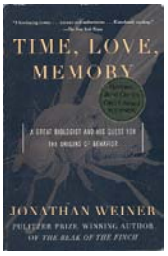
The Egg with Two Yellows

M K Chandrashekaran



Time, Love, Memory: A Great Biologist and his Quest for the Origins of Behavior
Jonathan Weiner
Knopf, New York, pp.314,1999
Hardcover Price:US $27.50

This book is about the life, times and work of Seymour Benzer, one of the founders of molecular biology. Whereas most biologists would recall, to varying degrees, the contributions of Francis Crick, Max Delbrück, Karl von Frisch, François Jacob, Konrad Lorenz, Jacques Monod, T H Morgan, H J Muller, Linus Pauling, Niko Tinbergen, E O Wilson and James Watson, Seymour Benzer's work and his place in the pantheon of biology are recognized adequately only by the community of molecular biologists. Benzer is possibly the most versatile, productive, self-effacing, and the least flamboyant of the founders of molecular biology. Inasmuch as that is the case, a book of this kind had to be written explaining, in popular language, the contributions of Benzer for over six decades which have changed the face of biology and neurobiology. The title of the book is somewhat misleading, for it would be denying honour properly due to Seymour Benzer to say that his work was limited to a quest to understand the origins of behaviour. His work has enriched biology in many other ways than by his discovering clock mutants, love mutants and memory mutants in *Drosophila*. After years of research on the genetics of virus, especially in the 1950s, he was able to present the world with the first 'fine structure map' of a gene. These early path-breaking contributions of Benzer would lead to the explosion of genetic mapping and genetic engineering that now dominate biology. Other biologists would have made much of this achievement and sought for themselves a place in the sun. But Benzer, as it emerges from this fine biography, was far too modest for such attention seeking. What he did in elucidating the fine structure of the gene was epochal and bridged the experimental gap between formal genetics and DNA. His old phage colleagues from Cold Spring Harbor days still talk about that phase of Benzer's work with something close to awe.

Trained as a solid state physicist in Purdue University, Benzer made some of the basic discoveries that led to the construction of the transistor. In 1946 a friend gave him the by now famous book by the Austrian physicist Erwin Schrödinger, *What is Life?*. In this book Schrödinger was trying to build bridges connecting the world of atomic physics with the world of genetics. The book's chief reference was some work that Max Delbrück had done before he left Germany on the idea that a gene mutation is like a quantum jump. Schrödinger's inspirational book had a tremendous effect on a whole generation of scientists

Reproduced from *Journal of Genetics*, Vol.78, No.3, December 1999.

(especially physicists) because it made the gene problem appear like *the* problem to solve. After reading the book, Benzer joined the molecular biologists who were just then readying themselves to crack the secret of the gene. He enrolled in the Cold Spring Harbor course on bacterial viruses in the summer of 1948, joined Delbrück's phage group, 'the fountainhead of molecular biology' (G Stent), and went to Delbrück's laboratory at Caltech as a postdoctoral fellow in the fall of 1949. Within one day of joining the phage group Benzer said, in the jargon of Delbrück and Luria, that he became instantly *induced, transformed, determined* and *committed* to be a biologist. In spite of the apparent lightness of that remark, it was not an easy decision to make. The Benzers had a one-year-old baby and both Seymour and Dotty came of very poor Jewish families, his from a *shtetl* west of Warsaw. Instead of coming to biology he could have thought of becoming rich on the six patents he had taken on his work on semiconductors. 'Benzer had believed' writes Weiner, 'that happiness is the pursuit of curiosity and a fall from pure science is a fall from grace', an ideal now out of vogue.

Benzer retained his base in the department of physics at Purdue and became peripatetic in the best sense of the word. In the late 1940s and early 1950s he spent a year at Oakridge National Laboratories in Tennessee, two years in Delbrück's laboratory at Caltech, a summer in the laboratory of Cornelius van Niel at Pacific Grove, and a year in André Lwoff's laboratory at the Institut Pasteur in Paris, doing all the while 'pretty and witty' experiments. Early in his career Benzer had accepted as his role model the ideal of a great scientist as portrayed in Sinclair Lewis's novel *Arrowsmith*: Max Gottlieb, 'the mysterious German biologist'. *Arrowsmith* was a great favourite of Benzer, because it showed science as an adventure, a romance, a pure faith and a way of life. This charming book about the idealism of science written in 1925 must be made compulsory reading for aspiring biologists, even in these days of 'venture capitalism'. Martin Arrowsmith and Max Gottlieb keep appearing and reappearing – like a refrain – during the various stages of the career of Benzer. As told in Weiner's book, Benzer was Martin Arrowsmith incarnate, his wife Dotty was likened to Arrowsmith's wife Leora, and Max Delbrück stood for Max Gottlieb.

