Seymour Benzer pioneered the use of the fruitfly *Drosophila melanogaster* as a model system for the study of nervous system function and development. His research spanned a range of topics from identification of mutations resulting in temperature-induced paralysis to neurodegenerative diseases and circadian rhythm. Benzer’s indomitable style as a scientist is well worth emulating; his work was driven by his curiosity rather than public pressure or fashion trends.

Can genetics be used as a tool to understand the brain and to decipher how it directs behavior? Today, studies on behaviors as esoteric as learning and memory, aggression and even sleep are being studied using the fruitfly *Drosophila melanogaster* as a model organism. The field of behavior genetics was pioneered by Seymour Benzer, who had the foresight to see that principles learnt from relatively simple systems, could provide a toolkit for understanding our own complex brains. By the early sixties, Benzer had already deciphered the fine structure of the rII region of bacteriophage T4 (discussed in the article by Jayaraman) and became increasingly interested in the question of how genes influence behavior. This was possibly inspired by his two daughters who were very different from each other in their behavior and mannerisms although they were nurtured in the same home. The relationship of genes to behavior was a rather contentious issue at that time and capable of generating heated debate often fueled by political undertones.

**The Start of *Drosophila* Neurogenetics at Caltech**

Benzer’s interest in neuroscience took him to the laboratory of Roger Sperry at the California Institute of Technology (Caltech) where he later moved his laboratory. Caltech was already the...
centre of *Drosophila* genetics; Thomas Hunt Morgan\(^1\) and his team had established the ‘fly room’ in the thirties and Alfred Sturtevant and later Edward Lewis, carried on this tradition. When Benzer went to Caltech, Lewis was actively involved in genetically dissecting the bithorax complex of the fly. Using elegant genetic strategies, Lewis showed that genes are made of components that could be separated and their position defined. These studies were comparable to Benzer’s own work on the fine structure mapping of the *rII* region of bacteriophage T4. After frequent discussions with Lewis, Benzer decided to use *Drosophila* as the organism to identify mutants affecting behavior [1-].

The rationale was similar to that used for genetically dissecting biochemical pathways in bacteria and fungi. Mutations that failed to produce the endpoint of the pathway were isolated. In the case of biochemistry, this was usually a metabolite, while for nervous system function, the endpoint was behavior. Benzer first set out to isolate mutants in an innate behavior – the movement of flies towards light (phototaxis). He designed a ‘counter current assay’ which could repeatedly test the fly’s ability to be attracted to light (Box 1). Following mutagenesis, he isolated a range of mutants that were defective in phototaxis – some that were not attracted to light (non-phototactic), others that were repelled by light (photophobic) and flies that were sluggish. Mutations with defective phototactic behavior were later found to affect the structure of the *Drosophila* compound eye or resulted in visual degeneration [2].

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**Box 1. The Counter Current Assay**

The basic principle of the assay consists of partitioning the flies by giving two choices. The apparatus consists of two glass test-tubes, one sliding into the other. Flies are deposited in one tube, covered by the other tube mouth to mouth and placed horizontally. The flies are given two alternatives (for example light or darkness by having a light source near the empty tube) and the tubes are tapped on a rubber mat. Flies can remain in the original tube or fly into the other tube (towards light). The partitioned flies are again tested by offering the same choices in six cycles. The apparatus allowed identification of non-phototactic (flies randomly distributed), photophobic (flies distributed preferentially in away-from-light tubes) or sluggish (flies found in the start tube) strains. For more details, see [8].
Soon, the Benzer-lab attracted a cast of students and postdoctoral fellows who saw the value of “ask(ing) a stupid question to (often) get amazing answers”. The question of how animals regulate their day-night activity rhythms is of great interest, as anyone who has taken a trans-Atlantic flight will readily agree. Ronald Konopka joined the lab as a graduate student and initiated studies on circadian behavior. Benzer’s laboratory designed a paradigm that could monitor the activity of the fly throughout the day and identified the first mutation-period with an abnormal activity rhythm. Stemming from these studies, the field of circadian rhythm genetics in *Drosophila* made rapid progress when the laboratories of Michael Rosbash and Jeffrey Hall at Brandies University on the one hand, and that of Michael Young at the Rockefeller University on the other took the field forward. Today we know a great deal about how cells use ‘molecular switches’ to encode time [3].

Other successes soon followed; the Benzer lab designed another simple test to ‘teach’ flies to avoid an odorant if it was paired with an electrical shock. Flies could learn and remember, and mutations in single genes such as *dunce* failed to learn; *amnesiac* could learn, but forgot very easily. This early work by Duncan Byers, Chipp Quinn, Yadin Dudai and others was the cornerstone of an exciting field of research on the mechanisms of learning and memory [4].

**Nerve Conduction and Synaptic Transmission**

The basis of the brain function lies in the properties of a single cell – the neuron – and how it conducts impulses. The biophysics of nerve cell membranes was well understood due to the pioneering work of Hodgkin, Huxley and Katz. However the molecular mechanisms underlying neuronal function and the nature of the channels were unclear. Failure of nerve conduction or synaptic transmission would be expected to result in paralysis. This possibility prompted Benzer and his students to isolate mutations that resulted in temperature-induced reversible paralysis and identify the corresponding genes. Obaid Siddiqi, then on a
sabbatical from the Tata Institute of Fundamental Research (TIFR, Bombay), and others isolated and studied a number of mutants in behavioral assays (Figure 1) and by electrophysiology. The electrophysiological preparation of the larval nervous system, standardized by Yuh-Nung-Jan, Lily-Jan and Bill Harris allowed the characterization of nerve conduction and synapse function in a range of paralytic mutants. Mutations such as paralytic affected neuron conduction, while comatose and shibire affected synaptic properties [5]. The Shaker gene was later cloned independently in the laboratories of Yuh-Nung and Lily Jan, Mark Tanouye, and Alberto Ferrus and shown to encode a component of K⁺ channels.

