Commentary

Successful beyond expectation: David Perkins's research with chromosome rearrangements in Neurospora

The Thomas Hunt Morgan Medal is awarded by the Genetics Society of America to recognize a lifetime contribution to genetics. The first awardees were Barbara McClintock and Marcus Rhoades, in 1981. In 1994 this Medal was awarded to David D. Perkins of Stanford University. By then David had shown that many different genetic properties of eukaryotes, including recombination, chromosome rearrangements and meiotic drive; can be studied using Neurospora as a model. David had also made compilations of all known Neurospora mutants, chromosome rearrangements, wild and multiply marked strains, linkage maps, methods, publications, and (more recently) useful websites. He was the prime mover in instituting an annual Newsletter and establishing the Fungal Genetics Stock Center (*www.fgsc.net*). In the Report of the Committee on Honors and Awards, David R. Stadler wrote – "For those of us who work on the genetics of Neurospora, a day seldom passes that we don't give thanks to a generous Providence for David Perkins. So many times he has made life's path a little easier for us" (Genetics 137:s12-s13, 1994).

David Perkins is best known for developing a simple and elegant method to detect and distinguish different kinds of chromosome rearrangements (inversions, intrachromosomal transpositions, reciprocal translocations and insertional and quasiterminal translocations). In a single-author tour-de-force paper that he dedicated to Curt Stern, his undergraduate mentor at the University of Rochester, David diagnosed >135 rearrangements by mere visual inspection of black (viable, non-deficiency) and white (inviable, deficiency) ascospores in unordered tetrads (actually, octads) from *Rearrangement* x *Normal* crosses (Perkins 1974). Five classes of tetrads are possible; 8B:0W; 6B: 2W; 4B:4W; 2B:6W and 0B:8W. Isosequential crosses produce 90-95% 8:0 tetrads but a *Reciprocal Translocation* (*RT*) x *Normal* cross produces equal numbers of 8:0's and 0:8's. The former result from 'alternate' segregation (which generates the parental types) whereas the latter are from "adjacent 1" segregation (which generates duplication/deficiency types). Any 4:4's signal a crossover between the centromere and the translocation breakpoint, and no 6:2's and 2:6's are expected. In *Insertional Translocations* (*IT*), a segment of one linkage group is translocated into another linkage group. If the 'recipient' chromosome from the *IT* parent of an *IT* x *N* cross (*N* = "normal sequence") segregates with the *N* homolog of the 'donor' chromosome, then the resulting segregant is duplicated (*Dp*) for the translocated segment and only the complementary 'deficiency' segregants are inviable. Therefore in *IT* x *N* crosses 8:0's = 4:4's, whereas 6:2's signal interstitial crossover, and 0:8's and 2:6's are rare. Thus the frequency distribution of the different tetrad classes is a characteristic of the kind of rearrangement and provides information as to the position of breaks relative to the centromere. None other than Barbara McClintock encouraged David to undertake this study, and in gratitude David exulted "The method proved successful beyond expectation …" (Perkins, 1992). David's 159 page review (Perkins 1997) describes more than 300 rearrangements that he analyzed over a 30 year period of work, and also discusses the pitfalls in identifying and diagnosing rearrangements. It remains to this day the definitive reference work on the subject.

David had a rare knack for doing crosses with carefully chosen markers to solve the structure of rearranged chromosomes, supplemented, when necessary, with cytological and molecular studies. Not surprisingly, his diligence led him to make novel and unexpected discoveries. For example, in his effort to determine the orientation of the transposed segment in the intrachromosomal transposition *Tp(IR>IL)T54M94*, David wanted to make a *Tp* x *Tp* cross heterozygous for markers both inside and outside the transposed segment. He had with "… great difficulty …" (by screening for a rare double crossover) inserted one marker *(cyh-1*) into the transposed segment, but needed two to obtain the needed evidence. My guess is at this stage a lesser scientist would throw in the towel (- why determine orientation of

Keywords. Meiotic silencing; quelling; repeat-induced point mutation; Spore killers

an intrachromosomal transposition anyway?). Not David, he decided to insert an *al-2* marker, but via repeat-induced point mutation (RIP); a genome defense process that occurs in the premeiosis of a sexual cross and efficiently mutates duplicated DNA sequences (presumably, to destroy transposable and other parasitic DNA elements). To duplicate the *al-2* gene sequence David and colleagues transformed the *Tp* strain with an as yet unpublished *al-2* clone (from T L Schmidhauser). To their surprise, many primary transformants showed an unstable albino phenotype; Perkins and colleagues had inadvertently (and independently) stumbled upon 'quelling', an RNAi-mediated gene-silencing phenomenon that silences transgenes and their endogenous homologues. Happily, they could also generate the *al-2* mutation and used it to establish that the transposed segment was "… inserted near the centromere in the opposite arm, in inverted order" (Perkins *et al* 1995).