From the beginning of his career Benzer kept a low profile, and worked mostly in the middle of the night, and worked very hard. The news of James Watson and Francis Crick putting together the double helix model of DNA arrived in early April of 1953. Weiner exclaims, 'Physics, chemistry and biology came together beautifully in one spiralling molecule, the staircase of DNA, now an icon of twentieth century science.' During the growth of classical genetics, the word 'gene' was defined as the unit of genetic material that is passed on from parent to progeny and identified operationally by its ability to mutate to alternative states. Genes recombined with other comparable units to impart to the organism a particular phenotype. Working with a

bacterial virus Benzer showed that there is no such thing as the 'classical' gene. The unit of recombination is a single DNA nucleotide base pair and the unit of mutation can vary from a single base pair to hundreds of nucleotide base pairs. And the unit of a function is a sequence of hundreds of nucleotides, which specifies the sequence of amino acids that make up the primary structure of a polypeptide. With these findings Benzer galvanized the reductionist revolution already under way in molecular biology of the 1950s. In 1959, when a geneticist put together a retrospective volume called *Classic papers in genetics*, he began his anthology with Mendel's peas, as a point of origin of classical genetics, and ended with Benzer's *r*II, as a signpost of the completion of the era.

Around 1965 Benzer turned his attention towards animal behaviour, especially as a result of reading about the split brain work of Roger Sperry at Caltech. He took a year's leave of absence from Purdue and visited Sperry's laboratory in Church Hall. Benzer thought that too many people were working on the central problem of molecular biology as defined by Schrödinger, namely the structure, self-replication and function of genes. Delbrück, who was also working at that time in the basement of Church Hall, was also entertaining doubts about his own work. By then Delbrück had spent fifteen years with *Phycomyces*. He wrote to George Beadle, who was studying the genetics of another fungus, that he was trying all kinds of things: '... from lunatic fringes to sober photochemistry. Perhaps I should train a duck (*sic*) to follow me around, that sounds like a very appealing way of life.' The remark may also be a subtle give-away of the intellectual condescension the reductionists showed for the work of ethologists. ('Ethology? Why, that's just bird watching, isn't it?')

Weiner is a bit long-winded in the part of the book narrating why Benzer finally decided to work on *Drosophila* and switched to the fly in 1966. He meanders through the work of Francis Galton and eugenics and enumerates the array of animals Benzer had, so to say, screened in his mind: anemones, starfish, octopi and tropical fish. The molecular biologists found the decision strange. His mother asked his wife, 'Tell me Dotty, if Seymour is going to examine the brain of a fly, don't you think we should have his brain examined?' But the choice of *Drosophila* would not strike other kinds of biologists as being exotic. Thomas Hunt Morgan chose it in 1907 and demonstrated the phenomenon of crossing over of genes in chromosomes and his student Alfred Sturtevant mapped the sequence of genes on the chromosome. More was known about the genetics of *Drosophila* than of any other metazoan. In 1967, when he was fortysix, Benzer published his first paper on *Drosophila*, 'Behavioral mutants of *Drosophila* isolated by counter current distribution', which is hailed as a landmark. Benzer designed his fly experiments on the lines of the 'counter current distribution' which chemists adopted to separate two compounds that were mixed together, such as oil and water. Using this principle

Benzer separated flies that moved towards light and flies that did not. Then he would look into the genes of these two sets of flies. The same year he left Purdue and joined the biology department at Caltech, where he still works.

