More Fun Than Fun: Is Evolutionary Medicine Coming of Age?

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Cell cultural flasks of Peyton Rous, circa 1936. Photo: Lubosh Stepanek, Courtesy of the Rita and Frits Markus Library, The Rockefeller University

RAGHAVENDRA GADAGKAR

This article is part of the 'More Fun Than Fun' column by Prof Raghavendra Gadagkar. He will explore interesting research papers or books and, while placing them in context, make them accessible to a wide readership.

I spent the second half of the 1970s at the Microbiology and Cell Biology Laboratory at the Indian Institute of Science, Bengaluru, immersed in studying the lysogenic mycobacteriophage I3. Bacteriophages are viruses that infect bacteria and use the host bacterial machinery to make copies of themselves. This one infects *Mycobacteria*, hence it is a mycobacteriophage. Lytic bacteriophages burst open the host-bacterium to release their offspring, infecting other healthy bacteria and continuing the cycle. On the other hand, lysogenic bacteriophages have a dual strategy: they can either follow the lytic life cycle right away or lie low for many bacterial generations before they make copies of themselves and burst the host cell.

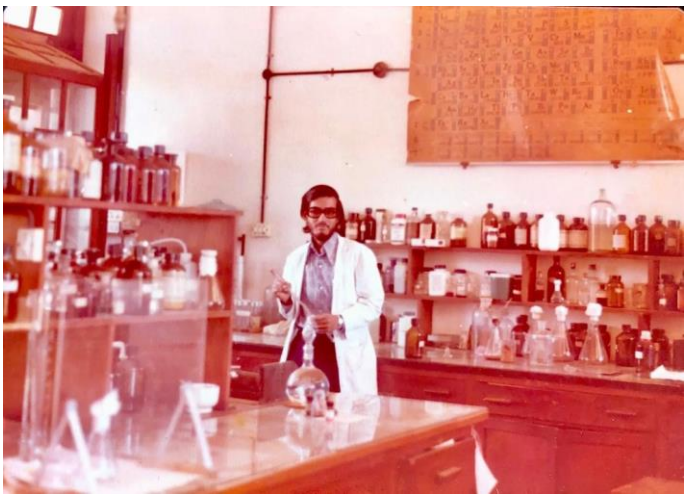
As I have described in more detail elsewhere and repeat here in part, as an undergraduate at Central College, I fell in love with two subjects – animal behaviour and molecular biology, neither of which were taught with any degree of passion or competence by my teachers. My love for animal behaviour was born from reading *King Solomon's Ring* by the Nobel laureate Konrad Lorenz and sustained by my discovery of many colonies of the Indian paper wasp *Ropalidia marginata* on the windows of the zoology and botany departments. And my love for molecular biology was born from reading *The Double Helix* by another Nobel laureate, James D. Watson and was sustained by my discovery in the

pages of journals in the library, of an exquisite organism, the lysogenic bacteriophage *Lambda*. But I soon found the local avatar of the bacteriophage lambda. One day, I jumped up from my chair in the library when I read in the pages of *Nature* [Vol 228, October 17, 1970] that C.V. Sunder Raj of the Microbiology and Pharmacology Laboratory of the Institute had discovered our very own Indian lysogenic bacteriophage. I promptly came to see him, and he showed me beautiful Petri plates in which the mycobacteriophage I3 had made transparent holes on a lawn of the bacterium *Mycobacterium smegmatis*. The 'I' in I3 was meant to denote Isolate 3, but I had no qualms about thinking of the 'I' as indicating India.

In November 1999, *Resonance – journal of science education* celebrated the bacteriophages I3 (left) and T4 (right) while paying tribute to Max Delbruck, the moving spirit behind the 'American Phage Group' (see below)

In 1974, at the age of 21, I was lucky enough to be admitted to a single vacancy in the interdisciplinary field of molecular biology. I joined the by-now re-christened, Microbiology and Cell Biology Laboratory for my PhD. I spent the next five years studying the alter ego of bacteriophage lambda, our own bacteriophage I3. Imagine my delight when I saw that the Institute campus was also home to innumerable colonies of my other love, the Indian paper wasp *R. marginata*. During the next five years, I made bacteriophage I3 the subject of my professional study, and the paper wasp *R. marginata* the subject of my hobby.

