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DROSOPHILA TEST SYSTEMS FOR MONITORING GENETIC DAMAGES

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Drosophila melanogaster provides one of the best worked out genetic system among higher organisms. This feature, together with its short life cycle, simple culture conditions and the low chromosome number, makes the fruit fly ideally suited for assessing genetic damages caused by various agents in vivo as well as in vitro. The techniques are simple and provide unambiguous information about the potential genetic hazards posed by the test chemical or other agents. In the present, some basic information about the methodologies for in vivo mutagen studies with Drosophila will be provided; besides the possibilities of in vitro mutagen studies using Drosophila cell and organ cultures will also be considered.

Literature on Biology and Genetics of Drosophila :

The literature on this subject is extensive. Reference numbers 1-3 are some of the invaluable sources of information for beginners in this field. The information on the genetic variations in D.melanogaster upto 1967 has been compiled by Lindsley and Grell (4). This work is now being updated. The annual Drosophila Information Service (DIS), currently being edited by P.W. Hedrick (Div. of Biological Sciences, University of Kansas, Lawrence, Kansas, USA), provides recent information in various fields of Drosophila Cytogenetics since every issue of DIS includes stock lists of various centres, information on new mutations, research and technical notes, recent bibliography and periodically a geographical directory of Drosophila workers. Literature on Drosophila till 1972 has been compiled in six bibliographies (5-10) and subsequent literature is compiled in various issues of DIS. A multivolume series, "Genetics and Biology of Drosophila", edited by M. Ashburner and E. Novitski and being published by Academic Press, provides information on a wide range of topics. The present note is essentially adapted from a paper by Würgler et al (11) in which all the relevant information for Drosophila test systems are nicely described for a beginner in the field.

Biology of Drosophila :

Inseminated adult female D.melanogaster lays eggs on surface of the food source. Eggs are white and about 0.5mm long and are provided with a pair of filaments on anterodorsal surface. Embryonic development which follows fertilization occurs within the egg membrane. Ultimately a larva hatches out of the egg and after 3 larval instars becomes a pupa which in turn develops into an imago or adult. At 24°-25°C, the embryonic development lasts 22-24h, the larval life about 96h while the pupal period lasts about 4.2 days. Thus at 25°C, fresh adults hatch in about 10 days after oviposition. It may be noted that if eggs are not laid immediately after

fertilization, embryonic development may progress while the eggs are still in mother's body. The adult flies may live for several weeks.

Recognizing the sex of the adult fly :

Wild type adult male and female D.melanogaster flies can be distinguished from each other on the basis of the shape of the abdomen tip (elongated in female and somewhat rounded in male), by darker markings on male abdomen and by the presence of "sex-comb" (a row of 9-10 stout black bristles on the distal surface of the basal tarsal joint of the fore-leg) in male. With experience, the sex of the fly may be ascertained without recourse to a microscope, although in doubtful cases the sex must be confirmed by a careful microscopic examination. Larvae can also be sexed from the gonads identifiable through the larval skin.

Handling of flies :

Flies have to be anaesthetised to keep them quite during examination and transfer to culture bottle for mating. A few drops of ether placed in an etherizer anaesthetises a batch of flies and they can be kept in that condition for half an hour or longer by re-etherizing at intervals. However, overetherisation must be avoided since this may prevent further use of the flies and may also hamper with diagnosis of the mutant character. Flies killed by overetherisation extend their wings at an angle of 45°. After examination and recording, the flies that are to be discared should be disposed of in a jar containing light mineral oil or alcohol. No flies should be allowed to escape in air.

Drosophila food :

A variety of recipes for Drosophila food have been suggested. One of the commonly used food preparation contains agar-agar, maize powder, brown sugar, dry yeast and anti-fungal and anti-bacterial agents like propionic acid and "Nipagin" (Na-para-benzoate). Fully cooked and hot-food in semi-fluid condition is poured in 250ml milk-bottles or small glass (or plastic) vials (all sterilized). Upon cooling, the food solidifies. The moisture condensed on the sides of bottles or vials is wiped dry with sterilized cotton wool and the bottles or vials are plugged with sterilized cotton or plastic foam plugs.

Stock Cultures :

Drosophila cultures should be maintained at 24°-25°C; higher temperature is harmful. The stock cultures may also be maintained at a lower temperature (18°-20°C) to need less frequent change of cultures. The experimental matings should generally be maintained at 24°C. Normally a stock culture needs to be changed every 3 or 4 weeks and at least two cultures of each stock should be maintained. For starting a fresh culture, the etherized flies of the required genotypes are put into a fresh bottle or vial. Since the etherized flies may stick to the soft food before their revival, the container should be kept horizontal or upside down till the flies revive.

Collection of virgins :

Sperms received by a female during a mating are retained and these fertilize a large number of eggs. Therefore, for any experimental mating, it is imperative to use "virgin" females. Generally, females do not mate within 6-8h of eclosion and thus if all the old flies in the culture bottle or vial are discarded, all the females collected during the next 6-8h will be virgins. Emergence of adult flies from their pupal cases usually occurs in the morning hours and thus it is convenient to discard all the flies early in morning and then collect all the females that hatch within the next few hours. These virgins may be stored in fresh food vials till they are needed for setting up the cross.