Soon after he arrived in Caltech, Benzer set up his own Fly Room, with the distant blessings of Sturtevant and active help of Ed Lewis. A graduate student, Ronald J Konopka from Dayton, joined him, hoping to use the genetic scalpel of Benzer to find and dissect the biological clock of the fruit flies. By then extensive information about the *formal* properties of biological clocks from fungi to humans was already available. Experiments carried out in 1932 by crossing strains of bean plants with different periods had indicated a genetic basis for circadian rhythms. In fact the circadian rhythm in the eclosion of adult *Drosophila melanogaster* had been reported (by E Bünning and H Kalmus, independently, in 1935) and the temperature compensation of circadian rhythms, which, more than any other characteristic, conferred the quality of reliable chronometers on them, was reported also for *Drosophila* (by C S Pittendrigh in 1954). The defining features of circadian rhythms are (i) persistence in constant light and dark in the laboratory with a circa 24-hour period, (ii) temperature compensation of period, and (iii) entrainability/phase shifting by light/dark cycles. These diagnostic characteristics are widely shared among organisms from fungi to humans, suggesting that circadian organization is evolutionarily an ancient and highly conserved process. Circadian rhythms might have arisen when life came from water to land and are known to be involved in seemingly unrelated physiological and behavioural processes. They are involved in events such as circatidal rhythms, lunar rhythms, orientation and homing in crustaceans and birds, and in seasonal phenomena such as flowering in plants and bird migration. No one knew how simple or complex the genetic basis of circadian rhythms was. In an analogy to mechanical clocks one reasoned that there may be hundreds of working parts. Any of hundreds of mutations may destroy the sense of 24-hour time in flies. Konopka patiently went about screening populations of flies in Caltech in an atmosphere of widespread scepticism about the Konopka–Benzer mission. What was momentous then, and for all time to come for molecular biologists, was that Konopka did find the first clock gene in *Drosophila melanogaster* (sometime in 1970) and fascinatingly it could be mapped to a single locus on the X chromosome. He found three mutants at the same locus: short period, long period and arrhythmic. Konopka was fortunate to have found the mutants after he had screened barely some two hundred strains. (Konopka's law: 'If you don't find it in the first two hundred, quit.') He had now earned the privilege to name the gene he had discovered. Because mutations of the gene had the power to alter the period of the fly's circadian clock or even abolish the clock Konopka called it the *period* (*per*) gene.

In 1971 Jeff Hall, who had a sound back-

ground in *Drosophila* genetics, joined Benzer as a postdoctoral scholar. It goes to Hall's credit for having recognized that *period* would turn out to be one of the most versatile genes ever discovered. It affects the totality of the flies' activity, rest, metabolism, physiology, and of course behaviour. In the early 1970s Hall was interested in finding the genetic cause(s) of male sterility in the fruit fly, and soon accumulated hundreds of mutants with interesting courtship problems. Hall gave them names like *celibate*, *coitus-interruptus* and *stuck*. The first courtship mutant had been discovered and reported by K S Gill, an investigator from India, then working in Yale, in 1963. This was before Benzer started working with *Drosophila*. Gill gave the mutant the luckless name *fruity* and apparently did not follow up his work. Jeff Hall read about this work and immediately recognized that this was a very interesting courtship gene. He renamed it *fruitless*. These mutant males courted other males and formed chains holding on to one another.

Hall's own breakthrough came later in 1973 in his own Fly Room in Brandeis University when he and a postdoc Charalambos Panyiotis Kyriacou, of Greek ancestry, British citizenship and a background in psychiatry (called Bambos by his friends), investigated the courtship 'songs' of *Drosophila* males. The males make a buzzing noise with the wing closer to the female. To us humans the songs, when amplified, sound alike but the female flies recognize males of their own species. Hall obtained a few test tubes of Konopka's clock mutants and Kyriacou recorded their love songs. The courtship songs of the male flies occur in brief bursts. There are two components, a hum followed by a train of pulses. The interpulse intervals (IPI) of the songs vary from 30 to 70 milliseconds. Kyriacou and Hall assayed IPIs of *Drosophila* courtship songs in a time-dependent manner. They discovered that the mean IPIs appeared to oscillate with a period of 50-65 seconds in the laboratory strains of *D. melanogaster*, and in *D. simulans* also a cycle was observed but the period was much shorter, about 35–40 seconds. These song periods are species specific. In play-back experiments with artificial songs *D. melanogaster* females clearly preferred songs with a 55-s cycle super-imposed on *D. melanogaster* IPI background of 30–3 8 ms, to a song with the same 55-s cycle but on a *D. simulans* IPI background of 43–53 ms. They also preferred this song to one with a 35-s cycle superimposed on *D. simulans* IPI background. However, the mating behaviour of *D. simulans* females was enhanced only when they were stimulated with this latter song, i.e., one with a 35-s cycle on a *D. simulans* IPI background. Consequently females of both species mate fastest when stimulated with the 'natural' song of their conspecific males, clearly indicating a function for the song cycle and the IPI length. The critical result here is that for each species both the IPI and the song cycle must be in the correct species range for the females to respond. For the record A W Ewing and H C Bennet-Clarke were the first to report in 1968 that *D. melanogaster* females mated best when stimu-

lated with an artificial song with 34-ms 'melanogaster' IPI compared to one with a 48-ms 'simulans' IPI.