The 1995 paper also illustrates the elegant use made by David and colleagues of the meiotic drive element *Spore killer-2* (*Sk-2*) to map the insertion site of the transposed segment with respect to the centromere. *Spore killers –2* and *–3* (*Sk-2* and *Sk-3*) are gene complexes (haplotypes), tightly linked to the centromere of linkage group III, that David and Barbara Turner identified in a subset of *Neurospora intermedia* strains David isolated from Borneo (Brunei), Java and Papua New Guinea (Turner, 2001). No killers were found in *N. crassa,* but the *N. intermedia Sk* factors are fully functional when introgressed into *N. crassa* and *N. tetrasperma* (Raju and Perkins, 1991). *Sk* x wild type crosses produce 4:4 tetrads; the black (viable) ascospores are ones that inherit *Sk*, whereas the white (inviable) ascospores are those that inherit the wild type linkage group III (figure 1). David and colleagues had earlier shown that *Sk-2* and *Sk-3* are useful for obtaining information from unordered asci that otherwise would require ordered asci (Perkins *et al* 1986). Since the *Sk* complexes are centromere-linked, they segregate at the first meiotic division. Ascospores are inviable in the half-tetrad that is devoid of *Sk*. Thus a marker gene is known to have segregated at the first division if the same allele is present in both ascospore pairs of the surviving half-ascus. If the ascospore pairs of the surviving half-ascus contain unlike alleles then the marker must have segregated at the second meiotic division, i.e., it must have undergone recombination with respect to its centromere. As the 1986 paper put it, "…(effectively), physically unordered asci are changed into ordered tetrads making it unnecessary to dissect ascospores from intact eight-spored asci. Availability of this easy method is not expected to produce any revolutionary new types of information. Rather, its significance is likely to be that the method will make it practical to obtain useful information that would previously have seemed too laborious to seek."

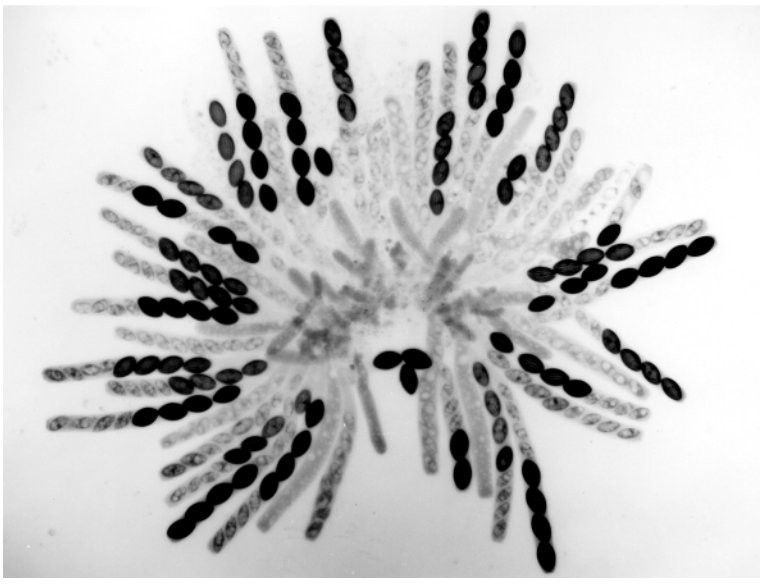

Figure 1. A rosette of asynchronous asci from the cross *Sk* x wild type. Each mature ascus shows four large black (viable) ascospores and four small aborted ascospores. All the viable ascospores are genotypically *Sk*. From Raju 1980, *Eur. J. Cell Biol.* **23** 208–223, reproduced here with permission from the author.

Figure 2. David Perkins with Prime Minister Indira Gandhi and M S Swaminathan at the International Genetics Congress in New Delhi, December 1983. (Photo from Susan Perkins.)

