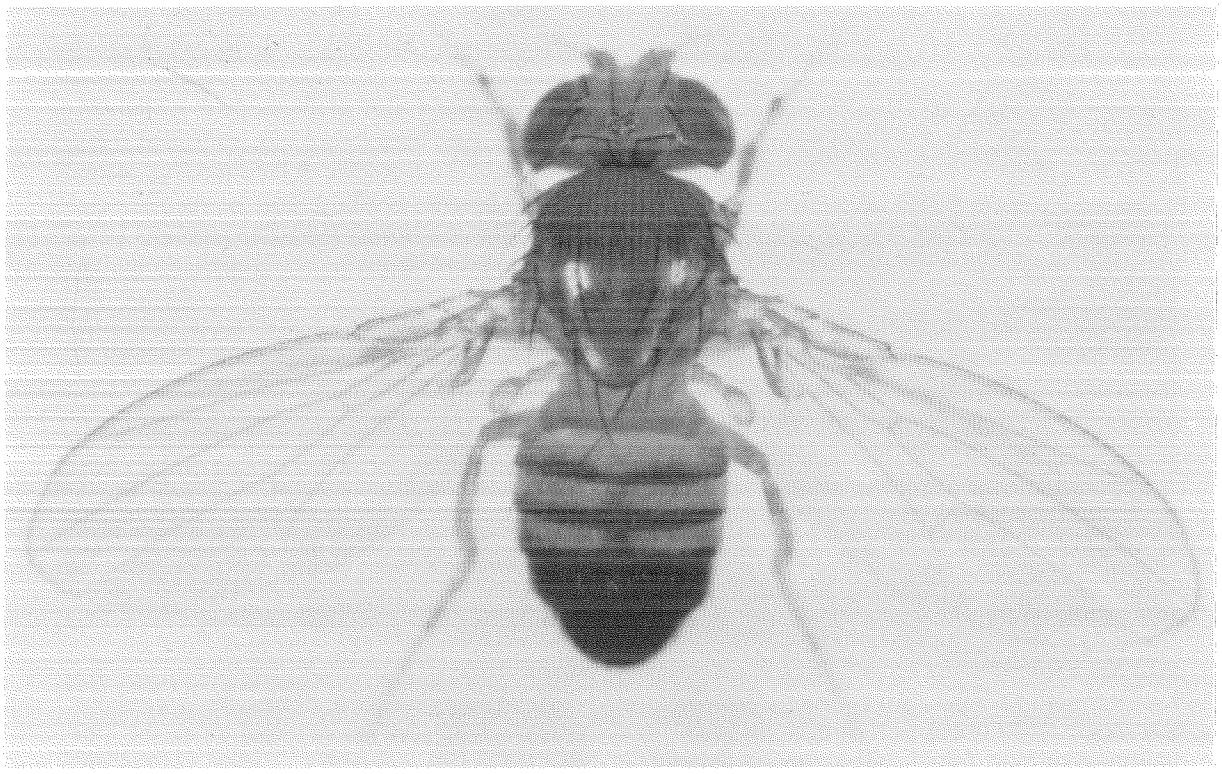

A Second Golden Age in Drosophila Research

by *Dennis Meredith*

How genes cause a single fertilized egg to almost magically erupt into a functioning, behaving creature is one of the central mysteries of biology. If we humans ever come to understand this stunning transformation and how it can sometimes go tragically awry, we will owe an enormous debt to a modest creature known as *Drosophila melanogaster*. Drosophila, better known as the fruit fly, is a delicate little insect about the size of a BB, usually found flitting about garbage cans. With its prismatic red eyes, its slim, translucent tan body, and its delicate many-veined wings, Drosophila is an aesthetically pleasing animal as insects go. And it is a benign creature. For instance, it is not really a "fruit" fly, for it does not feed on fruit like its fellow insect (but non-relative), the voracious Mediterranean fruit fly. Rather, Drosophila feeds on the yeast growing on rotting fruit. It is amiable, does not bite, and adapts well to captivity.

All these traits, plus the fact that it zips through its life cycle in a matter of ten days have made Drosophila ideal for the study of the genetics of development. Unlike bacteria, it is a full-fledged animal, with an embryonic stage of development, a full range of senses, and a repertoire of behaviors. However, it is a simpler animal than mammals such as the rat, and its genetics are easy for scientists to tinker with.

