THE USE OF DROSOPHILA MELANOGASTER FOR IN SITU BIOMONITORING

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INTRODUCTION

Drosophila melanogaster was the principal organism used for the development of diploid transmission genetics at the beginning of the century and more recently has been the subject of pivotal studies on eucaryote gene regulation, development and behavior. D. melanogaster is potentially valuable for in situ biomonitoring because it is convenient to test different life stages, it can be used for a multi-faceted analysis of environmental genotoxins and a rapidly expanding base of information on its molecular genetics facilitates the development of new methods for bioassays.

LIFE CYCLE ASSAYS

Drosophila melanogaster is relatively easy and inexpensive to rear which is important for genotoxicity tests that often require large sample sizes. Mass handling procedures and automatic counting devices will become increasingly useful in this regard. The short generation time of the flies means that investigators could rapidly identify health hazards.

With D. melanogaster it is possible to conduct toxicological surveys with different life stages. There are various ways to expose D. melanogaster to toxic compounds. These include adding compounds to fly food, leaving a compound residue in an otherwise empty vial, topical application, injection and absorption of airborne agents. Techniques are also available for mass collection of eggs and larvae (Roberts 1986). Numerous pupal stages can be identified (Roberts 1986) and it is possible to monitor metamorphosis after exposing either larvae or pupae to potentially teratogenic environments.

The adult stage can be used for tests of toxins on male and female reproduction, viability and senescence. It may be useful to determine

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reproductive effects since insect chemosterilants often are mutagens (Borkovec, 1973) and/or carcinostatic agents (Hayes 1968). Sterility can be assessed by the inability of females to lay fertile eggs or males to successfully inseminate females. Effects on female reproductive output (fecundity) can be measured by transferring inseminated females to fresh fly medium daily and counting the number of progeny that emerge from the set of transfers. Male reproductive output (virility) is more difficult to assess. One method is to place a male in a vial containing medium and virgin females. After one day the females can be transferred to individual vials with medium to determine their fecundity. The male can be repeatedly confined with females to measure reproductive output.

Adult mortality is a convenient parameter for in situ monitoring because it is possible to transport large numbers of flies to test sites and the proportion that die after exposure can be quickly determined. A more speculative approach would be to hold adults in suspect environments for several weeks and then examine them for evidence of accelerated aging. In this manner it may be possible to rapidly identify subtle physiological risks in the environment.

Various life stages of D. melanogaster can be used to evaluate terrestrial or aquatic samples from the field. Often contaminated materials are a complex mixture of compounds which are rarely evaluated (Pereira, 1983). Successive solvent fractions of the samples can be tested for cytotoxicity or genotoxicity with different life stages of D. melanogaster to monitor a range of possible biological effects.

GENETICS AND TOXICOLOGY

The widespread availability of commonly used laboratory stocks of D. melanogaster makes it possible to standardize the results of different investigations. In many in situ bioassays the genetic differences between the test organisms used by the same or different investigators are ignored. Yet there may be substantial genetically-based differential sensitivity to the effects of toxins. This makes it difficult to compare results from different studies or to be sure the differences between treatment and control organisms are due only to the environment.

The extensive genetics of D. melanogaster may be useful for toxicology studies. For instance, chromosomes with multiple inversions and a dominant mutation can be used to control the transmission of homologous chromosomes. It would be possible to place chromosomes with high or low detoxication enzyme activity into a common genetic background and thus construct lines with a range of detoxication potential. Such lines could facilitate study of detoxication or activation of toxins. Another possibility is to use low or no activity mutations in detoxication genes as a way of creating genotypes which are more sensitive or less sensitive to the presence of genotoxins or cytotoxins (Harshman et al., submitted manuscript).

There are numerous mutation tests available for D. melanogaster. This includes tests for sex-linked lethals, autosomal recessive lethals, sex-linked lethal mosaicism, recessive visible mutants, sex-chromosome aneuploidy, translocations, position effects produced by chromosome rearrangements and somatic mutations (Abrahamson and Lewis, 1971; Valencia et al., 1984; Zimmering et al., 1986). One D. melanogaster chromosome mutation test alone can detect non-disjunction, chromosome exchange and deletion (Valencia et al., 1984; Zimmering et al., 1986). In contrast, for most organisms chromosome aberrations must be detected by tedious cytological methods. The availability of efficient chromosome mutation tests in D. melanogaster may be particularly significant because half of the

spontaneous human abortions and many birth defects are a result of chromosome aberrations (Sankaranarayan, 1979). Chromosome mutation tests have been used to test volatile genotoxins (Sharkarnis, 1969; Verburgt and Vogel, 1977; Abraham et al., 1979; Zimmering and Kammermeyer, 1983). For assessment of human health hazards D. melanogaster may be particularly valuable when chromosome mutation tests are used to evaluate volatile genotoxins in situ.

A recent advance is to develop chromosome mutation tests in D. melanogaster based on defined molecular changes. The white-ivory eye mutation is a result of a 2.9 Kb duplication of a portion of this gene and reversions are the corresponding deletion. Green et al. (1986) constructed a quadruplication of the white-ivory locus to increase the frequency of reversions. Using this test they found that compounds which specifically promote deletions produce a high mutation rate (Green et al., 1986).

BIOASSAY DEVELOPMENT

There are opportunities to use the extensive genetics of D. melanogaster and molecular biology to increase the utility of this species as an indicator of human health hazards. For instance, the Pelement transformation system of D. melanogaster should prove useful for constructing new indicator strains. It might be possible to transform D. melanogaster with the human TCDD receptor and monitor P450 response for a dioxin bioassay (Jones et al., 1985). In general, it may be possible to construct D. melanogaster lines with mammalian receptors, regulatory elements and detoxication enzymes to parallel the human response to environmental health hazards.

It would be useful to develop mutation tests based on insecticide resistance. One way to employ insecticide resistance might be to expose adults in the field then bring them back to the laboratory for mass collection of eggs. The number of eggs collected could be determined by an automatic counting device or by weight. The eggs would then be transferred onto medium with insecticide and the few individuals surviving could easily be counted to determine a "mutation rate" to resistance per given number of eggs. The progeny of these individuals would also be tested to ensure the resistance is heritable. This type of test would allow the investigator to assess environmental genotoxicity without having to examine a large number of flies.

Induction of detoxication enzymes is a potential biological indicator of exposure to toxic substances. In D. melanogaster it has been possible to indirectly select for induction of a glutathione S-transferase activity (Harshman et al., submitted manuscript). The implication of this work is that it should be possible to select for modified inducibility of detoxication enzyme activities in D. melanogaster and other species to increase the specificity or sensitivity of the induction response.

In summary, D. melanogaster is potentially useful for in situ biomonitoring. In this capacity it may be especially valuable for monitoring volatile genotoxins in the environment. Drosophila melanogaster may also be valuable as a model for the development of new bioassay methods.

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