David D Perkins: In Memoriam

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Ramesh Maheshwari is a former Professor and Chairman of the Department of Biochemistry, IISc, Bangalore. This is his fourth article for *Resonance*.

I first met Prof. David Perkins in December 1983 in New Delhi where he had come to attend the International Genetics Congress. David was one of the Vice-Presidents of the Congress and for this reason had been lodged in a suite in the majestic Ashoka Hotel. He sensed that I was not feeling comfortable and suggested that we eat in some restaurant in Connaught Place. He enquired about my present and future research plans. I had previously corresponded with him for a thermophilic *Neurospora*, which I could use to cross to a normal strain to determine whether eukaryotic thermophily is a single- or multi-gene character. He pointed out that *Neurospora* does not grow above 42 ºC. However, he suggested that we go on an excursion the next day for a *Neurospora* 'hunt'. He was very friendly and easy to talk to. We scouted New Delhi Ridge and Buddha Jayanti Park, but didn't find any *Neurospora*. His boundless energy surprised me. The moment he spotted burnt vegetation or wood from bonfires – made by people for protection against the December cold – he would dash to the site and probe around the trash and the ash for any powdery orange or pink-orange growth, unmindful of the large number of people who gathered around wondering what this foreigner was up to. I learnt that burned vegetation is the favourite habitat of *Neurospora*. Although we didn't spot any *Neurospora*, he showed me nonetheless how, if *Neurospora* was to be found, as many as 100 or more cultures could be sampled using sterile tooth picks, conidia smeared on pieces of sterile filter paper inside small sterile glassine envelopes, which, he remarked, he always carried in his shirt pocket. He wished to explore congested areas, so we went to Chandni Chowk. All the while his eyes were focused on any rubbish dump or a pile of garbage, of which there were too many. I suffered embarrassing moments whenever a crowd surrounded us, undoubtedly wondering why a fellow citizen couldn't show better things to a foreigner.

Keywords
David D Perkins, *Neurospora*, burnt sugarcane, geneticist.

Figure 1. Prof. David Perkins meeting Prime Minister Smt. Indira Gandhi during his visit to India in 1983 to attend the International Genetics Congress.

During 1989-1990, David arranged for me and my family to visit Stanford. The very first day on our arrival he took us to his home to meet his geneticist wife Dorothy (Dot). Soon after our arrival in California, we were 'greeted' by the great earthquake in the Bay area. The gas supply and power were off. David, Dot and their long-time associate, Namboori Raju, saw to it that this nightmarish experience did not affect us mentally. The next morning, Raju and I walked to the Biology building. The police had cordoned off the building without being aware that someone had given them a slip and was working inside as usual. When alone, David was in the habit of not turning the lights on – the light from the Bunsen flame being sufficient for making crosses.

There was not a single day David missed coming to the lab. And everyday, by the time I came around 7.30 AM, he had already finished the lab upkeep, which he chose to do himself. This included the daily killing (autoclaving) of nearly a thousand test tube cultures, scores of Petri dishes, etc., discarded the previous day by people working in his lab. David could not risk contamination of one strain with another morphologically similar but otherwise a different strain. Surprisingly, colleagues could not tell me whether David ever went on a vacation, although, from reading his publications, I know that he did visit tropical countries for observing and collecting Neurospora in order that he could provide information on its natural history, distribution,

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population structure, and speciation. His vast collection provided genetic variants of the kind not obtainable by induced mutations in the laboratory. One example is the *spore-killer* mutant. This came from Perkins' collection of *Neurospora* from New Zealand and Africa. I should think that *spore-killer* would specially appeal to biologists interested in mechanisms of cell death or escape from death.

