New Approaches In *Drosophila* Genetics Make It More Useful

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The fruit fly or *Drosophila* is generally recognized as an ideal system for genetic studies. It was introduced to Genetics by T.H. Morgan in 1909. The basic advantages that led T.H. Morgan to introduce *Drosophila* for genetic studies were: (i) short life cycle with a new generation obtained within about 2 weeks; (ii) easy and relatively inexpensive rearing in laboratory; (iii) possibility of controlled mating for crossing between different genotypes and (iv) fewer chromosomes (N = 4). Also it was non-pathogenic. An understanding in the 30's of the structural basis of polytene chromosomes found in several tissues of *Drosophila* provided another immensely useful tool for cytogenetic and later molecular studies. Indeed the versatility of this organism was so great that already by 50's this organism had become genetically the best known eukaryote. However, the introduction of micro-organisms (*E. coli, Neurospora,* yeast etc.) for genetic and other molecular biological studies challenged the pre-eminent position of *Drosophila* and during 60's and early 70's it was beginning to be felt that the fruit fly may no longer be of much use in learning the secrets of cell and its genes. Nevertheless, the advent of recombinant DNA techniques and their rapid application to *Drosophila* not only revived the sagging interest in *Drosophila* genetics but has, in the last two decades, opened up very unorthodox and challenging avenues of studies. The pace of progress in recent years has been extremely rapid so that *Drosophila* is now being increasingly used for traditionally non-genetical cell biological studies.

Life Cycle of *Drosophila*

***Drosophila* is a dipteran (two-winged) fly which like many other insects undergoes complete metamorphosis (holometabolous) and thus within a short period of about two weeks passes through the embryonic, larval and pupal stages, prior to the imago or adult stage which survives for several weeks. It is remarkable that experimental biologists have turned even this rather complex mode of development to their advantage. The flies are reared in laboratory between 18°C and 25°C. The durations of different stages mentioned below apply to the flies grown at 24°C – 25°C on the standard food containing agar, corn-meal, molasses and yeast. Among the many species of *Drosophila*, most of the studies utilize *Drosophila melanogaster* and all description in the following refers to this species unless otherwise indicated.**

Fertilization occurs in the maternal genital tract by stored sperms as the eggs are laid. Embryonic development begins immediately. Very rapid (one division occurring in about 10 min) nuclear divisions ("cleavage") lead to syncitial (multinucleate) stage with nuclei migrating to the peripheral or cortical region of egg cell. Between 1.5 and 2.5h, all the nuclei are arranged as monolayer in cortical cytoplasm (the multinucleate or syncitial blastoderm stage). The only cells present at this stage are the "Pole Cells" derived from nuclei that lie in the "polar plasm" at the posterior end or pole of the embryo. These pole cells are the exclusive precursors of germ cells that would generate gametes in adult. Cellularisation soon occurs all over the embryo by formation of cell membrane around

each nucleus. Thus the syncitial blastoderm changes to cellular blastoderm which undergoes gastrulation and other events of embryonic development. By about 24h of oviposition, the embryo hatches out as a very small 1st instar larva. The larval period lasts for about 5 days and includes two moultings so that three larval instars (1st, 2nd and 3rd instar) are recognized. The third instar stage lasts for about 48h. It is the late third instar stage which is used to observe mitotic (from brain ganglia) or polytene (from salivary glands) chromosomes. About 8h prior to the cessation of larval life, the larvae stop feeding and begin to crawl outside the food (the wandering stage) and then to pupariate (formation of prepupa). This is followed, about 12h after, by pupation. The pupal stage lasts for abut 5 days. When metamorphosis is complete, the imago emerges out of the pupal case. As a consequence of the holometabolous mode of development, the larvae have two sets of cells in their body: (i) the larval cells, which form the functional organs of larva and would histolyse at pupation and (ii) the imaginal cells, which remain in larva as undifferentiated cells that are committed to specific paths of development and would begin to differentiate (as per earlier commitments) with pupal signal to form specific structures of imago or adult. A remarkable feature of these undifferentiated but determined imaginal cells in larval body is that according to their fate in the adult body, they remain discretely grouped. Thus, the cells destined to form different cuticular structures are present in larvae as distinct bags of cells, the imaginal disks: for each adult cuticular structure there is a separate imaginal disk (e.g. wing-disk, eye-antennal disk, labial disk etc.) each of which can be easily, dissected out from larval body due to their distinctive location, shape, size etc. The imaginal disks have contributed immensely to our understanding of processes involved in development and differentiation.