Probably the most exciting results to come from the early studies of Kyriacou and Hall of song cycles was the finding that *per* mutations affected the song cycle. Males carrying the per^S mutation have a 19-h circadian rhythm in locomotor activity and produce a 40-s song rhythm. Similarly per^L males with their 29-h rhythm of locomotor activity have 80-s song cycles. Finally per^0 mutants ostensibly arrhythmic in locomotor activity also gave apparently arrhythmic song cycles. Thus the *per* product was being used in two oscillating mechanisms, the circadian and the ultradian, in similar ways.

Hall, ever the versatile geneticist and the man with many ideas, had never learned cloning. Cloning genes turned molecular biologists into genetic engineers, who could excise a gene from one species and put it into another species and watch. A young molecular biologist named Michael Rosbash ('arrogant, always irreverent, extremely ambitious, and... a reputation for being cutthroaty – but also for being very smart' (a molecular biologist's description of Rosbash)) was then a close friend of Hall at Brandeis. Even before Rosbash and Hall got going with the business of cloning the *period* gene of *Drosophila* another young molecular biologist, Michael Young of Rockefeller University, had just concluded that cloning *period* may be a grand way to get started. In the post-double-helix phase the ethos of molecular biology was to race. The rival laboratories of Jeff Hall and Michael Young began racing their findings into print – Hall and Rosbash in the December 1984 issue of *Cell* and Young *et al*. in an issue of *Nature* that straddled December 1984 and January 1985. In 1988 Kathy Siwicki, a postdoc in Hall's lab, discovered that *per* gene expression was evident in a large number of cells and cell types in the adult visual system and brain. In 1990 a student in the Rosbash lab, Paul Hardin, discovered that *per* mRNA also cycles. In the 'rival' Fly Room at Rockefeller, two of Michael Young's postdocs, Amita Sehgal and Jeff Price, discovered the gene *timeless* on the fly's second chromosome. *period* and *timeless* made proteins that oscillated in a circadian pattern and when mixed in a Petri dish bonded strongly. The same year Sehgal and Price found *timeless*, Joseph S Takahashi, a molecular biologist at Northwestern University, found a clock mutant mouse, and the clock gene was named by him and his colleagues *Clock* (*c*ircadian *l*ocomotor *o*utput *c*ycles *k*aput). In 1999 Takahashi and colleagues reported successful molecular cloning and characterization of the human *CLOCK* gene and its expression in the suprachiasmatic nuclei. It now turns out that the human *CLOCK* gene is expressed not only in the suprachiasmatic nuclei and the brain, but in the pancreas, kidney, skeletal muscle, liver, lung and placenta. Given the multiplicity of circadian oscillations in the physiology and behaviour of humans, the human body has been likened to a 'clock shop'. The consequences of alterations in the *CLOCK* locus, however, need not be restricted to circadian

phenomena as strictly defined. The circadian organization has been experimentally shown to regulate hormone release and age-related human illnesses, as well as a number of psychiatric disorders such as schizophrenia and depression. In 1998, as all these findings on the genetic basis of circadian rhythms fell into place, a molecular biologist in Switzerland praised the clockwork story in the journal *Nature* for its heuristic value, a value that makes it a classic in the annals of molecular biology.

Many students of Benzer feel that it was Hall's vision and dynamism that made the field of *Drosophila* clock genetics come of age. In the late 1970s and 1980s Hall felt embattled, that he was holding the banner of molecular genetics of circadian rhythms aloft all by himself. Benzer, with his credentials and fame, could have identified himself more closely with the whole field he had started and stood more steadfastly by Konopka, especially in the earlier stages of research on the *period* gene.

Weiner's book gives a somewhat good account of the contributions to *Drosophila* genetics of Thomas Hunt Morgan and his student Alfred Sturtevant. It is moving to learn that Morgan, who was largely responsible for the impending immortality of *Drosophila* and had discovered crossing over, struggled to keep up with the findings of his students and, at least on one occasion, gave up saying it was too much for him. What a contrast to the 'superlatively arrogant' crew of molecular biologists, who even in the 1950s and 1960s began talking about 'the days of genetics' as though they were referring to prehistory. One of Sturtevant's star students Ed Lewis recalled, '*Drosophila* went into almost total eclipse.' Delbrück would pound the table: 'Genetics is dead! Genetics is dead! Genetics is dead! – molecular biology is the only biology.' (Manny Delbrück: 'You see, Max didn't *know* any other biology.') It is interesting, looking back, to note that if euphoria among the molecular biologists was very quick to set in, so was the subsequent ennui, barely one and a half decades after Crick and Watson's discovery. Gunther Stent began writing pessimistic essays ('That was the molecular biology that was', *Science*, **160**, 390–395, 1968) and was musing aloud if the genetic dissection of behaviour can reveal anything more about the origin of behaviour than dissection with a scalpel, and Delbrück was unhappy about the unsolved problem of the stalk of a fungus growing toward light.