As I began my PhD enthusiastically on my favourite kind of bacteriophage, while my supervisor was away on a sabbatical leave abroad, I already encountered a major roadblock. To obtain large quantities of any bacteriophage, it is routine practice to grow the host bacteria in flasks, infect them with a small quantity of the bacteriophage, and let the bacteriophage multiply.



The author conducting experiments during his PhD at the Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore, 1974-1979.

In my case, the host bacterium of I3 was *Mycobacterium smegmatis* which our laboratory was able to grow in flasks, but I3 would not grow in the flasks. The only way to grow I3 was on a lawn of the host bacterium on small Petri plates, but this was a very tedious and laborious procedure. So, I made it my first challenge to make I3 grow in the flasks. After some very exciting detective work and testing many hypotheses, I discovered that the culprit was the detergent Tween-80 which was routinely added to the culture medium to prevent *M. smegmatis* from clumping together. I was even able to show that in the presence of Tween-80, the bacteriophage was able to adsorb onto the bacteria but could not inject its DNA into the host. And once allowed to inject its DNA, it then grew well even if Tween-80 was added later. Thus, I was able to grow large quantities of I3 by delaying the addition of Tween-80 after

the bacteriophage had injected its DNA into the host bacterium. This was a great lesson in taking on a roadblock as a challenging part of the research. Being already of an ecological and evolutionary bent of mind, I went on to investigate such questions as how different individual bacteriophages cooperated and competed with each other when they were inside the host bacterium.

One floor below my laboratory, a dear friend Arun Srivastava was similarly engaged in studying the Rous Sarcoma Virus (RSV). While my main aim was to get my bacteriophages to grow as rapidly as possible, Arun's main aim, contrarily, was to find ways of inhibiting the growth of his viruses, at least in cell cultures. The reason for this is obvious, of course, because RSV is a tumour-causing virus, and the discovery of drugs that could slow or inhibit viral growth would have great potential in cancer treatment. Arun had a novel approach. His mentor professor T. Ramakrishnan had been working on Isoniazid, a potential anti-tubercular drug. A unique property of isoniazid is that it binds to metals. The enzyme reverse transcriptase (see below), which helps RSV to reproduce, had recently been shown to contain zinc. The idea was to see if isoniazid inactivated the reverse transcriptase by binding to its zinc. In a recent issue of *Resonance – journal of science education* published by the Indian Academy of Sciences, Arun Srivastava has given a moving account of that research. Arun Srivastava is now Division Chief and George H. Kitzman Professor at the Department of Pediatrics in the College of Medicine, University of Florida, US.

Dr Arun Srivastava at the lab workbench attempting to infect HeLa cells with a genome-modified adeno associated virus (AAV) in search of more effective gene therapy on March 22, 2023. Photo courtesy: Arun Srivastava

Arun and I, and a few other PhD-mates, were mesmerised by the viruses, both bacterial and animal. We lived and dreamed of phages and viruses and read every printed page we could find about λ, T4, ΦX174, RSV, RPV and NDV, as much in love with their names as with their biology and life cycles, their replication and coat proteins, their prophages and proviruses. In our youthful exuberance, we believed we knew nearly everything about these magical creatures, which were so charmingly neither alive nor dead. How wrong we were!

We did not know their tales. This I realised only after reading *A Tale of Two Viruses* by Neeraja Sankaran. Reading Neeraja's book was a fortuitous event because I got an invitation, out of the blue, from a little-known (at least to me) online publication called *Inference* to review Neeraja Sankaran's book, which I did with great pleasure. Some of what I write below is reproduced with permission from my article in Inference. The Oxford English dictionary defines a 'tale' as a "narrative or story, especially one that is imaginatively recounted". *A Tale of Two Viruses* fits this definition well. Neeraja Sankaran's principal imaginative contribution is to draw parallels between the tales of her two protagonists, the bacteriophages, and the Rous Sarcoma Virus (RSV), my and Arun's study subjects, as it happens. Her juxtaposition of these two tales adds value and colour to each protagonist's tale and creates a whole new tale.