Another remarkable aspect of *Drosophila* larvae is that most of the cells that constitute larval organs (as opposed to the imaginal cells) undergo endoreplication cycles (repeated cycles of chromosome duplication within the same nuclear envelope) with selective under-replication of certain classes of chromosomal DNA sequences. One consequences of the endoreplication cycles is formation of the giant polytene chromosomes containing thousands of parallely aligned sister chromatids. The most well analyzable polytene chromosomes in *Drosophila* are found in salivary glands of late third instar larvae. As is well known, cytogenetic and molecular studies using these chromosomes have been very useful in developing some of the fundamental principles of genetic organization and gene expression.

New methods for biological mutagenesis

An essential requirement for any genetic study is the availability of mutants so that "normal" function could be understood. Therefore, from the very beginning of *Drosophila* genetics, concerted efforts were made to isolate new mutants (spontaneous or induced). This required development of appropriate screening methods to identify new mutations at a higher frequency since spontaneous mutations were rather infrequent. As a result of sustained efforts of several generations of *Drosophila* workers since Morgan's days, very large number of mutations (spontaneous as well as induced) affecting a wide spectrum of phenotype have been isolated, systematically named, mapped, catalogued and maintained. This cumulative wealth of mutants is freely available, either from individual scientists or from stock centers established for the purpose, to the entire community of interested experimenters anywhere in the world. This free access to the ever increasing wealth of mutants has become the most remarkable advantage of using *Drosophila.* The available mutations include point mutations, deficiencies and duplications (involving very small to relatively large chromosome segments), inversions and translocations and in their phenotypic effects, they range from visible to lethal to those affecting very specific aspects of behaviour, circadian rhythm, cell determination and differentiation, mitosis, meiosis or other specific celluar functions. Over the decades, very ingenious genetic "tricks" have been developed for selection of specific types of mutants, their cytogenetic characterization and maintenance.

In spite of the accumulated vast wealth of mutants, the search for newer mutants affecting usual as well as exotic functions continues with more vigorous efforts. These efforts have been further encouraged by very novel methods of biological mutagenesis making use of very obliging transposable elements (P-element in particular) that also "happen" to be part of *Drosophila* genome.

Drosophila genome has a large number of transposable or mobile elements which as their name suggests have the propensity to move or "jump" from place to place in the genome. Among the diverse mobile elements, the P-elements have been particularly useful and their use has, in fact revolutionized approaches to molecular studies in *Drosophila.*