Collection of males :

Collection of males for setting up crosses does not require such stringent time schedule. However, the age of the males used for treatment should not vary widely. It is advantageous to first mate males with their sisters and then separate them for a day before the experimental mating : this helps to get rid of the Ist batch of sperms which show an increased rate of spontaneous lethals (12). As a standard procedure, 3-4 days old males may generally be used for experimental purposes.

Treatment of Adult Flies :

For general screening with test chemicals, it is advisable to use males rather than females since the latter have been found to be generally more readily sterilized by the treatment and show fewer induction of genetic changes (11).

Wild type males are treated with the test chemical by feeding or injection or by topical application or by gas or aerosol application. For water soluble and stable test chemicals, a solution in 5% sucrose is fed for 1 to 3 days (13). If not soluble in water, the chemical may be dissolved in DMSO or ethanol and then diluted with 5% sucrose. The larval stages may also be fed with the test chemical in the same manner. In specific cases, other methods of application (injection, topical application or aerosol) may be employed. For a description and references to some of these methods, see ref. 11.

Brooding :

Different stages of germ cell development may exhibit variable sensitivity to a test chemical and therefore, as a safeguard against a false negative result for a chemical having a pronounced effect on early stages of germ cell development, it is essential to examine different germ cell stages for induced genetic effects. This brooding technique usually consists of mating the treated males at regular intervals (2-3 days) to a succession of 2-3 fresh virgin females. With the knowledge of progression of spermatogenesis, it is possible to correlate each of the successive broods of progeny to specific stages of germ development and thereby, determine the sensitivity of these stages. It should, however, be noted that the successive broods may represent different stages of sperm development in different stocks and this also depends upon mating efficiency and other factors. Thus it may be useful to ascertain the

brooding pattern for a particular setup of the experiment (see ref. 11).

TEST PROCEDURES

For assessing the genetic or other damages caused by a given test substance in vivo, the following test procedure may be applied.

1. Toxicity Test :
The toxicity of the test compound may be ascertained by overnight feeding groups of males on different concentrations of the test compound. After this period, the flies are removed to fresh vials with standard food and live yeast and the number of flies surviving after 24h is counted and compared with that in control vials in which flies fed with identical solutions sans the test compound are kept. In these pilot experiments, relatively higher concentrations of the test chemical should be employed.

2. Sterility Test :
This is slightly more time-consuming than the previous one but is essential for planning further genetic experiments. Groups of males are fed overnight on selected concentrations of the test chemical and are later mated with twice the number of virgin females (any genotype but fertile and untreated). After every one or two days the treated males are removed and mated with fresh virgin females; this brooding is continued until 10-14 days. The females from each culture bottle are removed after 4-5 days. The possibility of partial or complete sterility induced by certain concentrations of the test chemical in certain broods can be easily ascertained by counting the number of flies in different bottles. For comparison, parallel control cultures have to be maintained. If there is some indication of sterility, it may be interesting to test if it is due to genetic or toxic effects and this can be done by applying the dominant lethal test.

3. Dominant Lethal Test :
In this test, the eggs deposited by the females are collected and the proportion of larvae hatching from the eggs or the adults emerging from pupae gives an indication of "lethality". It may be noted that true dominant lethals may also be mimicked by other phenomena like unfertilized eggs etc.
If embryonic lethality is being scored, Würger et al (11) have recommended that the scoring of "dead" eggs should be done between "white" lethal eggs and "brown" lethal embryos since the relative proportion of each type would indicate the nature of the damage : true genetic damages would increase the proportion of "brown" lethal embryos while conditions of unfertilized eggs or non-functional sperms etc would cause more "white" lethal eggs.

Sex-linked Recessive Lethal Test :
This tests for genetic changes that kill hemizygous or homozygous, but not heterozygous individuals somewhere between the zygote and adult stages. The recessive lethals can be induced on all chromosomes but their detection on X-chromosome is easier due to hemizygosity of the X-chromosome in male flies. The hemizygosity also ensures that the X-chromosome being tested does not contain any pre-existing lethals except those which arise during the development of the particular male fly.

Genetically, the sex-linked recessive lethal test in *Drosophila* is based on the fact that among the progeny of females heterozygous for an X-linked recessive lethal, half of the sons will die and thus a comparison of the female to male ratio in the progeny indicates the presence or absence of a sex-linked recessive lethal. This feature of the test makes the objective scoring a rule, particularly more so since in all the laboratories the same genetic strains are used. In these special tester stocks, recombination between the two X-chromosomes of a lethal carrying heterozygous female is prevented by having suitable inversions in the other X-chromosome and this ensures that the lethal induced due to treatment of the parental male is not transferred to the maternal X-chromosome due to recombination.

