Commentary

Sherlock Holmes, David Perkins, and the missing Neurospora inversions

It is now twenty years since David Perkins published his magisterial review 'Chromosome rearrangements in Neurospora and other filamentous fungi', in which he documented everything then known about 355 chromosome rearrangements in *Neurospora crassa* (Perkins 1997); and forty-five years since he published 'An insertional translocation in Neurospora that generates duplications heterozygous for mating type', wherein crosses of the insertional translocation *T(IL>IIR) 39311* led him to infer why no paracentric inversion was ever isolated in Neurospora although such inversions must undoubtedly occur (Perkins 1972). Perkins's reasoning brings to mind the dialogue from Arthur Conan Doyle's detective story 'Silver Blaze': 'Is there any point to which you would wish to draw my attention?' 'To the curious incident of the dog in the night-time.' 'The dog did nothing in the night-time.' ' "That was the curious incident," remarked Sherlock Holmes.' In this remembrance of Perkins on his tenth death anniversary, I will draw the parallel between Holmes and the dog that did nothing, and Perkins and the failure of evidence for crossovers that produce dicentric bridges and acentric fragments to materialize. Additionally, Perkins (1972) reported an exceptional strain of unexpected phenotype, that for want of an explanation he attributed to technical error. Recent results suggest, however, the error could be by Neurospora (Kasbekar and Rekha 2017). Perkins's research on Neurospora chromosome rearrangements was summarized previously in this journal (Kasbekar 2007).

1. The layabout dog

First, I summarize the plot of 'Silver Blaze' (Doyle 1894). The racehorse Silver Blaze, favoured to win the Wessex Cup, disappeared one night, and his trainer, John Straker, was found dead on the moor with his head bashed in, a knife wound in his thigh, and the bookmaker Fitzroy Simpson's scarf in his grasp. Ned Hunter, the stable hand, and Edith Baxter, the servant-maid, recognized Simpson as the stranger who had visited the stable that fateful night and offered £10 for a tip. Simpson was charged with drugging Hunter, abducting the horse, and murdering Straker. However, the curiousness of the dog's silence during the abduction, and the convenience of the curried mutton served for Hunter's supper to camouflage the added opium, struck Holmes that the horse-thief was no stranger but Straker himself, who the dog knew well. Straker plotted to win a large sum of money by betting against his own horse, but not without first impairing it by subcutaneously nicking its tendons with a delicate cataract knife, and he had spirited the horse away to perform the surgery surreptitiously on the moor, gathering Simpson's lost scarf presumably to secure its leg. However, before Straker could injure him, the frightened horse lashed out and struck Straker on the forehead with his steel shoe, and Straker gashed his thigh on the surgical knife as he fell. A bill found in his pocket hinted to a high-maintenance mistress, and established the motive.

2. Dyscentric translocation and dicentric bridges

The *Neurospora crassa* 39311 mutant strain can boast of as illustrious a pedigree as any thoroughbred. It was isolated in the laboratory of George W Beadle and Ed Tatum as a nutritional mutant requiring succinic acid for growth (Beadle and Tatum 1945; Lewis 1948). The succinate-requirement mutation (*suc*) was found to be linked to an insertional translocation, designated as *T(IL>IIR) 39311* (abbreviated henceforth to

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T(39311)). In an insertional translocation a segment from a donor chromosome is transferred to a recipient chromosome without any reciprocal exchange. The *T(39311)* strain, separated from the *suc* mutation by crossing over, had a wild-type vegetative phenotype. Homozygous *T(39311) A* × *T(39311) a* crosses were fertile, and as expected from an isosequential cross produced mostly (90%) viable black ascospores. Using appropriately marked structurally homozygous crosses (i.e., *T(39311) A* × *T(39311) a*) Perkins (1972) showed that a large segment of linkage group (LG) IL, including the loci *nit-2*, *ser-3*, *mat*, *suc*, *arg-1*, and *arg-3*, was transferred to IIR between the loci *aro-3* and *pe*, in inverted (dyscentric) orientation with respect to the centromere, and that markers within the translocated segment segregated independently of LG I markers located outside the segment, but were linked to markers in LG II. Decades later, my student Srividhya Iyer defined the breakpoints of *T(39311)* onto the genome sequence in the course of mapping of the *fmf-1* mutation, and the breakpoint junctions established by her were consistent with Perkins's predictions (Iyer *et al*. 2009; Singh *et al*. 2010).