Weiner says he had originally planned to write a book titled *A sense of time* concentrating on the happenings of Konopka and after, but fortunately realized that the story properly began at the turn of the century. Even so the dominant theme of the book, very unevenly unfolded by Jonathan Weiner, is the discovery of the *period* gene by Ronald Konopka. The narration weaves back and forth at a giddy pace in telling the story of the discovery of the clock gene and work that followed. Having mentioned *origins of behaviour* in the title of the book, and invoked *genes and*

behaviour in practically every chapter, Weiner, one expects, would touch upon the names and work of at least some prominent players in the field of biological clocks and behaviour research in *Drosophila*. In terms of physical space and without adding to the bulk of the book, this could have been achieved by doing away with the detailed description of the work of Francis Galton, Eugenic Fairs and mass sterilization programmes in America. For, as Weiner admits, even Benzer knew that he had to start with something simpler. Probably Weiner wanted to restrict the narration to describe only the contributions of those who were personally inspired by Benzer's work.

The only thing that is mystifying in this otherwise uncomplicated book is the myriad meanings the protagonists read into the word *behaviour*. Of course the word has always meant, and would always mean, many things to many people. It is not easy, nor even reasonable, for any one person to want to tie up the findings of Franz Boas, Frank A Brown Jr, Vincent Dethier, Henri Fabré, Jerry Feldman, Karl von Frisch, Sigmund Freud, Francis Galton, Karl Hamner, Konrad Lorenz, Ivan Pavlov, B F Skinner, Roger Sperry, Niko Tinbergen, John Watson, E O Wilson and Ludwig Wittgenstein and make them to relate intellectually. It is small wonder that Jeff Hall and C P Kyriacou got impatient when they were incessantly queried about how the clock gene could come to be the basis of *any* behaviour. It is in the meantime clear, at least to all chronobiologists (students of biological clocks), that many behavioural traits, from fungi to humans, have a *circadian* organization, and are therefore obvious expressions of the underlying clock gene(s).

One hopes Weiner would write his book *A sense of time* and that the next time around remember the pioneers of that field. Prominent among them are J Aschoff (contributor to our understanding of light relations of circadian rhythms in birds and mammals and in humans and formulator of *Aschoff*'s *rule*), E Bünning and Colin Pittendrigh, who laid down the coordinates of modern biological rhythms research. In fact Pittendrigh is directly in the line of *Drosophila* succession of T H Morgan, having done his PhD with Theodosius Dobzhansky, who was Morgan's postdoc. Konopka and Benzer had, in fact, acknowledged Pittendrigh in their paper of 1971, in which they announced the discovery of clock mutants of *Drosophila melanogaster*, for enlightening discussions and loan of his equipment with which Konopka monitored the eclosion rhythms. In 1970–71 Konopka was a frequent visitor at the laboratory of Pittendrigh at Stanford University. Unfortunately Konopka published only a few papers on the subject of the *period* gene and left it to others to continue. Konopka did not get tenure in Caltech and moved to a college called Clarkson and was in a kind of scientific exile.

Even though the stories Weiner tells are in themselves amusing, it is not clear what kind of readership he had in mind. There are innumerable anecdotes and Weiner often quotes the many scientists with whom he spoke. Some quotes are enlightening, others merely baffle.

The parts of the book on memory mutants are rather nebulously developed and do not tell a straight story.

Some of the stories, such as Hirsch's attacks and Lewontin's polemics against Benzer, are not well developed or explained. The competition between Gerry Rubin's laboratory in Berkeley and Benzer's group appears to be, in retrospect, a trivial matter. Weiner's book is certainly not a technical compendium of the researches of Seymour Benzer and his students. In fact there is so little written about Benzer's own researches in the second half of the book. For a lay reader the book does not explain adequately the main corpus of work that made Benzer famous in the first place. Even the central question of what genes can really tell us about behaviour is not properly examined. But as scientific reportage, which is what it is, this fine book is a fitting tribute to Seymour Benzer, a great and exemplary biologist and a scientist's scientist, who, at seventyseven, is still working through the night in Church Hall, hoping that in the course of the night (like Max Delbrück before him) he will 'find something worth telling Aurora, the goddess of the dawn'.

M K Chandrashekaran, Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore 560 064, India. Email:mkc@jncasr.ac.in

Seymour Benzer, Rita Levi-Montalceni, Ed Lewis and Sydney Brenner at Caltech.
(Credit: Caltech Archives)