And tinker they have, using X rays and chemicals to produce over the decades tens of thousands of mutants — a weird, fascinating menagerie of flies possessing a huge array of precisely defined colors, deformities, or quirky behavior patterns. More than just oddities, these mutations represent experimental probes into the genetic machinery that yield valuable clues about how that machinery functions.

An indication of the value to science of little Drosophila is the number of research papers pub-

Fly Repository

Edward B. Lewis

One of the world's most important repositories for Drosophila mutants is little more than a 20-foot-long rack of stoppered half-pint milk bottles kept in a cool room in Caltech's Kerckhoff Laboratories of the Biological Sciences. From this room, 1,500 stocks of flies from the lab's collection of more than 1,500 strains are shipped annually throughout the world. They range from strange color mutants like ebony, to exotic malformed flies with curly wings, to odd behavioral mutants like "Drop-dead," which true to its name drops dead at the sound of a hand clap.

Business has been booming for this National Science Foundation-sponsored repository, with the number of shipments rising about 10 percent a year. The steady rise in demand is just another example of the boom in research using the flies during what the Drosophilists call "The Second Golden Age of Drosophila." The First Golden Age began in the early 1900s with Thomas Hunt Morgan's use of Drosophila to develop the basic laws of genetics. In the 1950s, however, interest in the flies as research subjects waned, as biologists turned to cheaper, rapidly multiplying bacteria to discover the basics of DNA structure and function. But the 1960s saw a resurgence of Drosophila work, as scientists became interested in the molecular genetics of development and behavior in higher organisms.

Presiding over Caltech's collection for the last three decades has been Professor Edward Lewis, dubbed "Lord of the Flies" by waggish colleagues. But the responsibility is a serious business, for the mutants in this repository can offer scientists studying them valuable clues to genetic processes. And the Caltech center is one of only two in this country; the other is at Bowling Green, Ohio.

Maintaining the stock of mutants is a tedious, exacting task, for even in a cool room, the little flies breed like . . . well . . . *flies*. Technicians must periodically extract the insects from the bottles, anesthetize them with ether, and use a microscope and a fine brush to sort out the best to restock another new colony. In the course of a year, keeping the stocks fresh means preparing more than 100,000 half-pint milk bottles, which were chosen originally for their convenient geometry and sturdiness. They are sterilized; partially filled with Lewis's fly-food recipe of agar, cornmeal, mold inhibitor, and sugar; and seeded with a puff of yeast. And so it has been for over half a century for billions and billions of Drosophila.

It's no fly-by-night business.

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lished worldwide on the creature — currently about a thousand per year and increasing. Caltech scientists have been pioneers in Drosophila research since 1928. That was when Nobel Prize-winning biologist Thomas Hunt Morgan first used the fly to develop some basic theories of genetics. Caltech remains a major center of Drosophila research, for besides studies of the fly by Caltech scientists, the Institute continues to operate the oldest repository for Drosophila mutants — one of two in this country. (See box above.)

The current studies at Caltech using Drosophila are a fascinating carnival of experiments and ideas, and a look at them offers an excellent insight into the fly's value to science. Caltech scientists produce mutants with exotic names like "Dunce" and "Shaker." They insert microscopic electrodes into fly nerve cells, perform fly brain transplants, monitor the faint mating call of the lovesick Drosophila, waft odors past instrumented flies, plunge fly wings into solutions of radioactive tracer, heat-shock fly larvae, and even set a computer to watch over the flies' scurryings. All these activities provide basic insight into how genes create organisms, but they also help us understand an enormous range of disease — including cancer, learning disabilities, birth defects, neuromuscular diseases, and sleep disorders.

One veteran Drosophila researcher at Caltech is the Thomas Hunt Morgan Professor of Biology, Edward Lewis. Besides operating Caltech's Drosophila stock center for the last 30 years, Lewis has been studying the set of genes that control the

fly's body segmentation. To trace these genes, Lewis has produced a wide range of mutant flies with altered body segments. Ensconced in bottles in his lab are wingless flies, four-winged flies, eight-legged flies, and four-legged flies. Some flies are too abnormal to even make it to adulthood. One mutant that consists of a head and a chain of thoracic segments dies as an embryo. By studying such mutants, Lewis has found a set of about ten genes, called the bithorax complex, that seems to code for body substances that somehow regulate body segmentation. If the bithorax complex is thought of as a series of switches, Lewis's strange mutants, about 500 so far, are animals in which various of these switches have been clicked on or off.