The marked *T(39311)* strains were made by meiotic transfer of the *arg-1*, *suc*, *mat*, or *ser-3* markers in appropriate *T(39311)* × *N* crosses, which indicated that the translocated segment and its homologue can undergo meiotic pairing and two-strand double crossovers to achieve the transfer. Two-strand double crossovers are certainly outnumbered by single crossovers and, additionally, three- and four-strand double crossovers also can occur. Since the translocated segment was inserted in inverted orientation with respect to the centromere, exchanges of such types would produce dicentric bridges and acentric fragments (figure 1). Indeed, in a companion paper Barry (1972) confirmed cytologically that bridges and fragments were frequent in the *T(39311)* × *N* cross. Exchanges in insertional translocations in which the translocated segment is inserted in non-inverted (eucentric) orientation relative to the centromere do not produce dicentric bridges and acentric fragments. Before we address the fate of cells in which exchanges produce dicentric bridges and acentric fragments, it is instructive to outline Perkins's method to detect and characterize chromosome rearrangements.

3. Perkins's method to detect rearrangements

An *N. crassa* strain is the *mat A* or *mat a* mating-type mycelium produced upon an ascospore's germination. In a cross, *mat A* and *mat a* mycelia come into contact and form fruiting bodies called perithecia. Within the perithecia the parental *mat A* and *mat a* haploid nuclei fuse in a cell called the ascus to produce a diploid

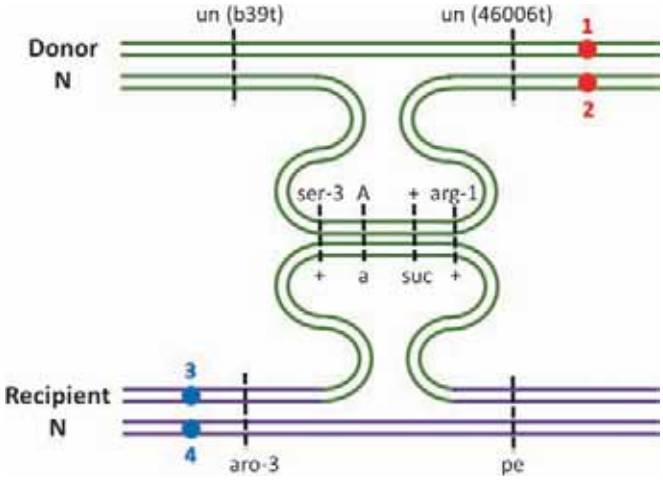

Figure 1. Dicentric bridge and acentric fragment formation in a *T(39311)*-heterozygous cross. In *T(39311)* a large segment from the left arm of chromosome 1 (orange centromeres), including the loci *nit-2*, *ser-3*, *mat*, *suc*, *arg-1*, and *arg-3*, is transferred to the right arm of chromosome 2 (blue centromeres) between the loci *aro-3* and *pe*, in inverted orientation with respect to the centromere. The translocation donor and recipient chromosomes are indicated, as well as their normal sequence homologues (*N*). Pairing is shown with synapsis between the translocated segment and its normal homologue. A dicentric bridge and acentric fragment will form if a single crossover occurs in the synapsed segment and centromeres 2 and 3 go to opposite poles at anaphase I. Adapted from Perkins (1972).

zygote nucleus that via meiosis and a post-meiotic mitosis generates eight progeny nuclei that are partitioned into the eight initially uninucleate progeny ascospores, four *mat A* and four *mat a*. A large fraction of progeny ascospores from crosses heterozygous for a rearranged chromosome have a deficient chromosomal complement as a result of independent assortment or crossover. Deficiency (*Df*) ascospores are inviable and remain white and are therefore distinguishable from viable, non-deficiency ascospores that turn black as the ascus matures. Ascospores, forcibly ejected from the perithecia as octets, can be collected on an agar surface. From the frequency spectrum of different ascus types one can infer whether a cross is heterozygous ($T \times N$) or homozygous ($N \times N$ or $T \times T$) for a translocation, and the distance of its breakpoints from the centromere (Perkins 1974).