I was fortunate to attend David's practical course on the care, feeding and breeding of *Neurospora*, which he gave every alternate year, mostly for the benefit of Charles Yanofsky's students. David had so influenced Yanofsky that half of the latter's large group of students worked on the molecular genetics of development in *Neurospora*; David provided them intellectual and emotional support and material. He showed me several innovative techniques that he had developed, such as how to preserve fungal stocks in silica gels, how to determine mating type of as many as 50 unknowns on a single Petridish culture of the *fluffy* mutant (just as a chemist does spot tests on a porcelain plate), how and when to make genetic crosses, and how to identify the species based on the mating test, (for example, his discovery of a rare *N. discreta*). He had developed tester strains for identifying species based on the criteria of mating compatibility (biological species recognition, BSR). Using his tester strains and learning how to make crosses and the criteria of species recognition, I identified the Indian *Neurospora* stains that I had taken to Stanford. Even with limited efforts, I had in my collection all five heterothallic species: *crassa*, *intermedia*, *sitophila*, *tetrasperma* and even the supposedly rare species *discreta*.

During a meeting of *Neurospora* workers at UCLA, David introduced me as "a visitor from India who has *Neurospora* growing in his backyard". Never once did David reveal the slightest irritation in my having come to his lab so unprepared in basic genetic tools and techniques. David demonstrated how to collect and analyze tetrads, how to analyze and interpret crossing-data for identifying the types of chromosomal rearrangements, or use *alcoy.csp* or *multicent* linkage tester strains that he had specially

constructed for rapid assignment of an unknown mutant gene to a particular linkage group. He instructed me on how to map an unknown gene using three-point crosses. These I did learn, but what I did not and could never learn was how he predicted the use of a particular genetic stock for solving a particular problem and often predicted the results with an uncanny accuracy. Under his guidance, within a short time, I characterized the two unknown genes in *Neurospora* strains that I had isolated in India. Using his tester strains, I identified a fragrant *Neurospora*. This strain, Vickramam – named after the place of its origin in India – is most unusual: its morphology on agar medium is perfectly normal like wild type but in liquid shake cultures it grows like bacteria; the conidia directly germinate to form more conidia, bypassing the usual mycelial phase, the microcycle conidiation. I worked out the genetic basis of microcycle conidiation. Namboori Raju, arguably the best fungal cytologist, showed me how the small fungal nuclei and chromosomes were visualized using DAPI stain. On my return I wrote up my Stanford work and sent the manuscript to David for approval; however, he politely refused to allow his name to be added as co-author. I found out that he never included his name in any publication, even from his own lab, unless he had equally contributed in the bench work.

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My success in finding *Neurospora* in my 'backyard' pleased David Perkins so much that he advised his associate David (Dave) Jacobson to visit Bangalore for two months. Dave, my students, and I had a great time collecting *Neurospora* cultures in sugarcane fields in and around Mandya (a place some 80 km southwest of Bangalore). We had an advantage: we could buy canes in the market, plant them in pots, cover them with straw, and burn the canes to simulate the situation in the field. The advantage of this reconstruction experiment or simulated burning was that we could daily monitor the development of *Neurospora*. Previous genetic and molecular data had

Figure 2. Neurospora growing on burnt sugarcane stubble in a field in Mandya near Bangalore

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predicted that *Neurospora* populations are outbreeding, but there was no proof of a sexual stage in nature. We discovered its perithecia and characteristic ascospores, implying that *Neurospora* reproduces sexually in nature: its asexual and sexual phase is separated in time and space. Our work suggested that *Neurospora* can survive between the burns in the form of sexually-formed, dormant, heat-resistant ascospores which are activated by furfural produced from burned hemicellulose in plant tissue. A puzzle had been solved. Dave Jacobson remarked, "Conducting experiments with burned sugar cane would be impossible in a lab in USA".

Though I did learn some basic microbiological and genetic techniques specific for research on *Neurospora*, I can't fool myself. Even if I had stayed in David's lab for the remaining years of my life, it would have been next to impossible to learn which marker strains to use, and how, in a particular genetic experiment, to predict the results with any accuracy. He was a walking encyclopedia of the more than 1000 genetic loci known in *Neurospora*, their distinguishing characteristics, and their positions in the seven chromosomes. He was able to provide immediately any mutant strain upon request from the stocks which he had constructed over the years and the precise application of which only he knew. Not surprisingly, investigators from all over the world came to Stanford, or regularly sought his advice, or for pilgrimage. There were senior visitors in his lab like me. But we mostly ended up learning ascospore – picking under the microscope; the interpretation of the data and inferences were left for David. Sharat Chandra has very aptly remarked, "The world has lost the last of the great geneticists".