A normal intact P-element has a 2907 base pair long DNA sequence with the two ends defined by characteristic terminal inverted repeats of 31bp each. The DNA sequence between these terminal repeats codes for the transposase enzyme which catalyses excision of the P-element from its original location and its insertion (transposition) at another site in *Drosophila* genome. Usually, the transposase is active only in germ cells and thus the "jumps" or transpositions of P-element/s normally occur in a limited manner and are inherited. Strangely, most of the strains of *Drosophila* maintained in laboratory for the past 30-40 or more years are free of P-elements in their genome. Crossing of P-carrying male (P-cytotype) with a P-lacking female (M-cytotype) leads to a rather unregulated mobility of the P-elements in germ cells of the hybrid progeny which causes mutations, chromosome aberrations and sterility, collectively termed "hybrid dysgenesis". When a P-element "jumps" from its original location, it may "land" anywhere in the genome. If this "landing" (insertion) happens to be in a "gene", the gene may be inactivated or otherwise modified in its expression due to presence of the P-element sequence within functional part of the gene. This results in an inheritable mutation (insertional mutation) of that gene. This "mutator" property has been elegantly adapted in schemes utilizing genetically engineered P-elements which would start "jumps" as and when desired. Knowing that for a successful "jumps", the transposase activity as well as the two terminal repeats are required, one of the more recent P-mediated mutator schemes utilizes two specially prepared (genetically engineered) strains or stocks of *Drosophila*: (i) the "mutator" stock: this carries a single defective P-element on its X-chromosome which cannot make its own transposase but is otherwise capable of "jumps" if another source of transposase is available and (ii) the "jump-starter" stock: in this case, a defective P-element is present on one of its autosomes which can make transposase but cannot itself jump due to its modified terminal repeats. The "mutator" P-element carries, in place of the usual transposase-coding DNA sequence, sequences derived from "marker" genes of *Drosophila* (e.g. w^+ or $rosy^+$ genes) and/or bacteria (e.g. *lac-Z* gene) coding for beta-galactosidase or the *neo (R)* gene which confers resistance against Neomycin related antibiotic, G418. Due to these markers, the movements of the "mutator" P-element can be unmistakably and easily followed through generations. These "mutator" and "jump-starter" stocks were prepared by germ-line transformation (see next section) of flies of appropriate genotypes with the *in vitro* constructed (genetically engineered) P-elements. The "mutator" and "jump-starter" stocks are stable since neither of the P-elements is capable of mobility by itself. However, when crossed with each other, transposase made by the "jump-starter" P-element in germ cells of the hybrid progeny causes the "mutator" element to "jump". This results in a high frequency of mutations due to insertion of the "mutator" element in any of the genes. The presence of "marker" genes on the "mutator" element permits simple selection procedures to be used to screen the progeny carrying new insertional mutations but which did not inherit the "jump-starter". Appropriate genetic crosses have been devised to maintain and map the newly identified mutations. These insertional mutations are stable as long as a source of transposase is not re-introduced in the genome. This property provides another convenient handle to revert the mutant gene by crossing the mutant stock back to "jump-starter" stock so that the inserted "mutator" P-element may "jump"

to another site and restore the mutant gene to its original wild-type status.

Unlike the chemical and radiation mutagenesis, the P-mediated mutagenesis does not pose any health hazards. Besides, P-insertion mutagenesis provides several distinct advantages over the other methods:

(i) Mutations frequency : the frequency of P-insertion mutations is at least as high as the most potent chemical mutagen.

(ii) Screening of mutation : selection of progeny with new mutant is very easy due to selectable markers present on the "mutator" element; e.g. if the "mutator" carries the neo gene, the progeny may be allowed to grow on food containing neomycin (G418) so that only the "mutator" element bearing progeny survives. Among the neo-resistant surviving progeny, identification of new insertional mutants is easy.

(iii) Precise mapping of new insertional mutations: mapping of the new insertional mutations can be done by the conventional genetic methods as well as by *in situ* hybridization of the P-element or its associated marker gene sequences with polytene chromosomes of larvae carrying the insertional mutation. The *in situ* hybridization procedure allows a very precise and quick localization of the P-insertion and thus new mutations occurring in a desired region of chromosome can be quickly identified without resorting to time-taking series of genetic crosses.

(iv) Cloning of genes: insertion of the "mutator" P-element in a gene provides a convenient DNA sequence marker during isolation of the gene sequence for cloning ("transposon tagging"). In recent years, special "mutator" elements have been synthesized which have appropriately placed plasmid vector sequence so that once a desired insertional mutation using this "mutator" is obtained, the gene in question can be directly cloned by transforming *E. coli* with genomic DNA isolated from the mutant strain: only the genomic DNA sequence associated with the "mutator" element carry the necessary plasmid vector sequence for successful cloning in *E. coli* and thus cloning of a specific gene becomes extremely simple. This strategy is especially useful when working with genes whose transcripts are not known or very rare so that cDNA cloning etc may not be possible.