Five different ascus types are possible, based on the number of black (B) and white (W) ascospores, namely, 8B:0W; 6B:2W; 4B:4W; 2B:6W, and 0B:8W. Most asci (>90%) from isosequential crosses are 8B:0W type, whereas heterozygosity for an insertional translocation (*IT*) results in equal numbers of 8B:0W and 4B:4W asci (8B:0W = 4B:4W), and 8B:0W = 0B:8W indicates heterozygosity for a reciprocal translocation (*RT*). In an *RT* two chromosomes reciprocally interchange their terminal segments. Regardless of the translocation type, alternate segregation recreates the parental genotypes, and produces the 8B:0W asci, whereas the equally likely adjacent 1 segregation generates non-parental genotypes. Adjacent 1 segregation in *IT*-heterozygous crosses generates 4B:4W asci, containing a viable non-tandem duplication (*Dp*) and the complementary inviable deficiency (*Df*), and in *RT*-heterozygous crosses it generates products with complementary duplications and deficiencies, thus yielding the 0B:8W asci. In *IT*-heterozygous crosses the 6B:2W asci signal crossovers between a breakpoint and its centromere and no 2B:6W and 0B:8W asci are expected; whereas in *RT*-heterozygous crosses the 4B:4W asci signal crossover and no 6B:2W and 2B:6W asci are expected. For figures explaining the genetic consequences of crosses heterozygous for the different translocation types, the reader is referred to Perkins's (1997) magnificent review article.

In general, *Dp* strains have wild-type morphology and growth, but their crosses with the wild type (i.e., $Dp \times N$) are barren, and produce very few progeny ascospores. The barrenness is due to silencing of duplication-borne genes, including those required for meiosis and ascus development, by an RNAi-based process called meiotic silencing by unpaired DNA. Suppression of meiotic silencing significantly increases the productivity of *Dp*-heterozygous crosses (Shiu *et al*. 2001).

When an inversion is crossed with normal sequence (i.e. $In \times N$), single crossover in the inverted segment generates products with complementary duplications and deficiencies of non-inverted loci (figure 2). If the inverted segment includes the centromere (pericentric), then crossover generates non-8B:0W asci, but if the inverted segment does not include the centromere (paracentric), then crossover produces a dicentric bridge and an acentric fragment. As can be seen by comparing Figures 1 and 2b, both paracentric inversions and dyscentric translocations, share the property that crossovers in them generate bridges and fragments.

4. Absence of evidence is not evidence of absence

We might expect 4B:4W type asci to be produced in a $T(39311) \times N$ cross in either of two ways: one is via adjacent 1 segregation, and the other is potentially by crossover in the translocated segment where a dicentric bridge and an acentric fragment should kill two of the four meiotic products, thus resulting in a 4B: 4W ascus. Since 4B:4W and 8B:0W types from adjacent 1 and alternate segregation are produced in equal numbers, asci containing bridges should have resulted in an excess of 4B: 4W over 8B: 0W. However, Perkins failed to find evidence for such excess in the 392 asci he examined from $T(39311) \times N$. The frequencies he obtained were 44% 8:0; 10% 6:2; 41% 4:4; 3% 2:6; and 2% 0:8, with the 2B:6W and 0B:8W frequencies not differing from the background values from structurally homozygous controls. That the fraction of 4B:4W asci did not exceed those of 8B:0W asci led Perkins to infer that bridge formation might lead to abortion of the entire ascus, and few asci containing bridges and fragments survive to the ascospore production stage to be detected as 4B:4W type. Barry's cytological observations also suggested that a bridge or fragment causes abortion of the entire ascus. Since the generation of bridges and fragments results in the failure of asci to survive or mature, Perkins reckoned that paracentric inversions also would not be detectable because recombination within the inverted segment would lead to ascus abortion. In