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Upon return to Bangalore, I gradually wound up my physiological and biochemical research on thermophilic fungi, and switched to *Neurospora*. We began collecting *Neurospora* from the Mandya District, where sugarcane is cultivated. Following the harvesting of canes for milling, the field is burnt to clear the enormous trash of cut leaves. The burnt stubbles are a substrate *par excellence* for *Neurospora*. Among the hundreds of cultures we collected over

the years since 1991, we could screen and characterize only a few. In this treasure trove of cultures, we identified a culture which died after 4-5 subcultures, regardless of the composition of the medium, unlike the wild-type which are 'immortal'. We called this mutant *senescent* and this was the material used by two of my graduate students. With David's guidance through electronic mail, the *sen* gene was mapped to the linkage group V, between *cyh-2* and *al-3*. I wish that, with *Neurospora* genome sequence being available, someone determines the identity of SEN protein product and elucidates the mechanism of death in fungi, which are supposedly immortal. The problem is how does one carry out genetics and biochemistry with a strain that dies in just a few subcultures? I recalled David's heterokaryon methodology of preserving strains harboring possible lethal genes. This and other mutants convinced me of the opportunities available in India for studies on natural populations. Our *sen* mutant would have been difficult, if not impossible, to produce in the laboratory. When we published on this, I quickly received a surprise invitation from Helmut Bertrand in East Lansing who was interested in senescence phenomenon in fungi – i.e., 'the death of the immortals'. Bert suggested that I come to East Lansing for three months. Instead I sent my last student to Bert's lab. The *sen* mutant dies because of short GC-rich repeats in mitochondrial DNA favouring intramolecular recombinations and deletions due to short repeats causing cytochrome deficiencies. I thus began to increasingly appreciate the study of natural populations. David Perkins' global collection of more than 4000 cultures is a resource for many types of genetic studies for generations to come. The cultures, laboriously collected by David over several years from warm and humid tropical regions of the world, are preserved by the Fungal Genetics Stock Centre.

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Perhaps I should record why I, despite being in a biochemistry department, never tried to become the so-called 'molecular biologist', even after the opportunity of a sabbatical in Stanford. Before I was to return to Bangalore, my students had sent me a list of biochemicals including restriction enzymes that they wanted

David Perkins' view on Post-genome *Neurospora* Genetics

I was very skeptical when genome sequencing was proposed and started thinking it would siphon resources and effort away from hypothesis-directed research. But I've been proved wrong. I find the new potentialities exciting and am ready to recant my pessimistic apprehensioenabled experimental genetics to transcend working within a single species. Comparative genomics has revolutionized evolutionary studies, provided evolutionary trees, and provided new and powerful ways of recognizing species and looking at the origin of species. Orthologies with homologs in other species enable the functions of new genes to be predicted, and genes specifying a desired product to be identified. The ease with which an interesting but previously little-studied species can be developed genetically has been increased dramatically – once the genome sequence is known, genetic maps based on orthology can quickly be constructed. Genes that specify missing links in biosynthetic pathways can be identified readily, as Radford has done for *Neurospora* in his Advances in Genetics paper. In silico genetics!

From the correspondence of David Perkins with the author

me to bring. David generously allowed me to take whatever I wanted from the store in the basement of Biological Sciences Department and have the cost charged to his grant. On the eve of my departure, with a twinkle in his eyes, David remarked at a meeting of lab colleagues, "We must plan on sending materials to Ramesh on a regular basis so that he can do molecular biology". Though undoubtedly a well-meaning remark, the message was clear. My research remained based on indigenous strains and on resources that could be generated for 'unfashionable' research. I decided not to make any more visits to foreign labs for the purpose of gathering research material or for material gains. This decision I did not regret. My students and I had fun and excitement in combining field work with investigations in the laboratory. We had opportunities of learning a bit of Nature even if all our observations did not result in publications.

I regard David as 'Guru'. In the cultural environment in which I was brought up, the position of a guru is above that of parents. Until recently, I could never address David by his first name. However he kept insisting in regular e-mails that, since I had known him for many years and was his colleague, he would feel happy if I called him David.

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