STUDY ON THE EFFECT OF BIOTIC AND PHYSICAL FACTOR MALATHION ON THE FITNESS AND LIFE CYCLE OF DROSOPHILA

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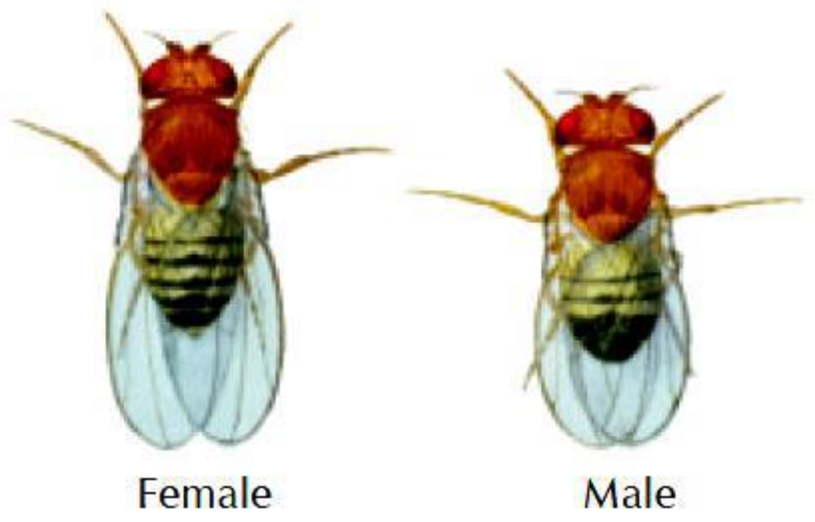
ABSTRACT:

Our current work under investigation aims at the study of the effect at the fitness history and the life cycle of Drosophila. For the purpose of studying the effects of different types of stress factors on stress factors or environmental pollutants, malathion is taken as our study material because of its wide range of applicability in diverse areas.The compound $C_{10}H_{19}O_6PS_2$ had been reported by different scholars as mutagenic in different studies under different conditions .It was found in the study that malathion is more toxic than indosulphan and male adults are more sensitive than females for both insecticides.It was also found to have a positive correlation among resistance to organophosphates and negative correlation between resistance to each of organophosphates malathion,prothiophos and fenitrothion.However,no attempt has been made to study the effect of this compound on the various developmental stages In our current study,therefore,attempt has been made to have a clear understanding of the relationship between toxicity and mutagenicity,various stages in life cycle and relative sensitivities of inbred ,outbred and different mutant strains . Drosophila is a small fly from the family Drosophilidae and its members are often called “FRUIT FLIES”. The entire genus, however, contains roughly 1,500 species and have wide diversity in terms of appearance, behavior, and breeding habitat. One species of Drosophila in particular *D. melanogaster*, has been heavily used in research in genetics and is a common model organism in developmental biology. Basic genetic mechanisms are shared by most organisms. Therefore, it is only necessary to study the genetic mechanisms of a few organisms in order to understand how the mechanisms work in many organisms, including humans. *Drosophila melanogaster*, the fruit fly a little insect about 3mm long, is an excellent organism to study genetic mechanisms. The general principles of gene transmission, linkage, sex determination, genetic interactions; molecular, biochemical and developmental genetics, chromosomal aberrations, penetrance and expressivity, and evolutionary change may all be admirably demonstrated by using the fruit fly *Drosophila melanogaster*. Discrete genes regulated different aspects of development. Many of these genes turned out to be homologous to those involved in human development and disease. These genes had been conserved over millions of years of evolution and could be studied easily and rapidly in flies. This led to a boom in the field as more and more researchers saw the potential of flies for asking basic and applied questions, and to the development of ever cleverer molecular tools to address these questions. For example, chemical mutagenesis was used for many years to generate new mutations that were screened for interesting phenotypes, followed by careful genetic mapping, a chromosome walk, and finally gene cloning . Currently, the MiMIC transposon system is being applied to target all genes in the *Drosophila* genome, providing null mutations and a platform to land protein tagging, gene expression tracking.

KEY WORDS:
Drosophila, Melanogaster, Malathion, Mutation, Prothiophos.