Neeraja's tale begins in about the second decade of the 20th century with the discovery of bacteriophages and RSV and extends forward by about half a century. But our journey with her is neither restricted to this period nor do we traverse the period chronologically. We are taken back several centuries before the present to set the context and ferried back and forth across time to benefit from hindsight. But because her narrative structure is so clearly explained in the Introduction, we are never lost.

Neeraja Sankaran and her book *A Tale of Two Viruses,* published by the University of Pittsburgh Press in (2021).

Neeraja opens with an inspired description of the birth pangs of RSV at the hands of Peyton Rous, an American pathologist working at Rockefeller University in New York, and of bacteriophages at the hands of Frederick Twort, a medical researcher in London and Félix d'Herelle, a kind of free-lance scientist working at the Pasteur Institute in Paris. With all these three pioneers, it appears that they themselves were more on the right track than most of their peers in understanding the nature of the substances they had found; so much for peer review! Two anecdotes especially struck me as they illustrate two contrasting benefits of paying attention to the history of science.

A young Peyton Rous, who won the Nobel Prize in Physiology or Medicine in 1966 for "his discovery of tumour-inducing viruses", was advised by his distinguished mentor William Welch "whatever you do, do not commit yourself to the cancer problem". A knowledge of history might make some of us a little more modest in our confidence in predicting the future and especially in second-guessing the abilities of our young mentees. In an apparent act of carelessness, Simon Flexner, the founding director of the Rockefeller Institute, attributed the early discovery of RSV jointly to Rous and his former assistant, James B. Murphy. Rous wrote in protest:

> "You said that Rous and Murphy demonstrated the existence of the filterable agent causing the chicken tumour. Now, the fact is that I carried out this work alone and published alone two papers that embodied its results… Murphy had no hand in the experimental episode which showed an 'infinitely little' agent to be the cause of the tumour…"

By paying attention to history, some of us might empathise with Rous's agony and take comfort in our sense of déjà vu, while others among us might become more sensitive directors.

The fact that a virus was an entirely new kind of entity, defying the boundary between the living and the non-living, adds much drama to the tales of RSV and bacteriophages, a drama that is captured in rich detail in *A Tale of Two Viruses*. If we put aside the benefit of hindsight, we can understand the incredulity of scientists and doctors of that era. They must have found it hard to imagine that an invisible substance that causes disease is not a mere protein or enzyme but rather a living agent that copied itself. I find it instructive to think how I might have fared in such a situation, which in turn makes me wonder whether I am already in a comparable situation regarding modern incredulities and future revelations.

Neeraja then takes us on a romp through the saga of the coming of age and the acceptance of bacteriophages and RSV as viruses. Neeraja is at her meticulous historical best in the chapter on bacteriophages, as this is based on her doctoral thesis. I particularly appreciated the light she shines on Frank Macfarlane Burnet's work on bacteriophages, which in my mind had been overshadowed by his Nobel Prize-winning work in immunology, predicting acquired immune tolerance and developing the theory of clonal selection. I was fascinated by Neeraja's refreshingly new perspective on the role of Max Delbrück and the American.

L-R: Peyton Rous, an American pathologist working at Rockefeller University in New York, 1911. Photo: Unknown; Non-Exclusive Unrestricted License, Courtesy: Olga Nilova; Frederick Twort, a medical researcher in London. Photo: Obituary Notices of Fellows of the Royal Society, Public Domain; Félix d'Herelle, a kind of free-lance scientist working at the Pasteur Institute in Paris. Photo: Service photo Institut Pasteur, Public domain.

