed in A.stephensi (Chapter II). The non-specific resistance could be possible through the reorganisation of the genes in the chromosome from $\frac{++-+}{--+-}$ order to $\frac{++++}{++++}$ order of organisation in a polygenic system (Chapter IV). Such a reorganization of genes into a system could be due to crossing over of the determinants. Once reorganized the determinants could be held together by their linkage bonds under the compatible enviornment. In case of weak linkage, the system could get disorganised through the mechanism of crossing over. Organization and disorganisation of the genes of DDT specific cluster, through crossing over, were attributed to the fluctuation in the level of resistance in Erode-strain of A.stephensi (Chapter II).

The non-specific DL resistance in A.stephensi was found to be associated with the specific DDT resistance. This was considered due to the linkage of non-specific system of determinants (genes) with the DDT specific R-cluster (Chapters II and IV). Further the studies on the inheritance of DDT-resistance in A.stephensi and C.p.fatigans showed significantly low mortality only in the lower concentrations of DDT than expected on the basis of monofactorial inheritance of the character in the segregating generations (Chapters III and V). Such an increased tolerance of low magnitude could be due to the contribution of the non-specific determinats closely linked with the DDT specific R-cluster.

The observations of Kalra (1970) could be considered as an example of the linkage between the structure specific R-clusters. The Rangoon-R-strain of C.fatigans was highly resistant to p,p' - DDT without any significant tolerance to o,p' - DDT. The resistance to

o,p' - DDT was inherited as a partially recessive character as against the dominance of the p,p'-DDT resistance. The bio-chemical protective mechanisms in those two types of resistance were also different. Hence the genetical determinants for the two isomer-specific resistance, could be considered distinct and different. The selection of resistance to both the p,p' and o,p'- isomers of DDT in the C.fatigans (normal strain) by the pressure of p,p'-DDT could therefore, be considered due to linkage of the isomer-specific resistance clusters. Studies with the heterogenous population consisting of linked and recombinant classes of different kinds of resistance clusters are more likely to give inconsistent informations with respect to gene transmission, expression and action.

The necessary but not sufficient cases of cross-resistance could be considered as an example of linkage between one type of R-cluster and some elements of another type of R-cluster. Selection with organo-phosphorous, O.P., compounds usually results in high level of cross resistance to chlorinated, CH, insecticides especially of DDT type but the selection with CH-insecticides does not ordinarily induce resistance to OP compounds (review by Perry 1966). The resistance to DDT due to selection by OP compound could be due to linkage of OP resistance cluster with some key elements of an incomplete DDT-resistance cluster. For a chromosomal mechanism to activate the elements of an incomplete DDT-R cluster it could be assumed that the OP-resistance cluster is polycistronic and hence activates more than one cistrons which include the cistron containing some elements of DDT resistance cluster. The long chain of RNA formed can code larger number of seperate enzymes

including those required for the protective mechanism against OP and CH groups of insecticides. The DDT-R cluster does not extend over the cistrons containing the key elements for OP-resistance and hence it is incapable of inducing cross-resistance to OP-compounds.

The resistance factors could, in certain cases, also be linked with factors for characters not concerned with resistance. In Ae. aegypti DDT resistance factor was found to be linked with the 'spot' locus on linkage group II (Cocker 1965).

The genetical factors located in different chromosomes could also show linkage due to translocation phenomenon. Sex-limited resistance in males of M. domestica was due to translocation from chromosome II to Y - chromosome (Kerr 1960, 1961 and Hiroyoshi, 1961).

From these considerations the following statement on chromosomal mechanism vis-a-vis resistance is made:-

Statement No.3

The determinants of different types of insecticide resistance being chromosomal are likely to be affected by the chromosomal activities like inversion, linkage recombination, translocation etc. The expression of resistance in varying magnitude and direction could be influenced by homozygous inversion due to change in the order of chromosomal material. In case of heterozygous inversion each block of chromatin could tend to become allelically different from the comparable blocks because of accumulation of given type of random mutants for resistance under insecticide pressure in absence of recombination. Selection in this case would be more effective than in the case of the resistance determinants constantly being reshuffled through crossing over. Linkage and recombination could result in evolution or integration of given resistance system/cluster. It could also give rise (1) to the necessary but not sufficient cases of cross-resistances(2) to the favourable condition for evolution of cross/multiple resistance and (3) to inconsistent information on expression and action of resistance determinants. Translocation would contribute to a new type of linkage system.

6.6 Position Effect

The position of the R-system(s)/cluster(s) can be defined with respect to its relation with the

(i) other genetical material in its own linkage group;

(ii) genetical material in other linkage groups;

(iii) associated cytoplasmic material and

(iv) the ecological factors constituting the insect's external enviornments.

The propagation, expression and action of the R-system/cluster being the resultant effect of its interaction with these neighbourhood factors are likely to be affected by the position(s) of the R-system(s)/cluster(s).

The R-system/cluster can occur at any locus in any of the linkage groups. In case of multiple gene complex the different R-systems/clusters can simultaneously occur at any locus in more than one linkage groups. The studies carried out with the visible mutant marker had shown that the specific factors for the DDT and DL resistance were located in the linkage groups II and III in each of Ae.aegypti and C.fatigans (Khan and Brown, 1961 and Tadano and Brown 1967). Linkage groups II and III were involved in malathion resistance in Ae.aegypti (Brown and Abedi, 1960 as quoted by Klassen, 1966). In his enlightening study of the polygenic inheritance of DDT-resistance, using mutant markers, Crow (1957) reported a regular correlation between the number of chromosomes from the resistant strain of Drosophila and the percentage of survival of flies exposed to DDT. Close analysis using crossover markers succeeded

in identifying particular chromosomal regions in which dominant mutant resistance factors were located, but the evidence indicated that very probably several genes on each chromosome concerned with the polygenic resistance to DDT.

The position of the resistance factor in a given chromosome was found to affect the expression of the character. The resistance factor at a locus 26 crossingover units (c.o.u) from the factor for 'SPOT' showed higher degree of resistance in Ae.aegypti than the factor located at the 16 c.o.u. (Cocker, 1965).

The position of the resistance factor in different linkage groups was found to affect the expression and action of the factor. Hoyer and Flapp (1966) reported that DDT and O - Chloro DDT types of resistance in house flies were recessive when the determinants were on chromosome II but the chracters were dominant when the determinants were on chromosome V. The expression of the determinant measured, by the degree of resistance, was moderate (100 to 200 fold resistant to DDT) and high (more than 3000 fold resistant to O-Chloro DDT) when the chromosome II was involved, but the degrees of resistance to DDT and O-Chloro DDT were moderate and nil respectively when R-factor(s) was located on chromosome V. The bio-chemical protective mechanism involved was the enzyme other than dehydro-chlorinase when the R-determinants was linked to chromosome II but dehydro-chlorinating enzyme was involved as a bio-chemical protective mechanism when the determinants were on chromosome V.