(v) In situ expression of a gene: "Mutator"-elements carrying the lac-Z gene of *E. coli* have also been constructed so that when this "mutator"-element gets inserted in a gene downstream of its promoter, the lac-Z gene may often express under the control of the resident promoter. If for example such an insertion occurs in a gene "m", the lac-Z gene may be expected to be under the control of the promoter of the "m" gene. If this happens, beta-galactosidase activity will be present in all those cells which normally express the "m" gene. A simple cytochemical staining procedure for localization of beta-galactosidase activity thus permits an *in situ* visualization of normal temporal and spatial expression of the gene "m". Such insertional-mutant strains of *Drosophila* are providing a simple system to obtain very useful information on the spatial and temporal activity of specific genes in different cells/tissues.

Considering the usefulness of insertion mutations in a variety of genetic, molecular and cell biological studies in *Drosophila*, a concerted approach has already begun to establish a library of *Drosophila* stocks carrying single inserts in different genes. It is expected that in few years' time, the insert library would have strains with a P-insertion at every 20-30 kb of *Drosophila* genome. It is obvious that this insertion library would provide unprecedented facility to physically map *Drosophila* genome at DNA level and to work with genes that may not otherwise have been known to exist. Since several different types of "mutator" and "jump-starter" stocks are readily available, one may select the mutator stock of one's choice and straightaway start a mutagenesis experiment without going through the pains of synthesizing one's own mutator-jumpstarter system.

P-element mediated germ line transformation of *Drosophila*

The remarkable property of P-elements to excise themselves from their genomic locations and to as neatly get inserted at some other site has been exten-

sively utilized to introduce other DNA sequences in *Drosophila* genome. Since in the presence of transposase, excision and insertion of P-element is governed mostly by its terminal repeat sequences, genetically engineered P-element plasmids have been prepared in which the original transposase encoding sequences are deleted and other desired DNA sequence placed between the two terminal repeats of P-element. Such *in vitro* constructed P-plasmids carrying the desired DNA sequence inserts are easily cloned in bacteria to yield sufficiently large amounts of DNA for transformation of flies. However, these genetically engineered P-plasmids lack the transposase activity and, therefore, cannot integrate themselves in *Drosophila* genome even if placed within the nucleus by micro-injection. To get over this, another class of P-plasmids ("helper-P") have been prepared that have lost or have modified terminal repeats but have retained the transposase activity. Thus when the "helper-P" and the foreign DNA inserted P-plasmid DNA are introduced in *Drosophila* cells, the former provides the transposase activity and the latter the terminal repeats. Thus the foreign DNA inserted between the terminal repeats gets transposed in *Drosophila* genome due to the transposase activity of the helper P-plasmid. As the helper P is not able to transpose itself (due to its defective terminal repeats), the transposase activity soon decays and the transposed P-plasmid with its foreign DNA insert gets permanently lodged in *Drosophila* genome. The mode of sex-cell formation in *Drosophila* embryo has turned out to be especially advantageous for inheritable germ-line transformation of *Drosophila*. As mentioned earlier, a few of the early cleavage nuclei come to lie in the "Pole plasm" at the posterior end of cleaving embryo to form "Pole cells", the exclusive precursors of future germ cells. Thus at a specific stage of early embryogenesis, precursors of germ cell nuclei lie free in a restricted area of embryo cytoplasm and therefore, are highly amenable to introduction for foreign DNA. Microinjection of P-plasmid (carrying the desired DNA sequence insert) and the "helper-P" DNA in the posterior region of early cleavage stage embryos results in a high rate of successful integration of the P-plasmid (with its foreign DNA insert) in germ line cells so that some of their progeny will be permanently transformed. To enable a distinction between transformed and non-transformed progeny, the embryos to be injected are mutant for an easily selectable marker (e.g. white or rosy eye mutants) while the transforming P-plasmid carries, in addition to the DNA sequence desired to be introduced in *Drosophila* genome, w^+ or $rosy^+$ gene sequence. Therefore, the transformed progeny also inherit a w^+ or $rosy^+$ gene sequence and lose their mutant (white or rosy eye, as the case may be) phenotype. They are thus easily selectable.

