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 artificially mating for crossing between different genotypes and (iv) fewer chromosomes (2n = 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 by 50's this organism had become genetically the best known eukaryote. However, the introduction of microorganisms (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 new and challenging avenues of studies. The pace of progress in recent years has been extremely rapid on this Drosophila is now being increasingly used for traditionally non-genetical cell biological studies.

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Life Cycle of Drosophila

Drosophila is a dipteran (two-winged) fly which like many other insects undergoes complete metamorphosis (holometabolism) and thus within a short period of about two weeks passes through the embryonic, larval, pupal and adult stages, prior to the imago or adult stage which involves 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 sperm as the eggs are laid. Embryonic development begins immediately. Very rapid (one division occurring in about 10 minutes) nuclear divisions ("cleavage") lead to syncytial (multinucleate) stage with nuclei migrating to the peripheral or cortical region of egg cell. Between 1.5 and 2.5h, all the nuclei are assigned as somatocytes in cortical cytoplasm (the multinucleate or syncytial blastoderm stage). The early 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. Cellulization 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 germline lasts for about 5 days and includes two moulttings so that three larval instars (1st, 2nd and 3rd instar) are recognized. The third instar stage lasts for about 48h. It is the last 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 about 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 signals 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 larva 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 consequence of the endoreplication cycles is formation of the giant polytene chromosomes containing thousands of parallelly aligned sister chromatids. The most well analyzable polytene chromosomes in Drosophila are found in salivary glands of late third instar larva. 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 scientist 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 cellular functions. Over the decades, very ingenious genetic "tricks" have been developed for selection of specific types of mutants, their cytogenetic characterization and maintenance.

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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 effects have been further encouraged by very novel methods of biologica] megengenesis 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 insert P-element has a 2507 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 catalyzes 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 "jump" or transpositions of P-elements normally occur in a limited manner and are inherited. Strangely, most of the strains of Drosophila maintained in the laboratory for the past 30-40 or more years are free of P-elements in their genome. Crossing of P-carrying male (P-cytops) with a P-lacking female (M-cytops) leads to a rather unrelated 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 inherited 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 "jump", 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 "jumping" 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 rasy genes) and/or bacteria (e.g. Iac-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" P-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 insertionional 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 the source of transposase is not re-introduced in the genome. This property provides another convenient handle for recrossing 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 mutagens, 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 mutagens.

(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 a medium containing ampicillin (G-18) 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 all new mutations occurring in a desired region of the chromosome can be quickly identified without resorting to time-consuming series of genetic crosses.

(iv) Mutating 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 cell lines.

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 different insertion types of "mutator" and "jump-starters" stocks are readily available, one may select the mutator stock of one’s choice and straightforward start a mutagenesis experiment without going through the pains of synthesizing one’s own mutator-jump starter system.

P-element mediated germ line transformation of Drosophila

The remarkable property of P-elements to excise themselves from their genomic locations and to actually 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-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 the 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 "embryonic 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 embryo 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 phenotypic can also be reversed by transformation using DNA sequence of the normal allele of the mutant. Different P-plasmids for transformation are now available so that the desired DNA sequence gets positioned next to a variety of promoters (e.g., promoter of a homoeologous gene like action) or promoter of an inducible gene like heat shock promoter or cecidine 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 cecidine-target cells (if under cecidine 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

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this article and Fig 1 of the article "Demonstration of Heat Shock Induced Gene Activity ....protocer" by S. C. Lakhtin). In yet another approach, the transformation

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resident gene) and anti-sense RNA (from transforma-
tics-inserted gene) are produced in the same cell, the
transformation of flies opens up unlimited possi-
bilities 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 tech-
niques. Application of these techniques in conjunction
with classical cytogenetical and histological techni-
ques is providing very detailed and specific informa-
tion 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 descrip-
tions 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 ap-
proach, 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
evaryl 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 under-
stand 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 oppor-
tunity.

In India, Drosophila is being used only in a few
laboratories. However, there is a great scope for its
wider use by those not directly involved in genetic
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 genetic and cytological experi-
ments, but also to demonstrate aspects of developmen-
tal 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 trans-
formed 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 pat-
tern) 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 Laboratory at Depart-
ments 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 pur-
poses. 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 function-
ing soon at the Department of Life Sciences, Devi
Aalaya Vishavavidyalyaya, Indore. These centers are
expected to provide stocks of Drosophila to different
institutions for their teaching and research require-
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.


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.