The position effect of the resistance determinant was also observed with Ae.aegypti. The bio-chemical protective mechanism was due to DDT-dehydro-chlorinase enzyme when the determinants for resistance

were located on chromosome II but enzyme other than dehydrochlorinase was responsible to make DDT inocuous when chromosome III contained the R-factor.

Cytoplasm as a neighbourhood factor was reported to affect the propagation of genes in mosquitoes (Review by Laven 1967). In insecticide resistance the cytoplasmic effect on quantitative expression of the R-cluster was observed with C.fatigans (Chapter V). Similar observations were also reported in case of Blattela germanica (Cochran et al 1952), Drosophila (Tattersfield et al 1953) and Musca domestica (Johnston et al 1954).

The cases of variation in the intensity of expression of resistance in insects when imported from another country and studied in different seasons could be considered as examples of effect of ecological factors on expression of resistance determinants.

In the light of above considerations the statement on position effect of resistance system/cluster is given as:-

Statement No.4

The position of the R-system(s)/cluster(s) could be defined with respect to its relation with genetical material in its own linkage group, the genetical material in other linkage groups, the associated cytoplamic material and the ecological factor constituting the external environment of the insects. Propagation expression and action of the R-system/cluster being dependent on the interactions with these neighbourhood factors would be affected by the position of the R-system/cluster.

6.7 Reversion and Reselection of Insecticide Resistance

The susceptible and specific resistant strains of mosquitoes were, usually, encountered in high frequencies in the un-sprayed and insecticide sprayed areas respectively. In few un-sprayed areas, however, resistant mosquitoes were found in significant proportions. These observations suggested that the direction of the forces of natural selection and a given type of insecticidal selection could, usually, be opposed to each other but in few cases they should either be positively correlated or mutually independent. It could, therefore, be argued that the effect of the natural selection, consequent to the withdrawal of the given insecticidal selection pressure, on the resistant population would be

(i) complete reversion of insecticide specific resistance in a given insect population in case of negative correlation between the directions of the forces of insecticide selection and the force of natural selection;

(ii) partial or no reversion of insecticide specific resistance in the given insect population in case of varying degree of positive correlation between the directions of the force of insecticide selection and that of the natural selection and

(iii) unpredictable status of resistance in the given insect population in case of mutually independent relationship between the directions of the forces of the two types of selections. The given state of resistance in the population would, in this case, be due to chance factor.

The reversion of the resistance in a given insect population would result from the operation of one or more of the following factors:

(i) Dis-integration of the R-system(s)/cluster(s) when it is not stable and not favoured by natural selection.

(ii) Selective elemination of the given type of stable R-system(s)/cluster(s) by the force of the natural selection if the directions of the force of the natural selection and that of the given insecticide selection are negatively correlated.

(iii) Dilution of the resistant population by the susceptible phenotypes, having better survival and reproductive values under the insecticide free environment, originating from

(a) migration;

(b) back mutation to susceptible genotype;

(c) existing heterogenous population and

(d) segregation of the susceptible phynotypes from the heterozygous resistant population.

The course of reversion could be expected to approximate the growth curve in the reverse direction i.e. from resistance to susceptible state. Re-selection of the reverted susceptible population by the same or other insecticides, having positive correlation in their effects, would result in quicker development of resistance than the initial one due to faster rate of reorganization of the R-system(s)/cluster(s). The reorganization of the determinants in to the R-system(s)/cluster(s) at a faster rate would be possible due to the loss of chromosomal inertia resulting from the frequent changes in the arrangements of chromosomal material under the demand of different types of selection pressures.

The field observations made in India, could be cited in support to some of the above assumptions and deductions. The populations of A.culicifacies in various parts of India were found to be susceptible to chlorinated hydrocarbon insecticides before and/or during the earlier part of their use for malaria control/eradication (Pal 1958). But

in 1958 the population of this species in certain localities of Thana District of Maharashtra State was observed 60 times resistant to DL (Patel et al 1958). The DL resistance was found to decrease to a low level within a year's time when spraying of DL was replaced by DDT (Rao et al 1960). The complete reversion of more than 60 times DL resistance in this species in two years time was also reported by Bhatia and Deobhankar (1963). Thus the reversion of DL resistance occured when DL spraying was substituted by DDT spraying. These findings indicated that the natural and DDT selection forces were partially/ completely opposing the DL selection. The quicker rate of reversion was probably due to the combination of events like disintegration of the R-cluster and dilution of the population by susceptible individuals with better survival efficiency in the DL-free enviornment. The susceptible individuals could migrate from the neighbouring areas. They could also be available due to segregation from the resistant heterozygous. Bhatia and Deobhankar (loc cit) also observed that more than 60 times DL resistance was reselected in a year's times with only 2 round of DL spraying. The faster rate of reselection with a higher degree of resistance could be due to weaker chromosomal inertia resulting from the frequent changes in organization of chromosomal material in the recent past under the demand of changing ecological conditions.

In view of these consideration the following statement on reversion and reselection of resistance is made:-

Statement 5

The status of resistant population of mosquitoes in absence of specific insecticidal selection would depend on the relation between the insecticidal specific selection force and natural selection force. The relation of the different selection forces could be opposing, partially supporting and neutral, causing complete reversion, partial reversion and no reversion of resistence when the specific selection force cease to operate. The mechanisms of reversion of resistance were likely to be due to disintegration of R-system/cluster or/and dilution of frequency of resistant gene by the susceptible gene due to immigration or/and from the local heterogenous heterozygous populations. The rate of progress of reselection of resistance was likely to be faster than the initial selection of resistance due to loss of chromosomal resistance to change in the arrangement of genetic material (chromosomal inertia). The loss of such chromosomal resistance would result from the stress and strain of selection and reversion of insecticide resistance.

6.8 Mechanics of evolution of resistance

Replacement of the susceptible strain of an insect species with the resistant one under the insecticidal selection pressure is a case of strain evolution. To understand the evolution of a resistant strain of insect species, therefore, the theories/hypothesis of

(i) post adaptation;

(ii) mutation;

(iii) selection of pre-adaptive mutants;

(iv) lingering modification and

(v) induction

are examined in the light of available information.