Phage Group in the history of the concept of bacteriophages as viruses, especially because my previous reading had been dominated by their role in the history of molecular biology. It is just as well that the coming of age of the bacteriophages and RSV are treated in separate chapters because the contexts in which the two fields matured are so different. The way I see it, bacteriophages (along with their host bacteria, of course) played a pivotal role in establishing molecular biology on firm ground, all the way up to Francis Crick's central dogma; the dogma states that information can only flow from DNA to RNA to protein and not in reverse. Perhaps we should call this the fairy tale stage. On the other hand, RSV (along with its eukaryotic host cells, of course), with the discovery of reverse transcriptase and violation of at least one part of the central dogma, took centre stage in taking molecular biology out of the fairyland and making it real, complex, and messy.

In what she describes as her second intermezzo, Neeraja shows how the development of new technology—ultracentrifugation, electron microscopy, X-Ray crystallography and more—helped the scientific community to select among previously held ideas about the nature of substances that somewhat mysteriously possessed the magical properties that define a virus. I admit to a sense of awe at what these technologies could do and how they were developed with great human ingeniousness and a running collaboration between scientists and engineers. Nevertheless, I must confess my prejudice—my greater awe at what scientists could imagine, postulate, and tease out in their minds without the aid of soon-to-be-available prosthetics. I have the greatest admiration for the developers of technology, the developers of ideas, and designers of experiments without the aid of technology, and a wee bit less for refining old ideas with new technology.

For me, one of life's greatest pleasures is to read the older scientific literature and admire how people thought about complex issues and designed ingenious experiments, using the kind of ingeniousness that seems obsolete in the light of present-day technology. Early experiments in classical genetics using the fruit fly *Drosophila melanogaster* yield some of the finest examples of ingenuity untrampled by too much knowledge and too much technology, elegantly described by Richard C. Lewontin in his *The Genetic Basis of Evolutionary Change* (1974). I believe that this very kind of 'obsolete ingeniousness' will be necessary for us to be creative today before the next-generation technology makes it obsolete again. I have a kind of 'supremacy of mind over instruments' prejudice. That is why I admire more the engineers who made the instruments than the scientists who use them. My twin heroes are the engineers who make sophisticated instruments and the scientists who make do without them!

I found Neeraja's chapter, 'Lysogeny as Linchpin,' the most interesting. This must be partly because of my great love for lysogenic bacteriophages, one of which, as I described above, was the subject of my PhD thesis. But there is more. As an evolutionary biologist, I can't help admiring the 'smartness' of lysogenic bacteriophages. The other kind, so-called lytic bacteriophages, inject their DNA or RNA into a host cell, subvert the host machinery to make more copies of themselves, burst open the host cell and escape to find more hosts. Lysogenic bacteriophages can and do all of this, but do so only if the host seems healthy enough to make this option profitable. If the host bacterium is a bit impoverished, it will lie low for a while and try later. Meanwhile, it will, of course, integrate its DNA into the host DNA so that as the host divides, all their daughters will carry a copy of its DNA – the so-called prophage. When some of the bacteria appear to be in good health, the prophage will exit and switch to the lytic mode, i.e., make more copies of the bacteriophage and burst the host cell.

I have long wondered why the host carries the burden of the prophage, including the cost of replicating it in every generation, not to mention the ever-present danger that it will exit and kill it one day. Not surprisingly, there is now growing evidence that the host benefits in many ways by harbouring the prophage. One somewhat obvious advantage is that because the ability to make copies of the phage is temporarily repressed, the host is also unsuitable for making copies of other super-infecting bacteriophages that might use this cell. The prophage thus confers immunity to the host from other bacteriophages. Even more interesting is the suggestion that the prophage might help the host to tide over conditions of low nutrition. This is interesting because the death of the host also means the end of the prophage. No wonder the prophage is especially concerned about the welfare of its host in a dire situation. So much for why I love the phenomenon of lysogeny. Neeraja's interest is very different but equally interesting.

In the early history of bacteriophages, the phenomenon of lysogeny appeared to be the strongest argument against the theory that bacteriophages were viruses. The Belgian microbiologist and Nobel Laureate, a staunch opponent of the 'virus' theory of bacteriophages proposed by Twort and d'Hérelle, claimed that "The invisible virus of d'Hérelle does not exist". One ground for his disbelief was that he found it "impossible to imagine that the lysogenic bacteria had harboured viruses for generations without manifesting any signs of infection and that it suddenly underwent lysis due to the action of those selfsame viruses."