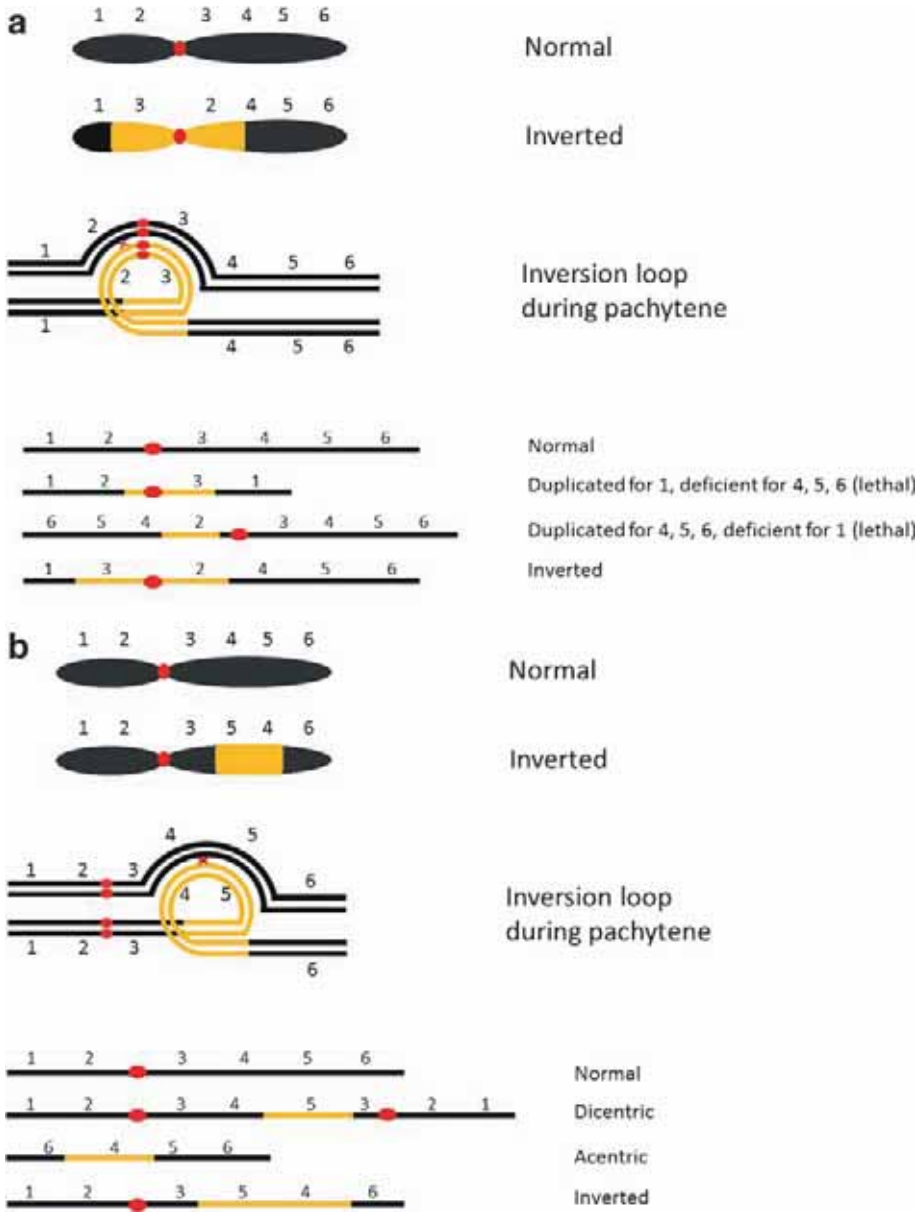

Figure 2. Consequences of single crossovers in inversion heterozygotes. The red ovals and circles represent the centromere. (**a**) Crossover in a pericentric inversion produces chromosomes with duplications and deficiencies of loci that lie outside the inverted segment. Ascospores that receive these chromosomes are inviable and remain white. (**b**) Crossover in a paracentric inversion produces a dicentric bridge and an acentric fragment, which have complementary duplications or deficiencies of the loci numbered 1, 2, 3, and 6 that lie outside the inverted segment. The dicentric bridge and acentric fragment cause abortion of the entire ascus.

contrast, recombination within a pericentric inversion does not generate bridges and fragments, but produces inviable nuclei with complementary duplications and deficiencies that result in the production of non-8B:0W asci. Thus dyscentric insertional translocations and pericentric inversions produce a significant fraction of asci with both black and white ascospores, whereas paracentric inversions produce mostly 8B:0W asci which makes them difficult to distinguish from structurally homozygous crosses. Unrecognized paracentric inversions in normal-appearing strains might in part be responsible for the high variability in recombination often observed between the same markers in crosses of different parentage.