According to the post adaptation theory, the evolution of the R-strain of an insect species could be considered due to acquisition of the ability to tolerate the increasing dosages of an insecticide under the direct impress of insecticidal contaminated enviornment. This theory, however, could not be supported by the

the available experimental results. Exposure to sub-lethal dosages of DDT for 55 generations did not result in the development of resistance in the pomacefly (Luers 1953). In another experiment seven different strains of Drosophila melanogaster were reared in DDT-treated media and exposed to sub-lethal amounts of DDT after emergence. None of the strain after 140-150 generations of sub-lethal contact with DDT showed any increase in tolerance to this toxicant (Luers and Bochrig (1963)). It could, therefore, be concluded that DDT-resistance in Drosophila was not due to post adaptation. Further the studies by Merrill and Underhill (1956) showed that even when dosages of DDT were increased to selecting levels resistance to DDT developed only when pre-adaptive genetic determinants were present. These conclusions were also confirmed by the studies carried out with houseflies using DDT, BHC, dieldrin and diazinon as selecting insecticides. (Hoffman et al, 1951; Hadaway, 1956; Harrison 1952 and Lavagnino; 1954).

According to the mutation Theory' insecticides could be considered to induce mutation in the chromosomal factors resulting in resistance. Treatment of adults and/or larvae with DDT failed to show any increase in the number of mutants in the X-chromosome (Luers, 1953). Pielon (1952) also could not detect any mutagenic effect of DDT and gamma BHC.

The theory of selection of pre-adaptive mutants states that the given type of resistant factor(s) could occur in a given species population by random mutation. The proportion of such factor(s)

in the field population is, usually, of low order. Insecticide favourably select these resistant population. The experimental information reported by Kikkawa (1964) could be considered to support this theory. Kikkawa (loc cit) induced pro-adaptive R-factor by irradiation. The resultant mutants after suitable re-organisation through breeding experiment when subjected to specific selection showed resistance to DDT, parathion and other insecticides. This suggested that the insecticides select the pre-adaptive mutants already present in the population of the species. Martin (1955), however, doubted the credibility of this theory on the ground that

(i) mutation is a pathological process and the embryology of mutants are not in harmony with the law of parallel embrological developments;

(ii) multifactorial basis of a character is questionable since in no case the factors were identified or discovered;

(iii) the theory failed to bridge the gap between unifactorial and multifactorial basis of character(s) and

(iv) environmental selection being non random above a certain point, and fluctuations was incapable of contributing towards evolution.

Perry (1966) also questioned the theory of selection of low frequency of mutants in wild population of insects by insecticide on the basis of current information on bio-chemical genetics and molecular biology. According to him the prominant drug-disposal mechanisms are microsomal enzymes having no specific substrate of their own. It is unlikely that all metabolic processes arose from low frequency mutations in only few individuals of each population for the sole purpose for detoxifying insecticides.

In view of the limitations of the theory of selection of pre-adaptive mutants Martin (1955) suggested the theory of lingering modification as probable mechanism involved in the evolution of R-strain. The theory of lingering modification states that the sub-lethal concentrations of a given harmful agent induces resistance with time (in generation) of contact with the toxic agent. The resistance thus induced will revert back to original level of susceptibility, sometimes, with rebound phenomenon, if the contact with insecticide is withdrawn. In this case the resistance in the offspring will be unpredictable, in a line in which it has been firmly impressed, when the resistance strain is crossed with the susceptible one. Simple mendelian segeragation could, however, be approximated when resistance has persisted for a longer generation. The experimental results by Luers (1953) and Luers and Bechinig (1963), mentioned earlier, could be considered against the credibility of the theory of lingering modification.

Perry (1966) suggested the hypothesis of induction as a mechanism of evolution of resistance. The information from the fields of biochemical genetics and molecular biology emphasize the nature of genes and proteins as dynamic process undergoing constant evolutionary changes. Insecticides were considered as one of the factors involved in bringing about these changes. The induction process could simulate activity of pre-adaptive microsomal enzymes having no specific substrates of their own. This hypothesis is unlikely to hold true for the evolution of specific types of insecticide resistant strain as well as non specific type of resistance since they were found to be determined by the chromosomal gene(s).

From the above discussion it could be concluded that none of the theories/hypotheses is sufficient to explain the phenomenon of evolution of insecticide resistant strain. In the light of the different statements given earlier an unified theory of induced integration organization and selection of available specific chromosomal material by a given insecticidal or its correlated forces is suggested as a mechanism for evolution of insecticide resistance. This theory is stated below

Statement No.6

The basic drive of survival of an insect species in an insecticide contaminated environment would initiate to such suitable actions, which would successfully meet the challange of its existence. Increased chromosomal activities like crossing over, translocation, homozygous inversion in one hand and linkage, heterozygous inversion on the other, induced by a given dose of insecticide in individual whose 'thresh hold' value of chromosomal inertia would be less than the dose of insecticidal and/or correlated environmental pressure, could be the actions for survival at gene level. These activities would result in integration, reorganisation and preservation of gene alleles, when available, in an effective genetic system and gene cluster. Such a reorganised system or cluster of genes would give higher survival value to the species in an insecticide contaminated ecosystem. The evolved resistance gene cluster could be of varying degree of complexities in its composition, stability and hence in expression, action and transmission. The individuals, in an insecticidal contaminated environment, with chromosomal inertia more than the dose of insecticide or correlated ecological pressure would be eleminated. The evolved cluster of genes could be unstable, moderately stable and highly stable at different points on the course of its maturity and evolution. The unstable, moderately stable and stable cluster would behave in transmission polygenic, multifactorial

multifactorial or monofactorial. Withdrawal of the insecticide specific force could result in the reversion of resistance through the disintegration of the R-system/cluster when unstable or moderately stable, in the elimination of the highly stable R-system/cluster when correlation between the directions of the insecticidal and ecological (when insecticide is withdrawn) forces are negative. The polymorphic state of resistance would depend on its state of dominance.

The theory considers that the

(i) induced integration and reorganisation of the chromosomal material into specific system/cluster, giving better survival value in the altered situation, is a healthy process;

(ii) multifactorial and monofactorial basis of resistance, which can be studied by suitable techniques, are due to variation in the state of maturity and stability of the R-cluster.

The theory also emphasises the importance of ecological forces, chromosomal inertia, availability of specific genetical material in the evolution of the effective genetical factor leading to the evolution of the resistant strain. It recognises the importance of pre-existance of genetical factors for selection and at the same time does not treat the exposed insects and the prevalent selection pressure as inert objects of fixed sizes and sieve respectively. It is hoped that the statement would be preferred as a good scheme to comprehend the phenomenon of evolution of R-strain of an insect species.