Mosaic Mapping of Mutants

Within a few years, mutant collections from the Benzer and a few associated laboratories included strains named to describe their interesting phenotypes; drop dead, bang-sensitive, ether-a-go-go, stoned and many others [6]. These mutations affected the whole animal. Was there a region in the brain that, when defective, triggered these abnormalities? Yoshiki Hotta, a postdoctoral fellow from Japan, decided to extend Sturtevant’s original idea of mapping external body parts of the fly to the primordial cells on the blastoderm. This analysis was based on the presence of mosaic individuals formed as a result of the loss of specific chromosomes in some cells during early mitotic division. In these individuals, one organ or body part is genetically different from the other. Hota generated mosaic animals and ‘mapped’ the mutant phenotype being studied with respect to easily-scorable visible phenotypes. As with conventional genetic mapping, the further apart two sites are, the more likely it is that the boundary of the mosaic will fall between them. These distances between foci were mapped in sturts (in honor of Sturtevant) in much the same way as genetic distances are mapped.
in morgans (in honor of Morgan). From these studies, it was possible to show whether the focus of a behavioral trait was 'submissive' or 'domineering' (analogous to recessive and dominant) and where in the animal the mutant trait had its effect.

**Drosophila Compound Eye and the Basis of Cancer**

Analysis of phototactic mutants led Benzer to embark on studies on the development of the *Drosophila* compound eye with Don Ready. The eye of *Drosophila* shows an exquisite organization leading Benzer to describe it as a neuro-crystalline lattice (Figure 2), this pattern allows for easy identification of mutants. Each of the approximately 800 ommatidia is composed of 20 cells, eight of which are photoreceptors. A number of mutations results in a lack of a subset of photoreceptors; perhaps the most valuable of those mutations was *sevenless* which lacks the R7 photoreceptor [7]. Subsequent molecular cloning of this gene showed that it encoded a membrane-associated protein with Tyrosine Kinase domains. The identification of this receptor tyrosine kinase and subsequent analysis of associated genes (bride of sevenless, etc) helped elucidate a pathway that is of great importance in development and cancer.

Cloning and expression studies on many of the genes discussed above showed them to be widely expressed in the nervous system. The specific behavioral effects seen were a consequence of 'partial loss-of-function' or hypomorphic mutations isolated in genetic screens. Null alleles, often resulted in lethality, as the genes are essential for neuronal function. Such 'Benzer-style' behavioral screens, in which the necessity for adult-survival ensured hypomorphic mutations in most cases, are invaluable in identifying genes required for neural function and helped enhance our understanding of neural physiology and cell biology.

**Use of Flies to Study Humans**

Benzer was always interested in humans. The transformation in

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**Figure 2. The compound eye of Drosophila melanogaster—a neuro-crystalline lattice.**

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the tools of biology makes results from *Drosophila* behavior genetics both meaningful to humans as well as central to the debate on how genes affect behavior. Interestingly, a survey of 287 human disease genes showed that 178 (67%) of them have possible homologues in *Drosophila*. Benzer, in collaboration with Carol Miller (his wife), tested 146 monoclonal antibodies raised against homogenates of *Drosophila* brains, and found that about half of them recognized cells in the hippocampus, cerebellum, spinal cord or optic regions of the human brain. The similarity between human and *Drosophila* neuroscience is heartening for the fly biologists.

With prescience that information from flies would be more generally applicable, Benzer had already begun studies on topics that appeared pertinent to human conditions. His interest in neurodegeneration led him to study the *dropdead* mutation, followed by isolation of a number of degeneration mutations including *swisscheese*. Many human genetic disorders are the result of mutations known as trinucleotide repeats, whereby the DNA sequences of functionally important genes carry several repeats of three nucleotides. In many cases, this results in the addition of multiple glutamate residues known as polyglutamate in the protein sequence encoded by these genes. Ectopic expression of polyglutamate repeat proteins in the compound eye was shown to induce degeneration; this phenotype therefore allows genetic analysis of the mechanisms by which polyQ proteins result in disease. There is no doubt that the disease with the highest prevalence is ageing. In an effort to understand mechanisms of ageing and longevity, Benzer and his colleagues screened for mutants with extended life spans. A spectacular mutation *Methuselah*, significantly increases lifespan of *Drosophila* and also renders them more resistant to environmental stresses.

His interest did not end here; Benzer also analyzed mechanisms underlying the sensation of pain, temperature and the alarm responses triggered by carbon dioxide. Seymour Benzer’s lifetime’s research has impacted many areas of modern neuro-
science. His legacy has set the ground for demystifying the role of genes in behavior and led to the identification of many components essential for cellular function and thereby circuit properties. Yet, our understanding of how network function relates to behavior and how this is put in place during development, remains poor. This is the great challenge of neurobiology, one where, inspired by Benzer, students of today have the opportunity to chart new paths without getting overwhelmed or swept by the tide of technology or information or popular opinion.

**Suggested Reading**