Lewis thinks of these genes as regulating other genes that actually produce the segments. What's exciting about this system, he says, is that it may provide a way of solving for the first time just what it is that regulatory genes make that affects other genes — whether it is a protein or an RNA. Nobody knows exactly what genes do to cause undifferentiated human cells to produce arms or legs or teeth or hair, so this system could offer an extremely valuable insight.

But what controls the "switches" of the bithorax complex? Lewis thinks he and his colleagues may have found an answer. By examining various mutants, his co-worker and wife, Pamela, discovered another gene, called Polycomb, that seems to control the bithorax region. Polycomb could be a "master regulator" — a gene which codes for a product that finds its way to the bithorax region and throws the correct switches to produce a normal fly. One clue, for instance, that Polycomb is a master regulatory gene, is that when the gene is removed by mutation, the bithorax genes are all unregulated, and all of the mutant's segments look like abdomens.

According to Lewis, the discovery of Polycomb is exciting because, although there are known cases of such genes controlling enzymes, there are no other examples of such a gene that controls development. Formerly, it was believed it would be years before such regulation would be understood, because nobody could trace its biochemistry. But in the Polycomb gene, scientists may have a tool that will aid them in coming to such understanding.

When genetic machinery is hard at work churning out proteins to create an organism, it is like a player piano, running through its roll of punched paper to spew out a song. To understand that machinery, biologists seek ways of freezing that genetic "paper roll" at certain points to "read the holes." Veteran Drosophila researcher Herschel Mitchell, professor of biology, has found that the development of wing hairs in Drosophila pupae offer a good model system for monitoring development. The 31,000 cells that make the hairs of a Drosophila wing all work conveniently in synchrony, and in a process that takes a mere 20 hours or so, all the hairs form and erupt at once, like a field of super-wheat springing up. To understand how this process operates, Mitchell and his colleagues carefully slit the pupal cases of flies at various points in hair development, clip off one wing, and immediately immerse it in a solution of radioactive amino acid, to label the proteins being produced. By separating these hundreds of proteins on an electrophoretic plate, the scientists produce a sort of snapshot of activity that tells what genes are turned off or on at any instant.

From the laboratory of Herschel Mitchell, a photo micrograph of a normal Drosophila chromosome (top). In the center, the same region on a chromosome from a fly that was given a heat shock and a pulse of tritiated uridine. Note the two puffs. The same chromosome is shown at the bottom after exposure to a photographic emulsion, and the silver grains appear as irregular black bodies in the scanning electron micrograph. There is rapid synthesis of RNA (messages for heat-shock proteins) at the puff sites.

Mitchell has used this technique to study various wing-hair mutants to understand the machinery of hair development, and has also embarked on studies of wing-hair abnormalities produced when pupae are subjected to heat shock. Such heat shock has been found to switch certain genes off and turn others on, producing abnormalities. In Drosophila, such abnormalities due to stress on an embryo are known as phenocopies. In humans, they are known as birth defects, and the two phenomena are thought to arise from the same basic mechanism of damage.

In numerous biochemical "snapshots" he has taken of the proteins of heat-shocked flies,

Mitchell has found certain genes that are especially sensitive to heat shock during critical periods of development. He is continuing to study the machinery in hopes of gaining fundamental insights into the process that causes birth defects.

Basic research often yields surprising applications, and this has been the case with Mitchell's work. Cancer researchers have long known that heat may be used to kill cancer cells selectively. Recently, Mitchell and collaborators at other institutions have found that the mechanism of heat shock is quite similar in both flies and cancer cells. In both cases, the shock appears to wreck the coordination of the genetic machinery by switching genes on or off prematurely.

But there are weird complications. In Drosophila, a mild heat shock before the main shock appears to protect the larva, perhaps by turning on still other proteins that protect the embryo. Mitchell and his colleagues are currently working to understand the complexities of the heat-shock phenomenon.