6.9 References

1. Abedi, Z.H. and Brown A.W.A. (1961): Mosquito News, 21, 1-4

2. Bhatia, S.C., and Deobhankar R.B. (1963): Ind.J.Mal., 17, 339-351

3. Brown, A.W.A. and Abedi, Z.H. (1960): Mosquito News, 20, 118-124.

4. Brown, A.W.A. (1967): In: Genetics of Insect vector of Disease Edited by J.W. Wright and R. Pal. Elsevier Publishing Company, Amsterdam.

5. Brown, A.W.A. and Pal, R. (19[illegible]1): Insecticide Resistance in Arthropods (2nd Edition). W.H.O. Geneva.

6. Bruce, W.N. and Decker, G.C. (1950): Soap. Sanit. Chem., 26, 122.

7. Covnick, A. (1966): Proc.Roy. Soc. (Series B), 164, 198-208.

8. Cochran, D.G., Grayson, J.M. and Levitan, M. (1952): J.Econ.Ent., 45, 997-1001.

9. Coker, W.Z. (1965): Ann.Trop.Med.Parasit., 59, 55-63.

10. Crow, J.F. (1957): Ann.Rev.Ent., 2, 227-246.

11. D'Alessandro, G, Frizzi, G. and Meriani, M. (1957): Bull.Wld.Hlth.Org., 16, 859-864.

12. Idem (1958): Riv.Parassit., 19, 67-72.

13. D'Alessandro, G., Mariani, M. Bruno-Sami-ragli, C. (1962): Ibid, 23, 227-234.

14. Davidson, G. (1958): Bull.Wld.Hlth.Org., 18, 579-621.

15. Idem (1964): Ann.Trop.Med.Parasit, 58, 180-188.

16. Davidson, G. and Jackson, C.E. (1961a): Bull.Wld.Hlth.Org., 25, 200-217.

17. Idem (1961b): Nature (London), 190, 364-365.

18. Garrod, A.E. (1909): Inborn errors of Metabolism. Oxford University Press.

19. Grayson, J.M. and Cochran, D.G. (1968): Wld.Riv.Pest Control, 1, 172-174.

20. Hadaway, A.B. (1956): Nature (London), 178, 149-159

21. Haldane, J.B.S. (1942): New Path in Genetics, London, Allen and Unwin.

22. Harrison, C.M. (1952): Bull.Ent.Res.,42, 761-768.

23. Harris, H. (1966): Proc.Roy.Soc.(Series B), 164, 298-3

24. Hiroyoshi, T. (1961): Genetics, 46, 1373-1380.

25. Hoffman, R.A., Roth, A.R. and Lindquist, A.W. (1951): J.Econ.Ent.,44, 734-736.

26. Hoskins, W.M. (1967): W.H.O./VBC/67/26.

27. Hoskins, W.M. and Gordon, H.T. (1956): Ann.Rev.Ent., 1, 89-122.

28. Hoyer, R.F., Flaff, F.W. (1966): J.Econ.Ent., 59, 499-501.

29. Johnston, E.F., Bogart, R. and Lindquist, A.W. (1954): J.Hored., 45, 177-182.

30. Kalra, R.L. and Joshi, G.C. (1962): Ind.J.Mal., 16, 327-355.

31. Kalra, R.L. (1970): Ind.J.Exp.Biol., 8, 112-117.

32. Kerr, R.W. ((1960): Nature (London), 185, 868).

33. Idem (1961): Aust.J.Biol.Sci.14, 605-619.

34. Khan, N.H. and Brown A.W.A. (1961): Bull.Wld.Hlth.Org., 24, 519-526.

35. Kikkawa, H. (1964): Botyu-Kagaku, 29, 37-42.

36. Klassen, W. (1966): Mosquito News, 26, 309-318.

37. Lavagnino, A. (1954): Memorie. Soc.Ent.ital, 22, 36-40.

38. Laven , H. (1967): In: Genetics of Insect Vector of Disease, Edited by J.W. Wright and R. Pal. Elsevier, Publishing Company, Amsterdam.

39. Luers, H. (1953): Noturwissenschaften, 10, 293.

40. Luers, H. and Bochnig, V. (1963): Zool Beitr., 8, 15-22.

41. Martin, C.P. (1955): In: Origin of Resistance to toxic agent. Edited by M.G. Sevag, Reger D.Reid. Orre E. Reynold. Academic Press Inc. New York.

42. Mather, K. (1949): Biometrical Genetics, Methuen and Co. London.

43. Matsumura, F. and Brown, A.W.A. (1963): Mosquito News, 23, 26-31.

44. Merrell, D.J. and Underhill, J.C. (1956): J.Econ.Ent., 49, 300-306.

45. Milani, H. (1956-57): Riv.Parasit., 17, 18, 233-246 and 43-60

46. M.I.T. (1957): W.H.O. Infom. Circular.

47. Mohan, B.N. (1960): Ind.J.Mal., 14, 175-178.

48. Mohan, B.N. and Singh, N.N. (1965): Ind.J. Gent.Pl.Breed., 25, 266-278.

49. Mosna, E. (1956): WHO.Mal.Soc.Progress Report No.4 May 28, 1956.

50. Mosna, E., Rivosecchi, L. and Aacher, K.R.S. (1958): Bull.Wld.Hlth.Org., 19, 297-301.

51. Mosna, E. Falmieri, C., Ascher. K.R.S., Rivosecchi, L. and Neri, I. (1959): Ibid., 20, 63-64.

52. Pal, R. (1958): Ind.J.Mal., 12, 383-390.

53. Pal, R. and Singh, N.N. (1958): Ibid, 12, 499-516.

54. Patel, T.B., Rao, T.R., Halgari, A.V. and Deobhanker, H (1958): Ibid 12, 367-370.

55. Patel, T.B., Rao, T.R. and Ambawani, G.J. (1961): Ind. J.Mal., 15, 71-90.

56. Perry, A.S. (1966): Mosquito News, 26, 301-309.

57. Pillai, M.K.K. and Brown, A.W.A (1965): J.Econ.Ent., 58, 235-240.

58. Pialon, D.P. (1952): Canad. J.Zool, 30, 375-377.

59. Rishikesh, N. (1959): Bull.Wld.Org, 20, 47.

60. Rozeboom, L.E. and Hobbs, J. (1960): Ibid, 22, 587-590.

61. Sharma, M.I.D. and Kalra, R.L. (1958): Ind.J.Mal., 12, 93-100.

62. Singh, N.N. (1961): Biometric study of Insecticide Resistance Phenomenon in Insect with special reference to mosquitoes A dissertation in lieu of practical papers on genetics and Probit analysis of the statistician's Diploma Examination of the Indian Statistical Institute, Calcutta.

