

Joshua Lederberg (1925–2008) and the pink–orange fungus *Neurospora*

Ramesh Maheshwari

Joshua Lederberg was awarded a Nobel Prize at the age of 33. He was one of the youngest biologists to receive a Nobel. Lederberg is widely remembered as one who discovered bacterial conjugation, and recombination between strains of bacteria using simple experiments. Few know that Lederberg had trained initially in fungal biology. He pioneered the heterokaryon approach for investigation of multinuclearity in filamentous fungi.

In a recent article Sachi Sri Kantha tells how researchers read science articles and send reprint requests¹. He cites Joshua Lederberg: how Lederberg came across the landmark paper by Oswald Avery on chemical transformation in bacteria. Lederberg's own discovery of bacterial conjugation and its implications in the spread of antibiotic resistance in bacteria is given in all textbooks. Few know that Lederberg had initially trained in fungal biology and had deep interest in natural history – undoubtedly the basis of his penetrating findings in microbiology. We received a request for a paper in the form of a letter (Figure 1) from Lederberg. We found that he was greatly interested in the natural history of the fungus *Neurospora*². The reprint request is what Sri Kantha¹ refers to as an 'intellectual calling card'.

Lederberg called our attention to F. A. F. C. Went³, who we had not cited in our publication. F. A. F. C. Went was the father of the plant physiologist Frits W. Went, who was a professor of biology at Caltech, and who discovered the plant hormone–auxin. Went Sr had worked in Java, where he was attracted by masses of pink–orange asexual spores or conidia of *Neurospora* (Figure 2). The Orientals prepare a fermented food from the soyabean cake by inoculating it with conidia of this fungus. Went Sr was also the first ever to exploit *Neurospora* for measuring the action spectrum of pigmentation (a photoresponse phenomenon). For this, he had used double-walled glass bell jar, filling it with different salt solutions which absorbed different wavelengths of light. Inside the bell jar he put bread contaminated with *Neurospora*. Thus, at a time when spectrophotometers did not exist, Went Sr was able to measure the action spectrum of conidiation; and found that the blue wavelength is the most effective! In 2000, during a visit to

Bangalore, two photobiologists from Munich, Till Roenneberg and Martha Merrow, told me that *Neurospora* has a remarkably sensitive photosensing mechanism – light from the moon is sufficient to induce pigmentation and conidiation in this fungus.

Another name that Lederberg found missing in our paper was that of David R. Goddard⁴. He felt that Goddard deserves credit for identifying furfural as an ascospore activator. In our paper we had credited Mary Emerson⁵, not Goddard. The ascospores of *Neurospora* are one of the most heat-resistant and dormant cells known. For this reason they were used by Alfred S. Sussman (my first postdoc mentor) as a model for in-

vestigations of mechanisms in dormancy. Through published literature and correspondence with experts I could confirm that we had, in fact, correctly cited² Emerson (1948). While working at Caltech, Emerson had discovered that ascospores of *Neurospora* consistently showed high germination on a synthetic medium which contained *d*-xylose as the carbon source. Upon autoclaving the medium, the pentose sugar xylose is converted into furfural (C₄H₃OCHO). That Emerson discovered furfural activation is also given in a book by Sussman and Halvorson⁶. Goddard had discovered heat activation, not chemical activation. I informed Lederberg about my literature 'hunt' through correspondence with David Perkins