INTRODUCTION:

Drosophila derived from the Greek word drósos meaning dew loving. *Drosophila melanogaster* is a fruit fly, of the kind that accumulates around spoiled fruit. It is also one of the most valuable organisms in biological research, particularly in genetics and developmental biology. Basic genetic mechanisms are shared by most organisms. Therefore, it is only necessary to study the genetic mechanisms of a few organisms in order to understand how the mechanisms work in many organisms, including humans. *Drosophila melanogaster*, a little insect about 3mm long, is an excellent organism to study genetic mechanisms. The general principles of gene transmission, linkage, sex determination, genetic interactions; molecular, biochemical and developmental genetics, chromosomal aberrations, penetrance and expressivity, and evolutionary change may all be admirably demonstrated by using the fruit fly. *D. melanogaster* and its hundreds of related species have been extensively studied for decades, and there is extensive literature available . The extensive knowledge of the genetics of *D. melanogaster* and the long-term experimental experience with this organism together with extensive genetic homology to mammals has made it of unique usefulness in mutation research and genetic toxicology. Many Drosophila genes are homologous to human genes and are studied to gain a better understanding of what role these proteins have in human beings . Much research about the genetics of Drosophila over the last 50 years has resulted in a wealth of reference literature and knowledge about hundreds of its genes. It is an ideal organism for several reasons: 1) Fruit flies are hardy with simple food requirements and occupy little space. In the current study it has been emphasized how *Drosophila* is currently being used, and what directions they think the system is moving in. From human disease modeling to the N-dissection of cellular morphogenesis and to behavior and aging, this work examines the current uses of flies, and the influence of fly research on other models.



Fig(1): Male and Female adult *D. melanogaster*

Methodology of Fitness in Drosophila

The concept of fitness has played a key role in the development of evolutionary biology as a discipline despite fundamental disagreement over what it means and how it should be measured. Recent investigations have served to corroborate the admonition of that it can be misleading to attempt to infer total fitness from individual components of fitness. For example, viability alone has been shown to be a poor indicator of fitness [and the simultaneous study of viability and fertility has proved unsatisfactory [6, 9] largely because of pleiotropic effects . Sexual selection, a component usually not distinguished from fertility, has been shown to be important to fitness . Moreover, the conditions under which fitness is estimated (such as density and temperature) can influence the results obtained . Clearly then, any study of fitness must include as much of the life cycle as possible. The assessment should be done, at least initially, under uniform environmental conditions. Also, one must have an operational definition of fitness, if only for comparative purposes. Lastly, these desires must be fulfilled within a manageable experimental regime. We have chosen to examine several experimental techniques that have been devised for estimating total or net fitness in *Drosophila melanogaster*. Because these are estimates of total or net fitness encompassing at least one complete generation, they can satisfy the above-mentioned conditions while avoiding the problems of component analyses. These techniques all operationally define fitness in terms of competitive ability, or reproductive success under competitive conditions. They are relative measures in that they assess the fitness of a strain or population relative to some standard. We treat the terms “strain” and “population” as interchangeable from an experimental point of view. The set of *D. melanogaster* strains subjected to these analyses include lines homozygous for chromosome 2, lines heterozygous for chromosome 2, wild type lines of varied geographic origin and lines that have been sib-mated for several generations. By subjecting the same set of strains to each of these techniques, comparisons can be made in an effort to determine what is being measured and if the same thing is being measured inthese types of analyses. Although the net parameter measured in each of these techniques is referred to as "fitness", at least for the strains tested they are not necessarily measuring the same thing.

Life Cycle of Drosophila: Stages and duration

Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membrane. The egg produces larva, which eats and grows and at length becomes pupa. The pupa, in turn develops into an imago or adult. The duration of these stages varies with the temperature. At 20 ^{0}C, the average length of the egg-larval period is 8 days; at 25 ^{0}C it is reduced to 5 days. The pupal life at 20 ^{0}C is about 6.3 days, whereas at 25 ^{0}C is about 4.2 days. Thus at 25 ^{0}C the life cycle may be completed in about 10 days, but at 20^{0}C about 15 days are required. Drosophila cultures ought to be kept in room temperature where the temperature does not range below 20 ^{0}C or above 25 ^{0}C. Continued exposure to temperatures above 30 °C may result in sterilization or death and at low temperatures the viability of flies is impaired and life cycle

The Egg **# The Larva #The Pupa # The adult stage (Appearance of sex comb ,External genitalia on sex comb ,Sex organs during larval stage)**