63. Singh,N.N. and Mohan B.N. (1965): Ind.J.Gent. Pl.Breed., 25, 279-290.

64. Spielman, A and Kitzmiller, J.B. (1967): In: Genetics of Insect vector of Diseases - Edited by J.W. Wright and R.Pal. Elsevier Publishing Co.,Amsterdam.

65. Swanson, C.P.; Merz, T. and Young, W.J. (1967): Cylogenetics: Princtice - Hall Foundations of Modern Genetics series.

66. Tadano, T and Brown,A.W.A. (1967): Bull.Wld.Org., 36, 101-111.

67. Tattersfield, F. Kerridge, J.R. and Taylor,J. (1953): Ann. Appl. Biol.40, 498-522.

68. Thomas, V. (1966): J.Econ.Ent., 59, 779-786.

69. Tsukamoto, M. (1963): Botyu - Kagaku, 28, 91-98.

70. Waldinton, C.H. (1966): Proc.Roy.Soc. (Series B)., 164, 219-229.

71. Wieb, G.A. (1968): Genetics Today: Proc. XI Int.Cong. Gent. Pergamon Press. Oxford,London.

72. Wiesman,R. (1947): Mitt Schweiz.Ent.Gen., 20, 484-504.

73. Weyer, F (1957): WHO.Mal.Soc.Progress Report No.3, May 13, 1957.

74. Yasutomi, K. (1956): Jap.J.Sanit. Zool., 1, 87-93.

VII GLOSSARY

Alleles	: Members of a pair of a unit of heredity e.g. R and S controlling resistance-susceptibility of insect to insecticide.
Autosomes	: Sets of chromosomes not involved in the control of sex of an insect.
b	: Symbol for expressing regression coefficient. It is inversely proportional to the variance of tolerance distribution over doses of insecticide in probit and log units respectively.
BIP	: Biparental progenies -- the third generation obtained from inbreeding of males and females of F_2 generation. The choice of a male partner is given to the female mosquito. A female mosquito once inseminated by a male will, usually, not be reinseminated in her life time.
Character	: It is applied to any heritable property of an organism in regard to which similarities or differences are recordable between individuals.
Chromosome	: It is an organised system of genes. A given system of organisation of the genetic units in making up a chromosome have a high selective value from the beginning of its existance.
Chromosomal inversion	: Change in the stable order of arrangement of genes e.g. from stable order ABCD to inverted order ACBD.
Crossing over	: Mutual exchange of segments within a pair of homologous chromosome.
Correlated selection forces	: Existance of correlation (+ve or -ve) between the resultant effects of two or more selecting forces.
Correlated response to selection	: Selection is aimed at for a character A but character B, is selected concomitantly. B is thus a correlated response to selection.
Cross resistance	: Selection by one member of a chemical group of toxicant results in extension of resistance to other member toxicants of the group e.g. extended resistance to methoxychlor due to selection by DDT.

Degree of resistance : It is the ratio of LD_{50} of the candidate population to the LD_{50} of the reference population (or base line LD_{50}). It is a measure of relative increase in resistance of a population.

Determinant : Heritable units (hypothetical) that determines resistance/susceptibility to insecticides.

Directional Selection : It is the selection that acts against (or in favour of) any one tail of the distribution of tolerance to insecticide. The result of such successful selection will cause a shift of the mean towards an optimum value.

F_1 : First generation obtained by crossing female P_1 X male P_2 or female P_2X male P_1. P_1 and P_2 are parents.

F_2 : Second generation obtained by inbreeding (F_1XF_1) F_1 generation.

Free genetic variance : It is the ratio of means of the susceptible and resista homozygotes, LD_{50} of RR $\div$ LD_{50} of SS.

Gene : A stable ultimate unit of heredity e.g. the element that controls the susceptibility-resistance to a given insecticide.

Genotype : An individual's genetic constitution.

Heterozygous : When the members of a pair of alleles are unlike the individuals are called heterozygous e.g. RS/SR.

Homozygous : When the members of a pair of alleles are like the individuals are called homozygous e.g. RR or SS.

Intensity of Selection : It is a measure of the severity of selection force expressed as LD_{10}, LD_{50}, LD_{90}.....

Insecticide Specific resistance : Resistance to a specific insecticide e.g. resistance to DDT or BHC. When resistance is to a specific group of insecticides it is called group specific resistance. e.g. resistance to DDT group of insecticides, resistance to CH (chlorinated hydrocarbon groups of insecticides) and OP (organophosphorous group of insecticides).

Isomer specific resistance	: Resistance to a specific isomer of an insecticide e.g. resistance to p,p' DDT or o,p' DDT.
LD_{50}	: Dose of insecticide expected to cause 50 percent mortality in a population from which groups of insects were taken for tests. It is an estimate of mean response rate of a population.
Linkage	: It is co-transmission of genes present in the same chromosome.
m	: LD_{50} in log unit.
Mendelian factor	Hypothetical factor determining the qualitative heritable character. Its transmission follows Mendelian Laws.
Major factor	: Genetical factor having major contribution in determining a character.
Multi resistance	: Resistance extending over more than one group of insecticides e.g. resistance extending over DDT and BHC groups of insecticides.
Multi-stage resistance	: Resistance extending over more than one stage of lifecycle of an insect species e.g. resistance in both adults and larvae of mosquitoes.
Non-specific resistance	: Resistance not specific to any insecticide - it extends over to all injurious chemicals and some times to ecological stress and strain.
Phenotype	: It refers to the appearance of an individual without having any regard to the individuals genetic constitution - e.g. RR and RS genetypes exhibiting resistance to say DL.
Potential genetic variance	: Hidden variance due to a given state of organisation of the genealleles e.g. $\frac{RSRS}{RSRS}$ or $\frac{RRRR}{SSSS}$ where contributions of R and S alleles towards resistance -- susceptibility to an insecticide are equal in magnitude but opposite in sign (+ & -), R contributing towards resistance and S contributing towards susceptibility. Free genetic variance is released from potential genetic variance through crossing over in heterozygotes (RS).