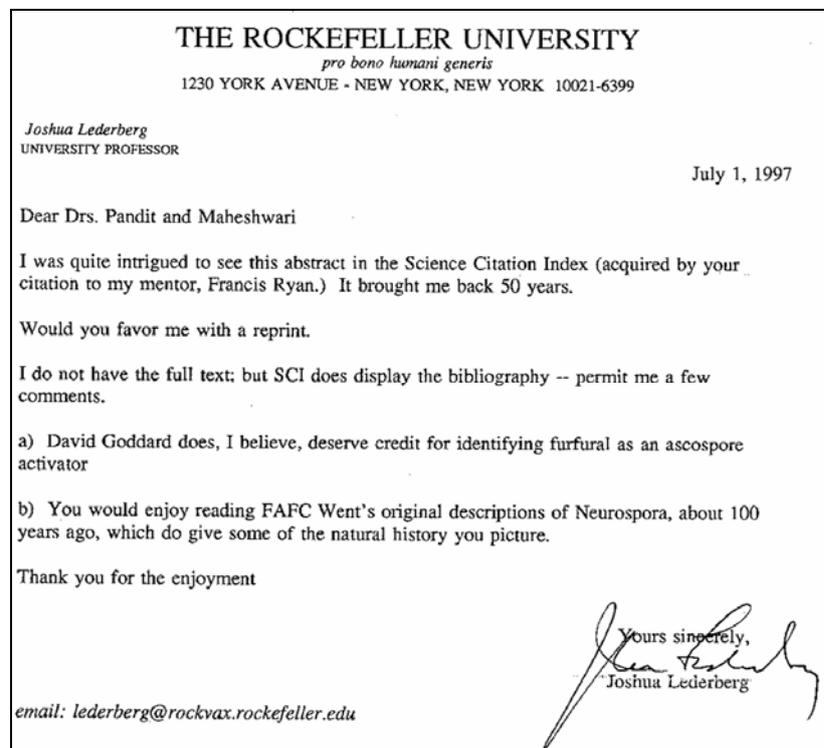


Figure 1. A letter written by Joshua Lederberg, illustrating the 'cherub functions' in passing information relating to natural history of *Neurospora*.

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(nicknamed, The Living Encyclopaedia on *Neurospora*) and Sussman. Lederberg assured me thus: 'Mine is just an old memory. If it is from Sussman, it must be right'.

Some writers credit Edward L. Tatum as being Lederberg's mentor⁷. Balaram⁸ credits Francis Ryan. In his letter to me, Lederberg himself acknowledges his debt to Ryan. Since my second postdoctoral

mentor, David Perkins too had earned his Ph D from Columbia with Ryan (1916–63), Perkins and Lederberg knew each other. I recall having received a one-liner from Lederberg, saying that he had received a mail about me from Perkins. I noted that one of the roles of a scientist is to facilitate networking. Talking to Lederberg via electronic mail was easy and stimulatory; he was most unpretentious and responded even if only in two- or three-line sentences. The email exchanges set to rest the image of Lederberg that I had carried through: At a freshers' party, an inebriated senior student in Wisconsin had told me that 'Josh is impatient. He threw his typewriter out of the window'.

During a sabbatical research in the laboratory of Perkins, I had learned that both Perkins and Lederberg had obtained their Ph D from Columbia under Ryan. I also learned that Lederberg began his Ph D research on the fungus (mold) *Neurospora*, and not on bacteria. However, it has been said (www.fgsc.net/fgn/n4/4obit.pdf) that when Lederberg became interested in the phenomenon of bacterial recombination, Ryan sent him to Yale to work with Tatum. The important point for a *Neurospora* lobbyist is that George W. Beadle, Lederberg and Tatum had employed the pink-orange bread mold *N. crassa* (Figure 3). Lederberg published his first paper when he was only 21 years of age.

For the uninitiated, the 1946 paper by Ryan and Lederberg⁸ is not an easy read; it required reading several times backwards and forwards, before it hit me that the authors were asking a profound question: Why are the fungi comprising only the single-cell thick filamentous hyphae multinucleate, whereas the cells of the more complex animals and plants are uninucleate? Until this work, the importance of multinucleate condition in fungi had not even dawned on biologists, let alone carrying out experimental work to resolve this issue. This paper of Lederberg remain as the first one to address to this question.