The applications of P-mediated transformation of *Drosophila* are enormous, particularly in understanding the role of flanking and intervening sequence that regulate the transcription and processing of transcripts of a given gene. Once a gene is cloned, different parts of the gene sequence and its flanking regions may be inserted in P-plasmids and transformation of flies with such constructs allows analysis of the role of various sequence elements in function of that gene. A mutant phenotype can also be reversed by transformation using DNA sequence of the normal allele of the mutant. Different P-plasmid for transformation are now available so that the desired DNA sequence gets positioned next to a variety of promoters (e.g., promoter of a house keeping gene like action or promoter of an inducible gene like heat shock promoter or ecdysone-promoter) so that when transformed, the gene would express in every cell (if under active promoter) or when heat shocked (if under heat shock promoter) or in ecdysone-target cells (if under ecdysone promoter). In another approach, the transformation P-plasmids carry a reporter gene sequence (like *lac-Z of E. coli*) in such a position that promoter region of the desired gene may be conveniently placed upstream of the reporter gene. Transformation with such constructs allows one to study the expression of the given promoter by staining tissues for beta-galactosidase activity since whenever this promoter gets activated, it leads to expression of the *lac-Z* gene and thus to synthesis of beta-galactosidase (e.g see Fig 1 in

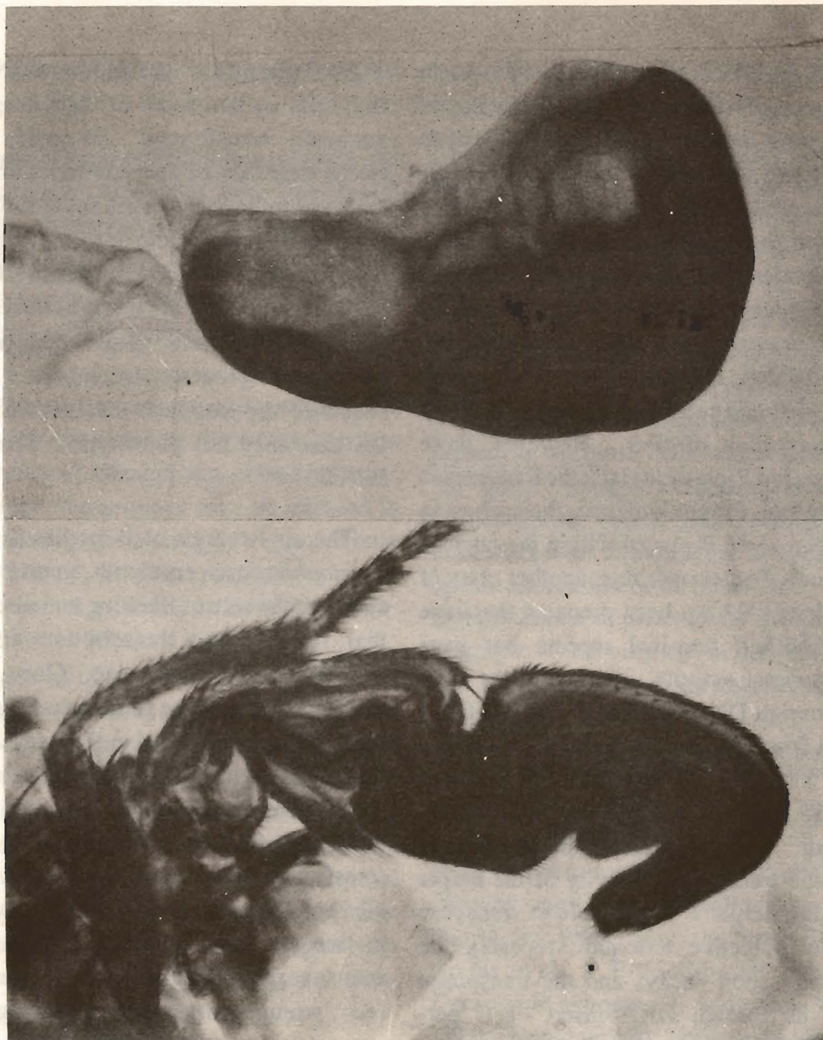

Figure 1: Posterior compartment specific expression of the engrailed gene in wing of *Drosophila melanogaster* : the expression of engrailed gene is revealed by staining for betagalactosidase enzyme activity in a fly stock made transgenic (through P-element mediated germ line transformation) for *engrailed* promoter *lac-Z* reporter fusion gene. The *engrailed* promoter is known to be activated in cells in the posterior half of every body segment (see the article by Chandrashekhar & Lakhotia "Genetics of body pattern .... etc" in this issue) and accordingly, in this transgenic stock, the *lac-Z* gene is also activated in cells in the posterior half of every body segment resulting in appearance of the beta-galactosidase enzyme activity in these cells. A blue colour is seen after histochemical staining, using X-gal as the chromogenic substrate, in all those cells which have beta-galactosidase activity (and therefore, active *engrailed* promoter in this stock). The upper panel shows a wing imaginal disk (the undifferentiated precursor of adult wing present within the body cavity of larva) while the lower panel shows a wing of fly (just before emergence from pupal case) from this transgenic stock. In both cases, the blue stained regions correspond to cells defining the posterior compartment of the wing and therefore, expressing the *engrailed* gene. This type of promoter-reporter fusion gene transgenic stocks permits simple and reliable identification of developmental stage- and tissue-specific patterns of expression of specific genes.