Mitchell believes there may be a better chance of understanding heat shock in Drosophila than in cancer cells because researchers have better control over the Drosophila system. It is much more uniform, homogenous, and synchronous than are cancer cells.

The new techniques of genetic engineering are also being applied to studies of Drosophila by such researchers as Professor of Chemistry Norman Davidson and Assistant Professor of Biology Elliot Meyerowitz. Basically, these techniques consist of using enzymes and other biochemical tools to isolate genes and to insert them into bacterial factories, so that the genes can be produced in large amounts for structural analysis.

For example, Davidson and his colleagues are in the early stages of a long-term project to understand the control mechanisms for the genes producing the several kinds of muscle proteins in Drosophila — including actin, myosin, and tropomyosin. The genes for these various proteins are turned on and off in precisely regulated ways in different cells to produce the different muscles of Drosophila, such as crawling muscles and flight muscles. By using genetic engineering techniques to fish these genes out of Drosophila cells and figure out their structures, these researchers hope to learn how muscle formation — a basic process in all animals — operates. Similarly, Davidson and his colleagues have begun isolating and characterizing the various genes governing the formation of the proteins of the insect's tough outer covering, called the cuticle.

Assistant Professor of Biology Elliot Meyerowitz could be said to be studying the "genetics of glue." Using mutants and recombinant DNA techniques, he has set out to understand a gene in Drosophila that codes for a protein glue called SGS-3, which is spit out by the fruit fly larva to hold it in place while it metamorphoses into the adult fly. Normally, spying on an operating gene might be difficult, because the strands are almost too small to be seen, even by a microscope. However, obliging Drosophila possesses a set of chromosomes in its salivary gland that are gigantic by biological standards. Unlike the usual chromosomes, which consist of a couple of DNA strands lined up, the salivary gland chromosomes are 1,000 or so DNA strands thick. Easily visible on these giant chromosomes are dark bands, which act as landmarks in searching out genes. Also visible are active sites of genetic activity called "puffs," which, true to their name, swell up like popovers in a hot oven when the genes are actively transcribing RNA.

Meyerowitz is, in effect, "stalking" the gene for this glue protein. By experiments on the chromosomal puffs, he has found that a region known as 68C3,4 on one of the chromosomes is the site of the SGS-3 as well as other glue protein genes. Now, Meyerowitz is using X rays to produce a range of fly mutants whose DNA is altered at sites closer and closer to the glue gene. His aim is to discover just how close he can come to this gene, and still have it function properly. This game of genetic "chicken" is valuable, for it will help determine just how much of the gene is needed for the vital process of control. The mystery of how much genetic material is needed for controlling development is a major one. While scientists are now unsure, they do know that the fraction of genetic material needed to actually specify the structure of living things is small. The function of the overwhelming amount of DNA in living things is still a mystery, and a significant amount may be control DNA.

Besides narrowing down the vital areas of the glue gene, Meyerowitz is also using recombinant DNA techniques to analyze the DNA sequence of the gene to detect patterns that might be control signals.

The researchers discussed so far are puzzling out how the machinery of the genes operates to control development. Assistant Professor of Chemical Biology Carl Parker, however, is studying some of the biochemical cams and springs and levers and pulleys of the machinery itself. Specifically, he is interested in the enzyme "DNA-dependent RNA polymerase," which is the basic device that copies information from the genes for use by the cell. While many researchers have studied this enzyme in bacteria, Parker