The most commonly employed test is the *Basc* or *Muller-5* test. Wild type males having normal round red eyes are treated with the test substance and mated with virgin homozygous *Basc* females. The *Basc* chromosome carries genetic markers *Bar* (causing narrow eye shape in homo- or hemizygous condition and kidney-shaped eyes in heterozygous females) and *white apricot* (eyes in homo- or hemizygous flies are light orange in colour); besides the *Basc* chromosome contains a combination of two *scute* inversions which effectively inhibit recombination over the entire length of the X-chromosome. The *Basc* test is diagrammed in fig. 1.

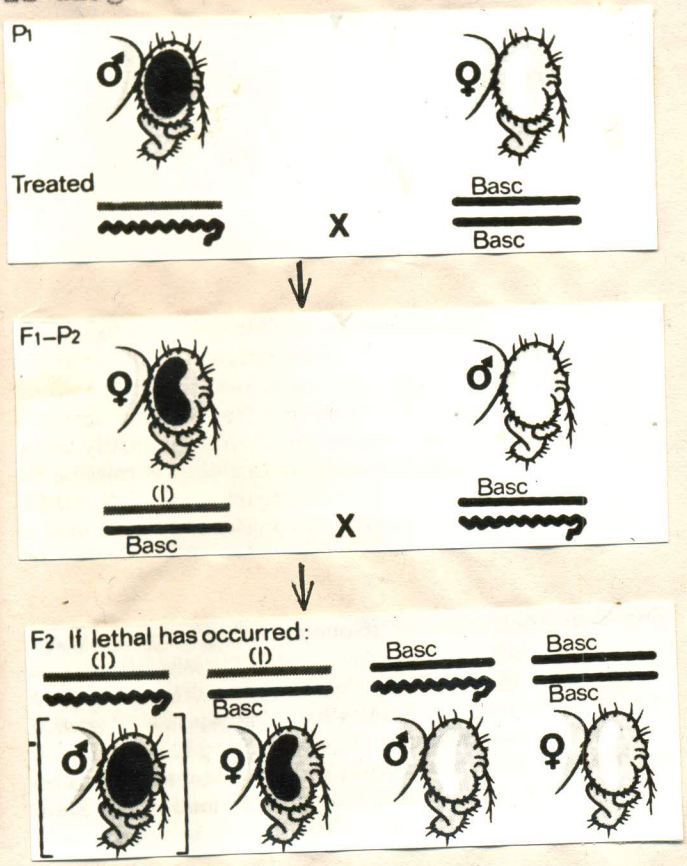

Fig. 1. The *Basc* Test. If a recessive lethal is induced in the X-chromosome of the treated P_1 male, the normal round red eyed males (shown in parentheses) are absent in the F_2 generation.

In the Basc test, it is absolutely essential that for obtaining the F_2 progeny, only one F_1 female is taken in a vial since each F_1 female represents one paternal X-chromosome treated in the male gametes. The vials of the F_2 generation are individually checked for the presence of males with round red eyes. If in a vial containing at least 20 progeny flies, no round redeyed males are found, it may be concluded (with $P \leq 0.05$) that the treated male gamete contained a sex-linked recessive lethal. Since the absence or presence of a well defined Mendelian class can be objectively and unambiguously determined without any personal bias in classification, these tests provide very reliable results.

As discussed by Würgler (14), the Drosophila sex-linked recessive lethal test provides the following types of additional information not obtainable by other in vitro tests :

i. Mutations in vivo germ cells of an eukaryot are detected;
ii. The test chemical is tested in the presence of intracellular xenobiotics metabolising enzymes;
iii. It records point mutations, deletions and aberrations.

In vitro Cytological Test Systems : Potential for future

While Drosophila has been extensively utilized for detecting genetic damage caused by various agents by using in vivo genetic test systems, there have been relatively fewer attempts to utilize the cytological test systems, mainly because of the poor cytological quality obtainable till recently. However, in recent years improvements in cytological techniques have made Drosophila mitotic chromosomes amenable to analysis in a favourable manner. In view of the small chromosome number and the availability of a large variety of genetic and cytological markers, Drosophila mitotic chromosomes should provide a test system of considerable promise and this field needs serious exploration.

Several established cell lines of D.melanogaster are now available and these could be used for mutagen testing in a manner similar to that in mammalian cells. Besides, short-term cultures of early embryonic cells and larval brain ganglia can be easily established and under optimal conditions they give a very good yield of mitotic cells. For these short-term cultures, very stringent sterility conditions are not essential. A variety of simple culture media formulations have been described in the literature for such short-term or cell line cultures.

The potential advantages that these short-term in vitro cultures of Drosophila cells and organs provide for mutagen screening studies are :

i. Relatively simple culture conditions;
ii. Low diploid chromosome number so that chromosomal aberrations can be easily scored; somatic synapsis of homologous chromosomes is also advantageous;
iii. Relatively short cell cycle periods, particularly in the embryonic cells, make the tests rapid;
iv. Use of bromodeoxyuridine-Giemsa technique permits analysis of sister chromatid exchanges also. Since the spontaneous frequencies of SCEs are extremely low (15-17), induced SCEs are easily scorable. This aspect has not yet been examined but appears to be very promising and should be explored.
v. The vast genetic information and the mutant strains available for D.melanogaster can be useful in specifically characterizing the nature of the damage caused by the test chemical.

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