The egg :The egg of *Drosophila melanogaster* is about 0.5 of a millimeter long. An outer investing membrane, the chorion, is opaque and shows a pattern of hexagonal markings. A pair of filaments, extending from the anterodorsal surface, keeps the egg from sinking into soft food on which it may be laid. Penetration of spermatozoa into egg occurs through a small opening or micropyle, in the conical protrusion at the anterior end, as the egg passes through the uterus. Many sperms may enter an egg, through normally only one functions in fertilization. The spermatozoa have been stored by the female since the time of mating. Immediately after the entrance of the sperm, the reduction (meiotic) divisions are completed and the egg nucleus (female pronucleus) is formed. The sperm nucleus and the egg nucleus then come into position side by side to produce the zygote nucleus, which divides to form the first two cleavage nuclei, the initial stage of development of the embryo. Eggs may be laid by the mother shortly after they are penetrated by the sperm, or they may be retained in the uterus during the early stages of embryonic development

The Larval Stages

The larva, after hatching from the egg, undergoes two molts, so that the larval period consist of three stages (instars).The final stage, or third instar may attain a length of about 4.5millimeters. The larvae are such intensely active and voracious feeders that the culture medium in which they are crawling becomes heavily channeled and furrowed .

The larva has 12 segments: the 3 head segments, 3 thoracic segments, and 8 abdominal segments. The body wall is soft and flexible and consists of the outer noncellular cuticula and the inner cellular epidermis. A great number of sense organs are spread regularly over the whole body.

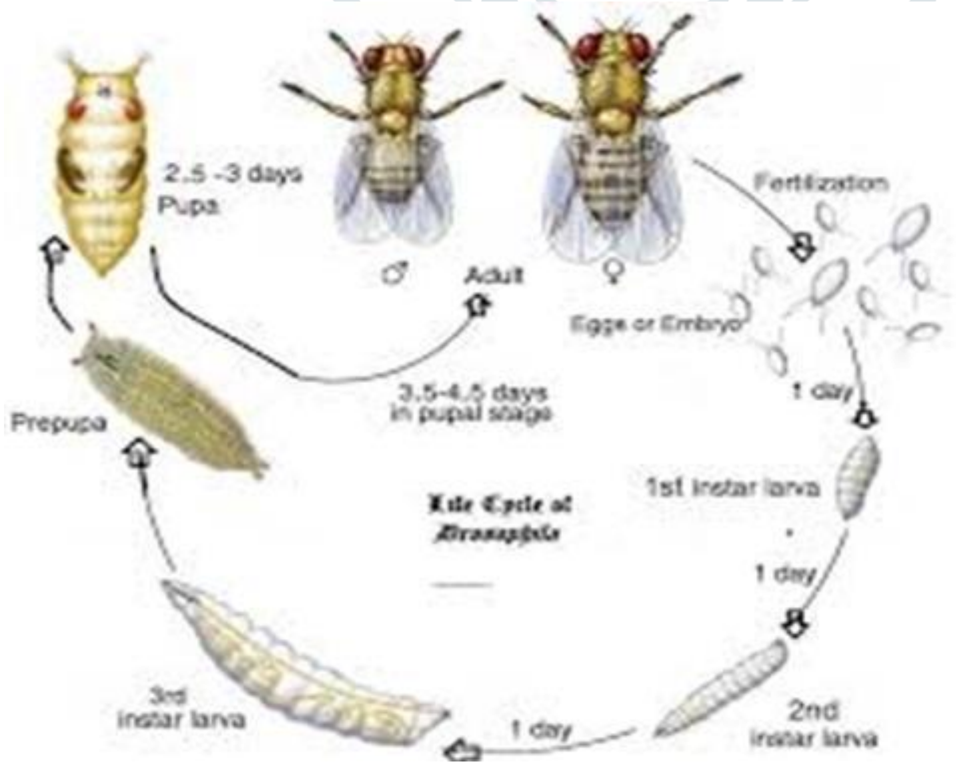
The pupa

A series of developmental steps by means of which the insect passes from the larval into the adult organism is called "metamorphosis". The most drastic changes in this transformation process take place during the pupal stage. Shortly before pupation the larva leaves the food and usually crawls onto the sides of the culture bottles, seeking a suitable place for pupation, and finally comes to rest. The larva is now very sluggish, everts its anterior spiracles, and becomes motionless. Soon the larva shortens and appears to be somewhat broader, thus gradually acquiring its pupal shape. The shortening of the larval cuticle, which forms the case of the puparium, is caused by muscular action. The puparium, which is the outer pupil case, is thus identical with the cuticle of the last larval instar. When the shaping of the puparium is completed, the larval segmentation is obliterated, but the cuticle is still white. This stage lasts only a few minutes and is thus an accurate time mark from which to date the age of the pupa. Immediately after the cuticle reaches the white prepupal stage, the hardening and the darkening of the cuticle begin and proceed very quickly.