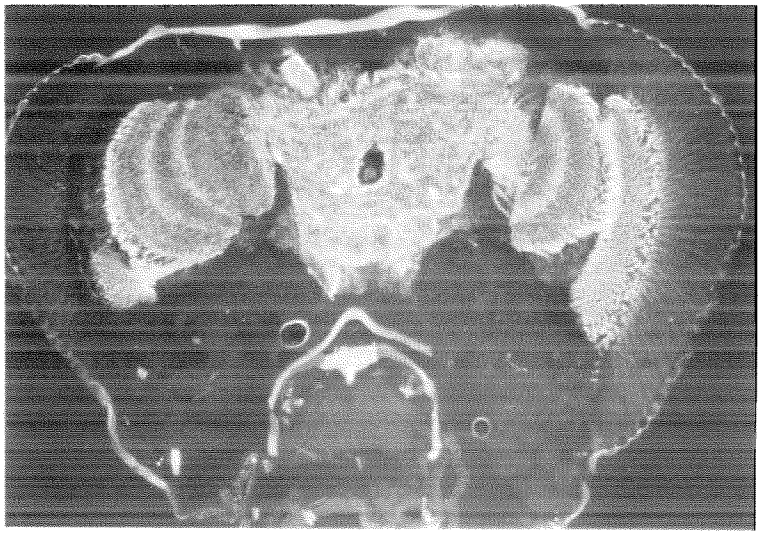
hopes to take his studies up the evolutionary scale to Drosophila, using the multitude of fly mutants that have been produced as a scientific proving ground to develop his theories. He will attempt to build in a test tube various working models of normal and mutant fly genetic machinery to discover how the components work. While many test tube studies have been done on the RNA polymerase machinery of viruses and humans, fly studies appear especially promising, says Parker. This is because the fly machinery appears to operate *in vitro* more realistically than do the human or virus test tube systems.

Parker is, incidentally, the latest addition to Caltech's Drosophila researchers, having arrived at the Institute but a few months ago. He was attracted, he says, because "the Drosophila research at Caltech is better now than any other place in the world. The huge range of work going on here makes the place most attractive."

While many researchers are concentrating on the genetics of the little fly's structure, others are taking advantage of the fact that Drosophila is a full-fledged *behaving* animal. For example, Seymour Benzer, the James G. Boswell Professor of Neuroscience, is a pioneer in Drosophila behavioral genetics. Current studies by him and his colleagues aim at tracing the genetic and physiologic basis of behavioral mutants.

Mark Tanouye in Benzer's group is studying Shaker mutants, which, as their name suggests, show uncontrollable trembling of legs and head parts. The defect in Shaker and other mutants may be analogous to various human inherited neuromuscular diseases, so the Caltech scientists believe that study of such mutants could lead to insights into these diseases.

Their studies have shown that the Shaker mutation appears to affect the electrochemical process in the nerve cell that triggers muscular contraction. By inserting electrodes into the "giant" axon of the fly — large nerve cells that are easily impaled — and recording action potentials, the Caltech researchers have narrowed the defect to a particular part of the nerve impulse mechanism known as the potassium channel. Basically, nerve impulses in both flies and humans consist of a wave of sodium inflow moving down the nerve cell, followed by a compensating wave of potassium outflow. The researchers have used drugs to selectively block different components of this process, and have implicated the potassium channel as defective. Also, using extremely delicate electrodes and recording procedures, Benzer's colleagues have managed to record the nerve signals in Shaker mutants to reveal electrical abnormalities in the cells.



From the laboratory of Seymour Benzer, a section (seen in a fluorescence microscope) of a section across a fly's head. The section was stained with one of the lab's monoclonal antibodies to reveal the fine structure of the fly's nervous system.

The fact that Shaker represents an abnormality in the potassium channel is important, because traditional biochemical techniques to study the structure of this channel are difficult, according to the scientists. While there are drugs that bind the sodium channel specifically and tightly, there are no such drugs for the potassium channel.

The other major mutant under study in Benzer's lab, called Dunce, does not learn to avoid odors associated with electric shocks, whereas normal flies can learn such avoidance. In their work with Dunce, the Caltech scientists recently experienced an example of the kind of serendipity that occurs when many scientists study the same organism. In discussions with researchers from the University of California at Davis, they discovered that the behaviorally defined Dunce mutant was affected in the same gene as another mutant with a biochemical abnormality in the enzyme cyclic AMP phosphodiesterase. This enzyme controls the breakdown of the substance cyclic AMP, a fundamental regulatory chemical in the body, which also seems vital to memory formation. Such breakdown is necessary to prevent undue buildup of cyclic AMP and thus, malfunction. Lawrence Kauvar of UC Davis and Caltech graduate student Sandra Shotwell are now tracing both Dunce's enzyme abnormality and its genetic abnormality.

Visiting Fairchild Scholar Obaid Siddiqi in Benzer's laboratory is studying the sense of smell, a poorly understood phenomenon at present. So far, mutant flies have been isolated that are behaviorally deficient in response to specific odors. These could be used to perform a "genetic dissection" of the spectrum of basic odor specificities. Siddiqi is testing normal and smell-insensitive mutant flies by inserting electrodes

into the odor-sensing antennae, and wafting odors past the flies, while measuring the currents produced by the smell receptors.