Pleiotropy	: One (set of) genetic factor(s) having effect simultaneously on more than one character in the off-srping.
Polygenes	: Large number of chromosomal genes, occuring in a system, each having very small effect but they may act together to produce big differences. They, usually, are responsible for quantitative character. Mendelian principls are applicable in their transmission.
Quantal Response	: Characteristic response (say kill) to a dose of insecticide (stimulus) is either present or not present.
Resistance	: Ability to tolerate significantly higher doses of insecticide. It is measured by R where R= LD_{50} of candidate population $\div$ LD_{50} of reference population or base line LD_{50} value. R is greater than 4.
Response to selection	: The effect of selection in the population of subsequent generations as measured by the increase (decrease) of the frequency of gene(s) controlling the character under observation.
Selection	: An action in which the insecticide susceptible genotype or phenotype leaves (or is permitted to leave), fewer progeny than the resistant genetype or phenotype.
Stabilising Selection	: Selection that acts against both tails of the distribution of a numerical character e.g. against highly susceptible and resistant types.
Segregation	: Clean seperation of the members of a pair of alleles from each other when germ cells (sperm and o are formed in an individual.
Sex Chromosome	: A pair of chromosome that contains the determinants for or that determines the male and female sexes of an insect.
Tolerance	: Increased ability (less than 4 times) to tolerate higher dose of an insecticide. It may or may not have a genetic basis.
Vigour tolerance	: Non-specific tolerance (less than or equal to 4 times) extending over large number of insecticides and also ecological stress and stran. It is polygenic.
Vector	: A strain of arthropod species in which a species of pathogene or parasite survive and under go biological development required for completion of its life cycle.

VITA

PERSONAL:

Name : Narendra Nath Singh

Date of Birth : December 1, 1926

Address : BA/13A, Ashok Vihar, Delhi – 110052

Field of Interest : Bio-Statistics and Genetics.

EDUCATIONAL:

Degree/Diploma/ certificate	Subjects	University/ Institutes
1. Statistician's Diploma	General: 1. Official and Descriptive & numerical analysis. 2. Probability Theory & Statistical Methods. 3. Sample Surveys & Design and Analysis of experiments. Applied Statistics: 1. Demography, Vital Statistics. 2. Educational Statistics and Psychometry. Specialisation: 1. Genetics. 2. Probit-analysis.	INDIAN STATISTICAL INSTITUTE, CALCUTTA-35
2. Certificate Course in Biometric Techniques (Post-graduate Course)	1. Statistical Methods 2. Medical & Health Statistics 3. Vital Statistics and Demography 4. Health & Nutritional Surveys 5. Epidemiology.	ALL INDIA INSTT OF HYGIENE AND PUBLIC. HEALTH CALCUTTA
3. Certificate in Vector Genetics.	Different aspects of Vector Genetics including Genetic Control.	UNIVERSITY OF NORTRE DAME, INDIANA, U.S.A.
4. Certificate in Malariology	Epidemiology, Parasitology, Entomology, Survey and Control of Malaria	MALARIA INSTITUTE OF INDIA, DELHI-6
5. Computor Programme	FORTRAN IV LANGUAGE	DATAMETRIC CORPORATION, DELHI.

WORK EXPERIENCES:

November 1945 to January 1948:
(At the Antimalaria office, Kanpur Cantt., Defence Ministry, Govt. of India)

1. Control of malaria in Defence Establishments and in Cantt. area in Kanpur, U.P.

March 1948 to February 1956:
(At the Antimalaria office Tarai Colonization Scheme, Govt. of Uttar Pradesh)

1. Control of malaria and its concurrent evaluation in Nainital Tarai area of U.P.

2. Field Research/studies on ecology and behaviour of vector mosquitoes in Tarai area.

3. Field Trial of different methods of malaria control and development of an efficient and economic method of control.

February 1956 to December 1975:
(At the National Institute of Communicable Diseases, Ministry of Health, Govt. of India).

1. Bioassay of drugs (insecticides and antimalarials) and their formulations.

2. Research in the fields of ecology, genetics and vector and disease control using biometric methods.

3. Development of sampling design for filariasis survey and their field trials.

4. Concurrent assessment of the National Filaria Control Programme.

5. Field investigation on some insect borne epidemic and endemic diseases and development of methodology for their control.

6. Writing of Scientific reports, notes and papers.

7. Teaching of Bio-Statistics to the participants of the courses in Malariology, Epidemiology and Advanced Medical Entomology.

December 1975 to Date:
(At the Central Statistical Organization, Ministry of Planning, Govt. of India).

1. Participation in the implementation of different Training Courses in Official Statistics and related methodology organised by the Central Statistical Organisation.

PUBLICATION:

Published, as coauthor, 50 scientific papers/reports/notes pertaining to medical entomology, chemotharapy, vector genetics and epidemiology of insectborne diseases. List of publication is annexed.

PARTICIPATION IN SEMINARS AND CONFERENCES:

1. International Seminar in Vector Genetics organised by the W.H.O. and the University of Notre Dame, Indiana, USA(1968).

2. International Seminar on Integrated Pest Control held at the Indian Agricultural Research Institute, New Delhi(1969).

3. National Filaria Workers Conference held at the National Institute of Communicable diseases, Delhi (1971). I served as a secretary of a sub-committee on 'Assessment and Mechanism of Evaluation of Results.

MEMBERSHIP OF PROFESSIONAL SOCIETY:

1. Member of the Indian Society of Genetics and Plant Breeding.

LIST OF PUBLICATIONS

The publications consist of scientific papers/notes and reports. The reports, marked by *, contain observational/experimental data without detailed analysis and with broad conclusions. They were published in the Annual Reports of the National Institute of Communicable Diseases (NICD).

The informations presented in the publications pertain to number of special subjects and they were collected by using different techniques. The subject(s) of each publication and the technique(s) used in each study were indicated by code number against each publication. The key for the code number is given below:

Subject	Code No.	Technique(s) used in the studies	Code No.
Bio-assay of drug/insecticide	B	Physical	1.
		Chemical	2.
Chemotherapy	Ch.		
Ecology and extinction/ Evolution of species/strain	E	Bio-assay	3.
Vector Genetics	G	Entomological Tech.(s) of population study in field	4.
Insect Colonization	IC	Toxicological/Susceptibility Test	5.
Psychology of Insects	P	Statistical methods/ inferences	6.
Survey of Resistance/susceptibility status of vectors	R	Breeding/crossing	7.
Toxicology	T	Other biological Tech.(s)	8.
Development of Techniques for quantitative estimation of insecticide resistance - Physiological/behaviouristic	Tech.	Review/Theoretical	9.
Vector Control	Vc.		
Epidemiology	Epi.		