At the time Lederberg was working, the *Neurospora* fraternity comprised of Ryan, Beadle, Tatum and Perkins. In the environment of free exchange of ideas, materials, methods and research findings, Lederberg must have known that in heterothallic fungus such as *N. crassa*, cells of two same-sex strains are bridged for passage of nuclei through the septa and



Figure 2. *Neurospora* growing on burnt sugarcane in an agricultural field in India. Ryan and Lederberg were the first to exploit the gene controlling the pink-orange colour in experiments on nuclear selection in heterokaryons. Left, conidiating pustules erupting through cracked epidermis at the internode of burnt sugarcane stump. Right, conidiating pustules erupting from the node of burnt sugarcane stump.

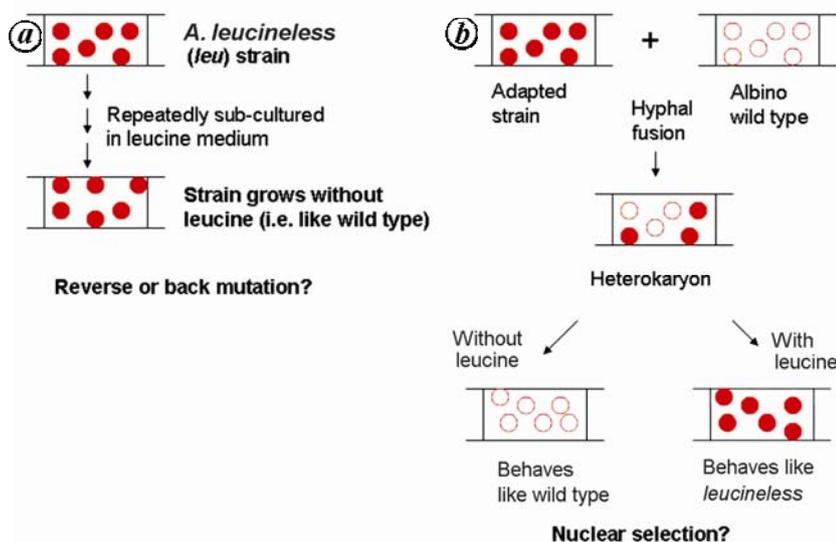


Figure 3. Diagram illustrating Ryan and Lederberg's 1946 findings on nuclear selection in artificially constructed heterokaryon of *Neurospora crassa*. The rectangle represents a hyphal compartment; the circles represent nuclei. Heterokaryon is represented with unfilled and filled circles. (a) A pink-orange-colored leucineless (leucine auxotroph) when repeatedly subcultured in medium containing leucine could grow without leucine. (b) To determine whether adaptation was due to back mutation of *leu* nuclei or the selective multiplication of wild-type nuclei, the pink-orange adapted strain was fused to an albino-coloured wild-type strain that could grow in the absence of leucine. The (*leu* + *leu*⁺) heterokaryon changed its color depending on presence or absence of leucine in the growth medium. Lederberg posed the question whether the reversible phenotypic change was due to nuclear selection or nuclear-gene silencing.

the nuclei become distributed in the hyphae. There is, of course, no nuclear fusion because the ‘parent’ strains, say (+) or (–) or *A* or *a* are of the same sex. However, hyphae of two same-sex parents spontaneously fuse to produce a mycelium. A heterokaryon constructed artificially from two strains would be an excellent system for studying whether the different nuclei residing in a common cytoplasm cooperate or combat. Lederberg’s experiment was the forerunner of the yet to come experiments on gene-silencing. Perhaps, because the title of the Ryan and Lederberg paper⁹ was not catchy, it has been missed by most workers. Lederberg mentions about this paper (<http://nobelprizes.com/nobel/medicine/conjugation.html>) as:

‘Well, I was working in Francis’ lab since 1942. In early ’44, Avery, McCloud and McCarty published their paper on the transformation of pneumococcus with DNA, and it just became obvious to me, and it was to a few people – it was resisted by some others – to put it in contemporary terms, that molecular genetics was about to begin, and that this was the medium for it, and that bacteria would be the object of that kind of investigation. Actually, my first experiments after that paper came out were to see if you could transform *Neurospora* with DNA (I was already skilled at working