Adult stage

When metamorphosis is complete, the adult flies emerge from the pupa case. They are fragile and light in color and their wings are not fully expanded. These flies darken in a few hours and take on the normal appearance of the adult fly . Upon emergence, flies are relatively light in color but they darken during the first few hours. It is possible by this criterion to distinguish recently emerged flies from older ones present in the same c bottle .They live a month or more and then die. A female does not mate for about 10 to 12 hours after emerging from the pupa. Once she has mated, she stores a considerable quantity of sperm in receptacles and fertilizes her eggs as she lays them. Hence, to ensure a controlled mating, it is necessary to use females that have not mated before. These flies are referred to as virgin females.

Sex combs in male fly



Life cycle of Drosophila.

Culture conditions

Timing & Lighting :

Fruit flies are "cold-blooded" so rate of growth and development varies with temperature. The duration of the different stages varies with the temperature. At 20 °C the average length of the egg-larval period is 8 days; at 25 °C it is reduced to 5 days. The flies are attracted to lights. Part of fly courtship behavior is visual, so it is probably a good idea to keep them in an area with good lighting most of the time .

CONCLUSION:

The toxic effect of malathion on the population peak is quite marked in the two mutant strains and between the two wild ones .In the two mutant strains the sensitivity for the peak may be due to the white eye and sepia eye mutant .

The wide known information of the genetics of *D. melanogaster* and the prolonged experimental results with this organism together with extensive genetic homology to mammals has made it of immensely useful in mutation research and genetic toxicology. Many of the Drosophila genes are homologous to human genes and are used to gain a better understanding of what role these proteins have in human beings. Much research about the genetics of Drosophila Metamorphosis Drosophila over the last 50 years has resulted in a wide variety of reference literature and knowledge about hundreds of its genes. Also, the offspring are produced in large numbers which provide statistically significant data and phenotypic mutant changes are easily recognizable under the microscope.

REFERENCES:

1. Demerec M, Kaufman P. Drosophila Guide: Introduction to the genetics and cytology of Drosophila melanogaster. Cold Spring Harbor Laboratory 1996, 4-8.
2. Milislav D. Biology of Drosophila. 10th ed. Cold Spring Harbor Laboratory 1950, 1-2. Available at: http://www.vguk.hr/novosti/akt/231-100.pdf (Accessed March 03 2009).
3. Williams M. UAB center for community outreach development – Drosophila Manual, University of Alabama at Birmingham Press 1998-2000, 2-4.
4. Available at: URL:http.//main.edu/cord/show.asp? dukre (Accessed February 12 2209)
5. Harry D, Mendelian genetics of Drosophila. Cambridge University Press 2001, 7-10.
6. Available at: http://alpha.sci.ege.edu.tr/~genetik/ alpagut/dr.pdf. (Accessed January 10 2009)

7. Marsh JL, Thompson LM, Drosophila in the study of neurodegenerative disease. Neuron 2006;52(1):69-78.

9. Whitworth AJ, Wes PD, Pallanck LJ, Drosophila models pioneer a new approach to drug discovery for Parkinson's disease. Drug Discov Today 2006;11:119-26.

10. Sang TK, Jackson GR, Drosophila models of neurodegenerative disease. NeuroRx 2005;2:438-466.

11. Baylies MK, Bate M, twist: A myogenic switch in Drosophila, Science 1996;272:1481-1484.

12. Bhanot P, Brink M, Samos CH, Hsieh JC, Wang Y, Macke JP, *et al.*, A new member of the frizzled family from Drosophila functions as a Wingless receptor, Nature 1996;382:225-230.

13. Brunner E, Peter O, Schweizer L, Basler K, Pangolin encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in Drosophila, Nature 1997;385:829-833.

14. Chawengsaksophak K, James R, Hammond VE, Kontgen F, Beck F, Homeosis and intestinal tumours in Cdx2 mutant mice, Nature 1997;386:84-87.

15.. Lawrence PA, The genetics of animal design. Oxford: Blackwell Scientific Publications. The making of a fly 1992, 240.

16. Metzendorf C, Lind MI, *Drosophila mitoferrin* is essential for male fertility: evidence for a role of mitochondrial iron metabolism during spermatogenesis. BMC Dev Biol 2010;10:1-17.

17. Ouyang Y, Andersson CR, Kondo T, Golden SS, Johnson CH, Resonating circadian clocks enhance fitness in cyanobacte