LIST OF SCIENTIFIC PUBLICATION AND REPORTS

Sl.No.	Title of the Publication	Code No.
1.	Studies of Chemical and Photodynamic deterioration of Dichloro-Diphenyl-Trichlorethane (DDT) when applied on solid surfaces by Srivastava, R.S., Chakrabarti, A.K., and Singh, N.N. (1955). Ind.J.Mal.2,27-32.	Vc. 1,2,3
2.	The probable causes of disappearance of A.minimus from the Terai area of Nainital Distt: of Uttar Pradesh by Chakrabarti,A.K., and Singh,N.N.(1957) Bull.Nat.Soc.Mal Mosq.Dis. 5, 82-85.	E. 4,6

3. Result of insecticide suscaptability tests on some anopheline Mosquitoes by Sharma, M.I.D., Krishnamurthy, B.S., Khamre, J.S. and Singh,N.N.(1957) - Bull. Nat. Soc. Mal. Mosq. Dis. 5, 198-199. R,5,6

4. Biometric study on susceptibility of some anopheline r aquities to technical DDT, gamma BHC and Dieldrin by Sharma, M.I.D., Krishnamurthy, B.S. and Singh,N.N. (1957), Ind. J.Mal. 11, 401-414. R,5,6

5. Note on the susceptibility of DDT of houseflies of Delhi and Rajasthan by Sharma, M.I.D., Krishnamurthy, B.S. and Singh,N.N. (1958) - Ind.J.Mal.12, 203-207. R,5,6

6. Inheritence of DDT resistance in C.p.fatigans by Pal, R and Singh,N.N.(1958) Ind. J.Mal.12, 499-516. G,5,6,7

7. A note on the study of morphology, prevalence and host preference of an ecocline of A.fluviatilis in Nainital Terai (U.P. - Rehman, J., Singh,M.V. and Singh,N.N. (1960) - Bull. Ind.Soc.Mal.Com.Dis. 8, 137-142. E,4,6

8. A note on a technique for quantitative estimation of susceptibility of bed bugs to contact insecticides by Sharma, M.I.D. and Singh,N.N. (1960), Ind. J.Mal 14, 311-327. Tech.,5,6

9. Studies on susceptibility of Pediculus Humanus Corporis DEG to DDT, gamma BHC and Pyrethrum by Sharma, M.I.D., Mohan,B.N. and Singh,N.N. (1961), Ind.J.Mal.15, 139-147. R,5,6

10. *Residual activity of DDT formulation with glue and potassium dichromate by Krishnamurthy, B.S. and Singh, N.N. (1961) Annual Report MIT. p.41-42. B,5,6

11. Further observations on the technique for quantitative estimation of susceptibility of bedbugs to contact insecticides by Sharma, M.I.D., Mohan, B.N. and Singh, N.N. (1962) Ind. J.Mal. 16, 1-16. Tech.P,5,6

12. A toxic reaction in male rat-fleas (Xenopsylla cheopis) and (Xenopsylla astia) exposed to DDT, gamma BHC and Dieldrin-protrusion of the intromittent organ by Mohan, B.N., Krishnamurthy,B.S., Joshi, G.C. and Singh,N.N.(1962) Ind. J.Mal 16. 143-152. T.5,6

13. DDT resistance in A.culicifacies Giles, 1901 and A. annularis Van Der Wulp. 1884 in a village of Meerut Distt.: U.P. by Krishnamurthy, B.S., and Singh,N.N. (1962) Ind. J.Mal. 16. 375-377. R.4,5,6

14. Evaluation of the WHO modified irritability test kit for mosquitoes by Krishnamurthy, B.S. and Singh, N.N. (1962) W.H.O. Expert Committee on Insecticides, Vector Control and Insecticide resistance - WHO/INS/13-Working Paper No.5-14.8 Nov. 1962. — Tech.F.5,6

15. Investigation of Plague in Kolar Distt: (Mysore State) Part II Rats and rat fleas by Krishnaswamy, A.K. Krishnamurthy, B.S., Ray, S.N., Singh, N.N. and Chandrahas, R.K. (1963). Ind.J.Mal. 17. 193-203. — R.4,5,6

16. A simple technique for rapid sepration of mosquite pupae by sudden chilling, by Ramakrishnan, S.P. Krishnamurthy, B.S. and Singh, N.N. (1963) - Symposium on colonization of insects held in Florida, USA under the World Health Organisation. Ind.J.Mal. 17. 119-121. — I0.1,6

17. *Effect of Sex-ratio and number of Female and Male on the successful mating in C. fatigans and Ae. aegypti by Krishnamurthy, B.S. and Singh, N.N. (1964). Annual Report NICD. p.43-44. — P.6,8

18. *Evaluation of Bromphos as an insecticide against C.fatigans, Ae. aegypti, A.subpictus, X. chopis and M.d.vicina-nebulo, by Krishnamurthy, B.S. and Singh, N.N. (1964). Annual Report NICD, p.49-50. — B.5,6,7

19. *Selection of Baytex-Resistant strain of houseflies (M.nebulo vicina). by Krishnamurthy, B.S. and Singh N.N. (1964). Annual Report NICD, p.56-57. — G.5,6,7

20. *Effect of larval density and food on development C.fatigans by Krishnamurthy, B.S. and Singh, N.N. (1964) Annual Report NICD, 1964, p.40. — E.6,7

21. *Effect of low temperature on the larvae of C.fatigans by Krishnamurthy, B.S. and Singh, N.N. (1964). Annual Report NICD p.41. — E.1,6

22. *Percentage of non-hatchability of eggs in the colony strain of C.fatigans, by Krishnamurthy, B.S. and Singh, N.N. (1964). Annual Report NICD p.41. — G.6,8

23. A study on the joint action of Diamino-Diphenyl Sulphone (DDS) and Pyrimethamine against Plasmodium gallinaceum and Plasmodium cynomolgi (Bastianelli). Potentiation between the two drugs by Basu, P.C., Singh, N.N. and Nawrihal Singh (1964). Bull. Wld. Health. Org. 31. 699-706. — Ch.5,6,8

24. Reconnaissance survey of Aedes mosquitoes in Delhi by Krishnamurthy, B.S., Kalra,N.L., Joshi,G.C. and Singh,N.N. (1965) - Bull. Ind. Soc. Mal. Com. Dis., 2, 56-67. E.4,6

25. *Investigations on intra and inter-species competition amongst mosquito larvae. Krishnamurthy,B.S.,Singh,N.N. and Surinder Kaur (1965) Annual Report NICD 1965-66, p.136. E.6,8

26. *Effect of larval development on the pH value of the media. Krishnamurthy, B.S., Singh,N.N. and Surinder Kaur (1965) Annual Report NICD, p.136-137. E.2,6

27. *Studies on Sex drive:- Part I - Investigations in respect of ratios and number of different sexes on a successful mating in C.fatigans and Ae. aegypti. Krishnamurthy,B.S., Singh,N.N. and Verghese, A.K. (1965-66) Annual Report, NICD, p.137. P.6,8