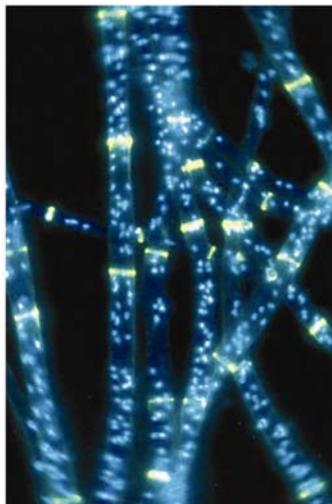


Figure 4. Multinuclear hyphae of *Neurospora crassa*. Nuclei were stained with a DNA-binding fluorescent dye. The cross walls were stained with a chitin-binding fluorescent dye. Photomicrograph under UV illumination. From Maheshwari¹¹.

with *Neurospora*). They didn’t work. Instead we found that some of the mutants of *Neurospora* that had been thought to be stable could actually rarely but spontaneously undergo reverse mutation. So, that was my first experiment with Francis. We published a paper about that.’

We are all taught that the cells in plants and animals contain one nucleus per cell, whereas fungi are multinucleated; each cell in the hypha containing tens or hundreds of nuclei (Figure 4) in cytoplasmic continuity. Ryan and Lederberg were the first to attempt a biological understanding of the multinuclear condition. Though Ryan and Lederberg have not stated this, they embraced the idea that a mixed population of nuclei harbouring different mutations could be a unique genetic system for adapting to almost any condition – the organism selecting from the mixed population of nuclei those which best allow for biochemical adjustment to fluctuating temperature and humidity, and nutrients. Ryan and Lederberg surmised that they could gain insight if they fuse cells of different genotypes (different mutations), subject the fused cells (heterokaryon) to changes of nutrient conditions, and analyse the selection of nuclei inside the heterokaryotic cell. Moreover, if they could mark the nuclei in the fusing strains by introducing marker genes controlling pigmentation in *Neurospora*, they might be able to visually monitor selection of nuclei in response to chemical conditions of growth medium. Ryan and Lederberg were among the first to experimentally study the phenomenon of reverse or back mutation and heterokaryosis as a mechanism of adjustment to the environment where conditions change.

Ryan and Lederberg provided the evidence that reverse mutation to the wild-type allele does ‘sometimes’ occur at the *leucineless* locus (which in *Neurospora* genetic terminology is abbreviated *leu*). When this heterokaryon (*leu* + *leu*⁺) was grown with leucine supplementation, the auxotrophic *leu* nuclei could not be demonstrated; the multinuclear mycelium became a *leu* homokaryon! Were the *leu*⁺ nuclei all physically eliminated or chemically silenced? The nuclei in the fusing strains were tagged by genetic colour markers (albino, *al*; and wild-type orange, *al*⁺), enabling to visually monitor

changes in the proportion of nuclei in heterokaryons. To quote Lederberg (from his e-mail): ‘it was “amusing” to see the mold changing color depending on the nutritive supplement in growth medium.’ Reading contemporary work, I believe that Lederberg was ahead of his time. Ryan and Lederberg had discovered a reversible gene-silencing phenomenon!

Lederberg provided a new insight into the significance of the unique multinuclear condition of the fungi. The multinuclear (coenocytic) cells allow the fungus to accumulate and to maintain mutations (variations) in different co-existing nuclei in the cytoplasm. A fungus with its mixed system of nuclei can rapidly adapt to any environmental conditions through rapid change in the proportion of different nuclei. It is no surprise that the American mycologist, Dodge (1872–1960), who discovered *N. crassa*, had said¹⁰: ‘The fungi...are progressive, ever changing and evolving rapidly in their own way, so that they are capable of becoming readily adapted to every condition of life. We may be rest assured that as green plants and animals disappear one by one from the face of the globe some of the fungi will always be present to dispose of the last remains.’

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Ramesh Maheshwari (formerly at Indian Institute of Science, Bangalore) lives at 53/13, Sriethertha Apartments, 4th Main, 17th Cross, Malleswaram, Bangalore 560 003, India.
e-mail: ramesh.maheshwari01@gmail.com