5. Treasure Perkins's exception

Dp ascospores in which both the *mat A* and *mat a* alleles are present in the same nucleus produce germinating hyphae that show sparse spidery growth and produce a dark pigment that diffuses into the medium, giving the so-called Dark Agar phenotype (DA). All four viable progeny from the 4B:4W asci from *T(39311)* × *N* were expected be *Dp* type, and display the DA phenotype, since *Dp(39311)* duplicates the *mat* locus. In contrast, the 8B:0W asci are not expected to give rise to any *Dp* progeny, because crossover products complementary to the *Dp* would be *Df* that should result in white spores. The latter prediction was fulfilled by 70 of 71 8B:0W asci examined by Perkins, which gave all four spore-pairs non-DA. The one exception, with 7 non-DA, and 1 DA ascospore, was attributed by Perkins to technical error. However, it is conceivable that the DA ascospore indeed was a [*mat A* + *mat a*] heterokaryon that was produced by interposition of an additional mitosis following the post-meiotic mitosis but before nuclear partitioning. Such rare 8B:0W asci that included DA ascospores could reflect a background rate (here 1/71 = 1.4%) with which ascospore partitioning is uncoupled from the post-meiotic mitosis. Recently, we obtained similar evidence for uncoupling in *N. tetrasperma* crosses heterozygous for hybrid translocation strains (Kasbekar and Rekha 2017).

6. The yeoman service of the FGSC

The *T(39311)* strain of Perkins (1972) and the *fmf-1* mutant of Johnson (1979) were maintained for decades by the Fungal Genetics Stock Center before their molecular analysis by Iyer *et al*. (2009), by which time even the laboratories in which the earlier work was done had closed down, which illustrates the tremendously important yet often overlooked role played by stock collections such as the FGSC for researchers across the world.

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References

Barry EG 1972 Meiotic chromosome behavior of an inverted insertional translocation in Neurospora. *Genetics* **71** 53–62

Beadle GW and Tatum EL 1945 Neurospora. II. Methods of producing and detecting mutations concerned with nutritional requirements. *Am. J. Bot.* **32** 678–686

Doyle AC 1894 'Silver Blaze' in 'The memoirs of Sherlock Holmes', reprinted in 'The Penguin Complete Sherlock Holmes'(Penguin Books, Harmondsworth) 1981, pp 335–350

Iyer S, Ramakrishnan M and Kasbekar DP 2009 *Neurospora crassa fmf-1* encodes the homologue of the *Schizosaccharomyces pombe* Ste11p regulator of sexual development. *J. Genet.* **88** 33–9

Johnson TE 1979 A Neurospora mutant that arrests perithecial development as either male or female parent. *Genetics* **92** 1107–1120

Kasbekar DP 2007 Successful beyond expectation: David Perkins's research with chromosome rearrangements in Neurospora. *J. Biosci.* **32** 191–195

Kasbekar DP and Rekha S 2017 *Neurospora tetrasperma* crosses heterozygous for hybrid translocation strains produce rare eight-spored asci bearing heterokaryotic ascospores. *J. Biosci*. **42** 15–21

Lewis RW 1948 Mutants of Neurospora requiring succinic acid or a biochemically related acid for growth. *Am. J. Bot.* **35** 292–295

Perkins DD 1972 An insertional translocation in Neurospora that generates duplications heterozygous for mating type. *Genetics* **71** 25–51

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

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

Shiu PK, Raju NB, Zickler D and Metzenberg RL 2001 Meiotic silencing by unpaired DNA. *Cell* **107** 905–916

Singh P, Iyer SV, Naga Sowjanya T, Kranthi Raj B and Kasbekar DP 2010 Translocations used to generate chromosome segment duplications in Neurospora can disrupt genes and create novel open reading frames. *J. Biosci.* **35** 539–546

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