28.* Studies on Sex drive Part II- *Effect on age on mating drive. Krishnamurthy,B.S., Singh,N.N. and Verghese,A.K. Annual Report NICD, (1965-66) p.137-138. P.6,8

29. *Study of the comparative susceptibility of C.fatigans larvae, colony strin (v/c), Field strain (Karkarmanden) and Fresno-strain to DDT, BHC and dieldrin. Krishnamurthy, B.S. and Singh,N.N. Annual Report (1965-66) NICD, p.143. R.5,6

30. *Laboratory evaluation of Methyl Bromophos (Nexition) 25% W.D.P. and folithion 25% emulsion concentrate. Krishnamurthy,B.S., Joshi,G.C. and Singh, N.N.. Annual Report NICD (1965-66). p.147. R.3,6

31. **Studies on selection and inheritance of insecticide resistance in A.stephensi, Part I Selection of DDT Resistance strain in the laboratory by Mohan,B.N. and Singh,N.N. (1965). Ind. J.Gent. and Plant Breeding, 25, 266-278. G.5,6,7

32. **Studies on selection and inheritance of insecticide resistance in A.stephensi Part II Inheritance of resistance to DDT by Singh,N.N. and Mohan,B.N. (1965). Ind. J.Gent. and Plant Breeding 25, 279-290. G.5,6,7

33. *Estimation of resistance potential in C.fatigans of Delhi (sprayed village) against malathion and pyrethrin. Krishnamurthy, B.S., Singh,N.N. and Surender Kaur. Annual Report NICD (1965-66) p.138-139. G.5,6,7

** Papers were adjudicated as "the best contribution in the field of genetics" for which the authors were awarded the Indian Journal of Genetics Medal.

34. *Population dynamics of mosquitoes under varying ecological conditions. Raghavan,N.G.S., Wattal,B.L. Kalra,N.L., and Singh,N.N. Annual Report MICD (1967) p.72-73. E.4,6

35. *Evaluation of trapping devices for sampling mosquito populations of malaria vectors in flight. Wattal,B.L. Kalra,N.L., Kaul,H.N. and Singh, N.N. Annual Report MICD (1967) p.87-91. E.4,6

36. A note on DDT resistance in Anopheles culicifacies giles in Terai and Bhabar Tract of District Nainital, Uttar Pradesh by Wattal,B.L., Singh,N.N., Kalra,N.L. and Bihari,R.C. (1967) Bull. Ind.Soc.Mal.Com.Dis.4. p.373-374. R.5,6

37. *Studies on the susceptibility of insects of public health importance to insecticides. Wattal B.L., Bhatnagar, V.N, Joshi, G.C. and Singh,N.N. Annual Report MICD (1967) p.101-102. R.5,6

38. Genetic basis of differential susceptibility of A.aegypti to Dirofilarial infection: Part I-Preliminary observations on selection of Aedes aegypti strains susceptible and refractory to Dirofilaria immitis infection by Raghavan, N.G.S., Das, M., Mammen,M.L., Singh,N.N. and Wattal,B.L. (1967). Bull. Ind.Soc.Mal.Com.Dis., 4. 318-323. G.6,7,8

39. *Detection, Isolation and Establishment of morphological mutations in A.stephensi. Bhatnagar,V.N., Singh, N.N. and Wattal, B.L. Annual Report MICD, (1967) p.108-110. G.7,8

40. Concept, scope & feasibility of inegrated Pest Control against Arthropods of Public Health Importance by Raghavan N.G.S., Wattal, B.L., Das, M., and Singh,N.N. (1970) Proc. Int. Semi. Integrated Pest Control held in Delhi (1962) Jour. of Com. Dis. 7-15. VC.9

41. The Building Blocks for an integrated Pest Control Programme in Public Health, by Raghavan, N.G.S., Singh,N.N., Das,M., and Wattal,B.L. (1970) Proc. Int. Semi-Integrated Pest Control held in Delhi (1969) Jour of Com.Dis. 16-24. VC.6,9

42. Eco-Genetical Basis for Integrated measures against Culex fatigans by Wattal,B.L. and Singh,N.N. (1970). Proc. Int. Semi. Integrated Pest Control - held in Delhi in (1969) Jour. of Com. Dis. 2, 46-52. VC.9

43. Time table for integrated control measures against Culex pipiens fatigans in India by Wattal,B.L., Kalra, N.L. Kaul, H.N. and Singh,N.N. (1969), Proc. Int. Semi. Integrated Pest Control. VC.9

44. *Inheritance of refractoriness of Aedes aegypti to Dirofilaria immitis infection by Raghavan,N.G.S., Das,M. Singh,N.N. and Wattal,B.L. Annual Report NICD (1969). p.87-88. G.7,8

45. *Effect of withdrawal of selection pressure on refractoriness of Ae. aegypti to D.immitis infection by Raghavan,N.G.S. Das,M, Singh,N.N. and Wattal,B.L.Annual Report NICD. (1969) p.88. G.7,8

46. *Studies on mutant markers:-

I Inheritance of WHITE EYE in Ae. aegypti
II Inheritance and interaction between RED EYE and WHITE EYE
III Distortion of Sex ratio in different genotypes
IV Search for new mutants of Ae. aegypti
V Susceptibility of some markers to filaria infection
VI Isolation, Selection and inheritance BLACK and WHITE occiput mutants of Ae. aegypti. by Drs. Raghavan,N.G.S., Wattal,B.L., Singh,N.N. and Das,M. Annual Report NICD (1969), p.90-97. G.6,7,8

47. *Selection of D.repens susceptible and refractory strains of Ae aegypti by Raghavan,N.G.S., Wattal,B.L., Singh,N.N. and Das,M. Annual Report NICD (1970) p.92. G.7,8

48. *Studies on mutant marker in Ae. aegypti G.7,8

I Search for new mutants in Ae. aegypti
II Selection of PINCER mutant.
III Selection of BLACK TARSI mutant.
IV Inheritance and Dominance of PINCER and BLACK TARSI characters by Singh,N.N., Das,M, and Wattal,B.L. Annual Report NICD (1970) p.93-98.

49. Epidemiological study of an Epidemic of Japanese Encephalitis in Bankura District of West Bengal during-1973, by Arora,R.R., and Singh,N.N. (1974). J.Com.DIS.6, 310-319. Epi.6

50. A Sample Design for Filariasis Survey and the Results of its Field Trial by Aikat,T.K., Sen,Tapan and Singh,N.N. (1974). J.Com.Dis.6, 284-289. Epi.6