this article and Fig 1 of the article "Demonstration of Heat Shock Induced Gene Activity ......promoter" by S. C. Lakhotia). In yet another approach, the transformation P-plasmid may be constructed in a manner that would allow production of anti-sense RNA for the given gene in fly: if the sense RNA (from the normal

resident gene) and anti-sense RNA (from transformation-inserted gene) are produced in the same cell, the sense RNA may not get translated, resulting in a mutant condition (simulation of a null mutant allele).

Thus in summary, the ready availability of a wide variety of P-plasmids and the relatively easy technique of transformation of flies opens up unlimited possibilities to play around with gene sequences with a view to understand their organization and function.

Concluding Remarks

The P-element mediated mutagenesis and term-line transformation in *Drosophila* are very powerful techniques. Application of these techniques in conjunction with classical cytogenetical and histological techniques is providing very detailed and specific information relating to various biological systems. These and the other molecular and cell biological techniques have made *Drosophila* much more useful for experimental studies than ever before. A most illustrative case is in the field of developmental biology. Embryology of *Drosophila* was difficult to understand because of its several unusual aspects. Thus while excellent descriptions of events of embryological development of higher eukaryotes have been available for some time, little was known of these events in *Drosophila* till about a decade ago. However, using a genetic approach, mutants affecting development of *Drosophila* were systematically identified. Thus unlike the usual practice of going from phenotype to genes, in this case the genes that affect development were identified first. It is testimony to the power of techniques available with *Drosophila*, that within the short span of one decade or so, it has been possible to more or less completely unravel the process and mechanism of early development in *Drosophila:* scientists, instead of trying to get the secret of embryonic development by attacking from outside, worked from within, i.e., they started with the genes themselves. Now the genes isolated in *Drosophila* are being used to really understand the process of early development in higher eukaryotes like sea-urchin, amphibians, mammals etc, although as stated earlier, descriptive embryology of these organisms had been very well known for a long time. This gives a clear message that a biological process would be more easily and comprehensively understood if we can get at the genes that govern the process. Among the higher organism, *Drosophila* is most advantageously placed to provide this opportunity.