David and colleagues reported many other interesting results on Spore killers. Turner and Perkins (1979) showed that no killing occurs in homozygous *Sk-2* x *Sk-2* and *Sk-3* x *Sk-3* crosses and that all ascospores are killed in *Sk-2* x *Sk-3* intercrosses. And in both *Neurospora intermedia* and *N. crassa* they identified resistant strains that neither kill nor are killed. But my favourite was the demonstration of a Spore killer-like programmed ascospore death in the homothallic species *Coniochaeta tetraspora* (Raju and Perkins 2000). In homothallic species the mycelium from a single, initially uninucleate ascospore is capable of completing the sexual cycle. Thus all the nuclei participating in a cross are identical. Nevertheless, of the eight ascospores cut out in each ascus, four die and only the other four are viable, just as in a Neurospora *Sk-2* x WT cross. What maintains this system of apparently willful waste?

David crafted rearranged chromosomes into versatile, Swiss Army Knife-like tools. For example, to increase the efficiency of linkage detection he marked three reciprocal translocations with "easy to score" markers and brought them together in the *alcoy* linkage-tester strains (*T(I;II)4637 al-1; T(IV;V)R2355, cot-1; T(III;VI)1, ylo-1*; *csp-2*). Except for the marker *csp-2*, which marks linkage group VII, each of the other markers (*al-1*, *cot-1* and *ylo-1*) tags two linkage groups (Perkins *et al* 1969). If a point mutation in a *mutant* x *alcoy* cross shows linkage to one of the three *alcoy* markers, one only needs to do a follow-up cross with a normal-sequence tester to determine which of the alternatives is correct. A mutation that shows linkage to two markers very likely signals a new translocation.

Dp progeny from crosses of the type *IT* x *N*, *RT* x overlapping *RT*, or *Inversion* x overlapping *Inversion*, were used for a variety of purposes, including establishing correct gene order among closely linked loci (Perkins 1986), localizing vegetative incompatibility loci (Perkins 1975) and to detect escape from

vegetative incompatibility via chromosome breakage (Perkins *et al* 1972). *Dp* segregants are recognizable by the barrenness of *Dp* x *N* crosses. Barren crosses make perithecia but produce very few ascospores. The barrenness of *Dp* x *N* crosses is now known to be due to the silencing of duplication-borne genes by another RNAi-mediated gene-silencing process, which silences genes unpaired in meiosis as well as their homologues. Robert Metzenberg and colleagues, including David's associate N B Raju, discovered meiotic silencing and also isolated semi-dominant suppressors of this process (Shiu *et al* 2001). Much of this work was done during Metzenberg's stay in David's lab in the late 90's and early 00's. The suppressors of meiotic silencing have now made it easier to obtain large numbers of progeny from the otherwise barren *Dp* x *N* crosses.

Much of my own research was sparked by David's demonstration of RIP in large duplications (Perkins *et al* 1997). This prompted us to test, and thus discover, that a small gene-sized (~1-3 kb) duplication escapes RIP when a large (>300 kb) duplication is present in the cross (Bhat and Kasbekar 2001), possibly because the large duplication titrates out the RIP machinery. Armed with the knowledge that one can screen for genotypes that confer a dominant RIP suppressor phenotype, we screened for this phenotype among all the >400 wild-isolated strains of *N. crassa* obtainable from the Fungal Genetics Stock Center, most were collected by David on trips made around the tropical world (Turner *et al* 2001). Seven wild isolates (including one naturally occurring duplication strain) had the dominant RIP suppressor phenotype (Bhat *et al* 2003; Vyas *et al* 2006). David encouraged me to use *Neurospora tetrasperma*, a pseudohomothallic species, to screen for recessive mutants defective in RIP and meiotic silencing. Many years previously Francis Ryan took David, then a graduate student at Columbia University, to the New York Botanical Garden up in the Bronx, to visit B. O. Dodge, the discoverer of both *N. crassa* and *N. tetrasperm*a. *N. tetrasperma* was Dodge's favorite species and he tried (without success) to get his visitors interested in using it. Dodge even offered all his slides related to *tetrasperma* cytology to anyone who would use them. David confided to me that Dodge would have been pleased to see what we were doing (Bhat *et al* 2004). David also contributed more directly to our articles. I treasure the detailed handwritten comments and suggestions that he made on our manuscripts. These were sent both by fax (because he knew we were impatient) and by regular mail. Often he redrew our figures to make them more understandable to a larger audience and once even re-wrote the abstract!