In another highly promising project, Benzer and his colleague Shinobu Fujita are developing extremely specific stains for various parts of the fly's nervous system, using a new technique of producing monoclonal antibodies. This technique involves injecting mice with fly brains and then fusing sensitized spleen cells with myeloma cells to produce hybridoma clones, each producing a single antibody type. The many different antibodies thus obtained can be used to trace the development of the nervous system, perhaps ultimately detailing not only how the nerves connect, but how they got that way.

Besides having the ability to learn, Drosophila are like humans and other higher animals in that they possess a built-in daily rhythm of activity. For about 12 hours a day, they actively go about the business of feeding and mating, and for another 12 hours they "power down," sitting quietly. (Whether flies actually sleep, nobody knows.) Assistant Professor of Biology Ronald Konopka has been searching for the genetic and neurological basis of this cycle by studying "clock mutants" that have shorter or longer cycles, or are arrhythmic. For instance, a 19-hour mutant tends to settle into a natural cycle of 7 hours of activity and 12 hours of inactivity; a 29-hour mutant is active for 17 hours, inactive for 12. These mutations occur on a gene known as the *per* locus, and in collaboration with Meyerowitz, Konopka is currently trying to isolate the gene and clone it, producing huge numbers of copies to deduce its structure.

A fly-brain transplant recently led to one important discovery about the fruit fly clock. After the brain from a short-period mutant fly had been carefully removed and inserted into the abdomen of an arrhythmic mutant, the two-brained fly assumed a short-period cycle. This was the first proof that a humoral substance produced by the brain governs periodicity.

Konopka and collaborators at Brandeis University are now puzzling over the surprising discovery that the fly's clock also governs the rhythm of the animal's courtship song. This modest ditty, sung by the male to a likely female, consists of a rapid series of low-frequency clicks made by vibrating the wings. By recording this song in normal flies, the Brandeis University researchers discovered that the interval between clicks naturally lengthens and shortens in an oscillation that takes about a minute. However, in the short-period mutant this oscillation takes about 40 seconds, and in the long-period mutant, approximately 76 seconds.

Still other long-period mutants are slow learners in the game of Drosophila mating. When normal amorous male flies are rejected by a female, they quickly learn to seek companionship elsewhere. However, certain long-period mutants are persistent-but-dumb suitors — unable to learn to "buzz off" when rejected.

The production of "mosaic" flies is one technique Konopka and his colleagues use to find out which brain cells are responsible for such behavior. Mosaics are insects possessing genetically different cells in the same animal. These cells can show the scientists which fly-brain cells are the key clock cells. Thus, if a mosaic fly with short-period cells and arrhythmic cells assumes a short-period rhythm, the scientists know that the brain clock cells are of the short-period type. The scientists program special marker genes into these mosaic cells that enable them to use a little detective work to narrow down the exact brain cells responsible for the clock.

Incidentally, the onerous duty of figuring out the day-night cycles of hundreds of flies is not heaped upon some poor student; it has been taken over by a fly-watching computer. A fly to be monitored is first placed inside a small tube with a supply of food. The tube is then placed in a rack, along with hundreds of others within a special temperature- and light-controlled chamber. Beneath each tube are photoelectric cells, and each time the fly moves over a cell, the computer counts the pass. By statistically analyzing the number of passes, the computer can trace the activity cycle of the fly.

From these studies, Konopka has advanced a tentative theory to explain the phenomenon of circadian rhythm, found throughout the animal kingdom, including man. According to this theory, the clock genes may code for an ion pump found in the cell membrane. This pump may operate during the day, moving the ion out of the cell like the sand running through an hourglass. By nightfall, the ion is exhausted, activity slows, and the pump begins a recharge cycle. Slow cycles like the circadian rhythm may employ slow pump systems; fast cycles such as the courtship song may employ fast pumps.

These of course, have been only brief descriptions of the sophisticated studies being carried out using Drosophila. However, they do show clearly what this little fly means to the science of biology.

So, while that tiny fly flitting about an overripe peach or a past-its-prime banana may be a nuisance in your kitchen, in the laboratory it's a treasure. □