As Neeraja Sankaran has argued, the true meaning of lysogeny could not be fathomed by all but the most astute or the luckiest of scientists before the chemical nature of the genetic material and the basics of molecular biology were understood. In any case, after the famous Avery, MacLeod, and McCarty demonstration of DNA as the hereditary material in 1944, it became clear, especially from the work of the French microbiologist André Lwoff that "the invisible virus does exist" in the form of a prophage. It also became clear how the host cells "suddenly underwent lysis due to the action of those selfsame viruses". Ironically, this clarification thus became the strongest argument in favour of the virus theory of bacteriophages. As if this were not enough, understanding that lysogenic bacteriophages remain dormant as prophages in the DNA of their host bacteria paved the way for accepting the idea that tumour viruses such as RSV could do the same – by making proviruses instead of prophages. And because RSV is an RNA virus, its RNA has to be first copied into DNA before it can be integrated into the host DNA – an invitation to violate the central dogma of molecular biology and the inevitable discovery of reverse transcriptase. Little wonder that Neeraja calls lysogeny the lynchpin of her tale of two viruses. I find all this incredibly beautiful and enriching.

Almost everything Neeraja Sankaran describes in *A Tale of Two Viruses* (not including the new knowledge she has created in retelling these tales) had already transpired before the mid-1970s. How I wish Arun and I had heard these tales while studying bacteriophages and RSV for our PhD. Rich as it was, our intellectual life would have been so much more enriched by studying the history of our study objects. I am surprised that scientists pay little more than lip service to the history of science. Personally, I find that while textbooks, monographs and research papers give me the bricks to build, only the history of science and biographies and autobiographies of scientists can provide me with the cement to glue the bricks together and construct a stable and coherent edifice. This truth is brought home to me repeatedly when I read books such as *A Tale of Two Viruses* by Neeraja Sankaran, *The*

Monk in the Garden by Robin Marantz Henig, *Unravelling the Double Helix* by Gareth Williams, *The Transforming Principle* by MacLyn McCarty, *Defenders of the Truth* by Ullika Segerstrale, *Genes, Germs and Medicine* by Jan Sapp, *The Atomic State* by Jahnavi Phalke, to name some of my most recent pickings.

History informs practising scientists of how and why the questions and techniques they pursue came to be privileged over others and how ideas and theories rise and fall with time. Even more importantly, a historical perspective gives us a sense of purpose and a feeling that we are part of a grand narrative. It helps make the pursuit of science a hobby and a passion rather than a mere job. I believe science pursued without the benefit of the kind of historical perspective gained from reading *A Tale of Two Viruses,* for example, is significantly impoverished.

Lorraine Daston, director emerita at the Max Planck Institute for the History of Science, Berlin has argued most persuasively and with welcome provocativeness that scientists need to pay attention to the history of their discipline. She said in a recent interview:

> "… because of the combination of the narrowness of research specialization and the intense pressure to produce results quickly, they [scientists] have no overview of their field. Or perhaps to put it more provocatively, they don't know why they're working on what they're working on. Moreover, they don't know what the alternatives are…. The history of science has always served two purposes. One purpose has been to give that kind of orientation … Here's how the field has developed; this is why it has taken this path rather than another path … Another use, of course, is to prepare scientists for decisions that no science textbook can prepare them for, namely, ethical decisions."

If I complain about scientists not paying attention to history, I also sometimes complain about historians of science paying too much attention to who writes history. They make too big a deal of what they call the 'insider-outsider' problem. I can see that those formally trained in history and teach themselves science will write a different kind of history than those formally trained in science and teach themselves how to do history. But I believe we need both types of histories unless we are fortunate to have someone like Neeraja Sankaran, who first trained as a microbiologist [BSc (Hons)], Punjab University) and later trained as a historian (PhD, Yale). Let there be more of her kind.

Raghavendra Gadagkar is a Department of Science and Technology (DST) Year of Science Chair Professor at the Centre for Ecological Sciences at the Indian Institute of Science, Bengaluru.

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