In India, *Drosophila* is being used only in a few laboratories. However, there is a great scope for its wider use by even those not directly involved in genetical studies, since these flies can be profitably used as the model organisms for investigating a variety of basic biological issues. The advantages offered by *Drosophila* far outweigh the initial hurdles in setting up a *Drosophila* lab. Even for teaching purposes, *Drosophila* would prove to be a useful system, not only for classical genetical and cytological experiments, but also to demonstrate aspects of developmental biology (like effect of specific mutations on early embryonic development to show the hierarchy of genes controlling sequential developmental events), gene expression (especially through use of transformed stocks in which a given promoter is linked to a reporter gene like *ADH* (alcohol dehydrogenase) or *lac-Z* (beta-galactosidase) so that the gene expression can be monitored by simple cytochemical staining patterns) or of behaviour (through use of some of the many available mutations that affect specific aspects of behaviour) etc. In this context, a recent welcome development is the establishment of Stock Centers of *Drosophila.* The Cytogenetics Laboratories at Departments of Zoology at Calcutta University and at Banaras Hindu University and the Molecular Biology Division of TIFR had already been maintaining many stocks of *Drosophila* for teaching and research purposes. To help growth of *Drosophila* genetics in the country, a new stock center has now been established at the Department of Zoology, University of Mysore, Mysore and another one is expected to start functioning soon at the Department of Life Sciences, Devi Ahilya Vishwavidyalaya, Indore. These centres are expected to provide stocks of *Drosophila* to different institutions for their teaching and research require-

ments. It is hoped that the potentials of *Drosophila* for teaching and research in biology will be more widely exploited by our academicians and researchers.

Sources of Information on *Drosophila*

Scientific literature on *Drosophila* is enormous and is increasing exponentially. Fortunately, however, a number of sources are available for obtaining comprehensive information on specific aspects of *Drosophila*. Some of these are noted in the following.

1. Strickberger, M.W. 1962. Experiments in Genetics with *Drosophila* (Wiley & Sons, NY).
2. Demerec, M. & Kaufman, B.P. 1973. *Drosophila* — A guide (Carnegie Inst. Wash. Publ., Washington)
3. Roberts, D.B. (ed.) 1986. *Drosophila* a practical approach (IRL Press, Oxford): provides details of several classical and modern molecular biological methods in *Drosophila* research.
4. Ashburner, M. 1989. *Drosophila* a laboratory handbook (Cold Spr. Harb. Lab. Press, USA): provides a detailed single source for updated information on various aspects of *Drosophila* biology and techniques.
5. Ashburner, M. 1989. *Drosophila* a laboratory manual (Cold Spr. Harb. Lab. Press, USA): a companion of the above handbook by Ashburner which provides step-by-step details of a large variety of laboratory techniques used in *Drosophila* research.
6. *Drosophila* Information Service (DIS): an informal periodic (mostly annual) publication founded by C. Bridges in 1934. It is a very useful source of information on new research findings, new mutants, stock lists from different laboratories, geographical directory of *Drosophila* workers etc. In earlier years, it also used to include a section on Bibliography of *Drosophila*. Currently, the DIS is edited by Dr. J.N. Thompson, Jr. Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019, USA) and is available from him at a nominal price. In recent years, DIS has also become available through E-Mail.
7. The Genetics & Biology of *Drosophila*: a series of edited volumes published by Academic Press (London, New York, San Francisco) between 1976 and 1986. These volumes are a very important source of comprehensive information on various aspects of genetics and biology of *Drosophila*.
8. Lindsley, D.L. and Grell, E.H. 1968. Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627: this is a detailed catalogue of all mutations (gene mutations as well as chromosomal rearrangements) known in *D. melanogaster*. This is being revised by Lindsley and G. Zimm to make it updated: draft versions of the revision have been published in DIS 62, (1985), DIS 64 (1986), DIS 65 (1987) and DIS 68 (1990).