David Perkins passed away on January 2, 2007, four months short of his 88th birthday. His wife Dorothy Newmeyer Perkins (Dot) passed away peacefully on January 6, and joined David Perkins. Dot was 84 years old. They are survived by their daughter Susan and her husband John.

Acknowledgements

Writing this tribute would not have been possible without the reassurance and critical inputs I received from Namboori Raju, Sharat Chandra, Ramesh Maheshwari, K Dharmalingam, P Maruthi Mohan, D Balasubramanian and Imran Siddiqi.

References

Bhat A and Kasbekar DP 2001 Escape from repeat-induced point mutation of a gene-sized duplication in *Neurospora crassa* crosses that are heterozygous for a larger chromosome segment duplication; *Genetics* **157** 1581–1590

Bhat A, Noubissi F K, Vyas M and Kasbekar D P 2003 Genetic analysis of wild-isolated *Neurospora crassa* strains identified as dominant suppressors of repeat-induced point mutation (RIP); *Genetics* **164** 947–961

Bhat A, Tamuli R and Kasbekar D P 2004 Genetic transformation of *Neurospora tetrasperma*, demonstration of repeat-induced point mutation (RIP) in self-crosses, and a screen for recessive RIP-defective mutants; *Genetics* **167** 1155–1164

Perkins D D 1974 The manifestation of chromosome rearrangements in unordered asci of Neurospora; *Genetics* 77 459–489

Perkins D D 1975 The use of duplication-generating rearrangements for studying heterokaryon incompatibility genes in Neurospora; *Genetics* **80** 87–105

Perkins D D 1986 Determining the order of chromosomal loci in Neurospora by tests of duplication coverage; *J. Genet*. **65** 121–144

Perkins D D 1992 Neurospora chromosomes: in *The dynamic genome: Barbara McClintock's ideas in the century of genetics* (eds) N Federoff and D Botstein (New York: Cold Spring Harbor Laboratory Press) pp 33–44

Perkins D D 1997 Chromosome rearrangements in Neurospora and other filamentous fungi; *Adv. Genet.* **36** 239–398

Perkins D D, Newmeyer D, Taylor C W and Bennett D C 1969 New markers and map sequences in *Neurospora crassa*, with a description of mapping by duplication coverage, and of multiple translocation stocks for testing linkage; *Genetica* **40** 247

Perkins D D, Newmeyer D and Turner B C 1972 Nontandem duplications in Neurospora and restoration of the euploid condition by chromosome breakage; *Genetics* **71** s46–s47 (Abstr.)

Perkins D D, Raju N B, Pollard V C, Campbell J L and Richman A M 1986 Use of Neurospora Spore killer strains to obtain centromere linkage data without dissecting asci; *Can. J. Genet. Cytol*. **28** 971–981

Perkins D D, Turner B C, Barry E G and Pollard V C 1995 Cytogenetics of an intrachromosomal transposition in Neurospora; *Chromosoma* **104** 260–273

Perkins D D, Margolin B S, Selker E U and Haedo S D 1997 Occurrence of repeat induced point mutation in long segmental duplications of Neurospora; *Genetics* **147** 125–136

Raju N B and Perkins D D 1991 Expression of meiotic drive elements Spore killer-2 and Spore killer-3 in asci of *Neurospora tetrasperma; Genetics* **129** 25–37

Raju N B and Perkins D D 2000 Programmed ascospore death in the homothallic ascomycete *Coniochaeta tetraspora; Fungal Genet. Biol*. **30** 213–221

Shiu P K, Raju N B, Zickler D and Metzenberg R L 2001 Meiotic silencing by unpaired DNA; *Cell* **107** 905–916

Turner B C 2001 Geographic distribution of Neurospora Spore killer strains and strains resistant to killing; *Fungal Genet. Biol*. **32** 93–104

Turner B C and Perkins D D 1979 Spore killer, a chromosomal factor in Neurospora that kills meiotic products not containing it; *Genetics* **93** 587–606

Turner B C, Perkins D D and Fairfield A 2001 Neurospora from natural populations: a global study; *Fungal Genet. Biol.* **32** 67–92

Vyas M, Ravindran C and Kasbekar D P 2006 Chromosome segment duplications in *Neurospora crassa* and their effects on repeat-induced point mutation (RIP) and meiotic silencing by unpaired DNA; *Genetics* **172** 1511–1519

Durgadas P Kasbekar
Centre for Cellular and Molecular Biology,
Hyderabad 500 007, India
(Email, kas@ccmb.res.in)

*e*Publication: 31 January 2007