Neurospora tetrasperma crosses heterozygous for hybrid translocation strains produce rare eight-spored asci-bearing heterokaryotic ascospores

Durgadas P Kasbekar* and Selvam Rekha

Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500 001, India

*Corresponding author (Email, kas@cdfd.org.in)

*This paper is dedicated to the memory of Prof. Bharat Bhushan Chattoo (1949-2016),
a distinguished fellow fungal geneticist and friend.*

During ascogenesis in Neurospora, the ascospores are partitioned at the eight-nucleus stage that follows meiosis and a post-meiotic mitosis, and the ascospores that form in eight-spored asci are usually homokaryotic. We had previously created novel T^{Nt} strains by introgressing four *Neurospora crassa* insertional translocations (EB4, IBj5, UK14-1, and B362i) into *N. tetrasperma*. We now show that crosses of all the T^{Nt} strains with single-mating-type derivatives of the standard *N. tetrasperma* pseudohomothallic strain 85 (viz. $T^{Nt}a \times 85A$ or $T^{Nt}A \times 85a$) can produce rare eight-spored asci that contain heterokaryotic ascospores, or ascospores with other unexpected genotypes. Our results suggest that these rare asci result from the interposition of additional mitoses between the post-meiotic mitosis and the partitioning of nuclei into ascospores, leading to the formation of supernumerary nuclei that then generate the heterokaryotic ascospores. The rare asci probably represent a background level of ascus dysgenesis wherein the partitioning of ascospores becomes uncoupled from the post-meiotic mitosis. Ordinarily, the severest effect of such dysgenesis, the production of mating-type heterokaryons, would be suppressed by the *N. crassa tol* (*tolerant*) gene, thus explaining why such dysgenesis remained undetected thus far.

1. Introduction

The *Neurospora crassa* insertional translocations *T(VR>VII)EB4*, *T(VIL>IR)IBj5*, *T(VIR>VL)UK14-1*, and *T(IVR>I)B362i* were described by Perkins (1997). Recently, these translocations were introgressed into *N. tetrasperma* to create the strains *T(VR>VII)^{Nt}EB4 a*, *T(VIL>IR)^{Nt}IBj5 a*, *T(VIR>VL)^{Nt}UK14-1 A*, and *T(IVR>I)^{Nt}B362i A* (Giri *et al.* 2015). The new strains will henceforth be referred to as *T(EB4)^{Nt}a*, *T(IBj5)^{Nt}a*, *T(UK14-1)^{Nt}A*, and *T(B362i)^{Nt}A*. An insertional translocation (*T*) transfers a donor chromosome segment into a recipient chromosome and thereby creates three breakpoint junctions: 'A' on the donor chromosome and 'B' and 'C' (proximal and distal) on the recipient chromosome (figure 1). The introgressions were done by first crossing the *N. crassa T* strains with the *N. crassa/*

N. tetrasperma hybrid strain C4T4 *a*, distinguishing the *T* progeny from this *T* × *N* cross from their *N* and *Dp* siblings (*N* = normal sequence strain, *Dp* = duplication strain) by PCR with breakpoint junction-specific primers (Singh 2010; Singh *et al.* 2010), and then backcrossing them once with C4T4 *a*, followed by two or three backcrosses with the *N. tetrasperma* standard strain 85, until we obtained the first self-fertile [*T* + *N*] heterokaryotic progeny (Giri *et al.* 2015). The T^{Nt} strains were isolated from the [*T* + *N*] heterokaryons as homokaryotic (self-sterile) conidial derivatives (see below), and verified to contain the translocation breakpoints. A T^{Nt} strain's genome is nominally from *N. tetrasperma*, except at the *N. crassa*-derived translocation breakpoint junctions. However, based on the number of backcrosses we estimate that ~14% of the T^{Nt} strain genome is *N. crassa*-derived. In principle, even more of the *N. crassa* genome

could have been eliminated by recombination had we done additional backcrosses using the homokaryotic *T*-type conidial derivatives of the [*T* + *N*] progeny.

In an *N. tetrasperma* sexual cross, karyogamy between the haploid parental *mat A* and *mat a* nuclei produces a diploid zygote nucleus that undergoes meiosis and a postmeiotic mitosis to generate 8 haploid progeny nuclei (4 *mat A* + 4 *mat a*), that become partitioned into 4 initially binucleate (dikaryotic) ascospores, each ascospore receives a pair of non-sister nuclei (1 *mat A* and 1 *mat a*) (Raju and Perkins 1994). Occasionally, a pair of smaller homokaryotic ascospores (1 *mat A* + 1 *mat a*) replaces a dikaryotic ascospore. Thus, *N. tetrasperma* produces mostly [*mat A* + *mat a*] dikaryotic ascospores as well as smaller numbers of homokaryotic *mat A* or *mat a* ascospores. The dikaryotic ascospores generate mycelia that can self-cross, whereas the homokaryotic ones generate self-sterile mycelia. In contrast, *N. crassa* produces only homokaryotic *mat A* or *mat a* ascospores (Raju and Perkins 1994). A subset of conidia (vegetative spores) from the dikaryotic *N. tetrasperma* mycelia can be homokaryotic by chance, and they produce self-sterile mycelia. Mycelia from homokaryotic ascospores and conidia can out-cross with like mycelia of the opposite mating type (Raju 1992; Bistis 1996).

In a *T* × *N* cross, the chromosomes can segregate either via alternate (ALT) or adjacent-1 (ADJ) segregation (Perkins 1997; figure 1). ALT produces 8 parental-type nuclei per ascus (4 *T* + 4 *N*), whereas ADJ produces 8 non-parental-type nuclei (4 *Dp* + 4 *Df*). Since heterokaryotic ascospores receive a pair of non-sister nuclei, they acquire either the [T^{Nt} + *N*] or [*Dp* + *Df*] genotype. The homokaryotic (self-sterile) conidial derivatives from

the [T^{Nt} + *N*] mycelia can be of either mating type, whereas those from the [*Dp* + *Df*] mycelia can be of only the mating type of the *Dp* nucleus, since the *Df* homokaryons are inviable. Self-cross of either heterokaryon can again generate [T^{Nt} + *N*] and [*Dp* + *Df*] heterokaryotic progeny. Additionally, a T^{Nt} × *N* cross can produce five-, six-, seven-, and eight-spored asci via the replacement of one or more heterokaryotic ascospore by pairs of homokaryotic ones. A T^{Nt} and *N* pair can replace a [T^{Nt} + *N*] ascospore, and a *Dp* and *Df* pair can replace a [*Dp* + *Df*] ascospore. The T^{Nt}, *N*, and *Dp* types are viable and become pigmented (B = black), while the *Df* type is inviable and remains white (W). Replacement of all four heterokaryotic ascospores results in the production of an eight-spored ascus. Among the eight-spored asci, the 8B:0W type is derived from ALT segregation, the 4B:4W type from ADJ segregation, and the 6B:2W type from crossover between the centromere and a breakpoint followed by ALT or ADJ segregation (Perkins 1997). Although [*T* + *N*] heterokaryons can also be made in *N. crassa*, by 'forcing' heterokaryosis between *T* and *N*-type strains, obviously the [*Dp* + *Df*] heterokaryons cannot be obtained in this way.

Interestingly, the T^{Nt} × *N* crosses showed two novel 'ascus dysgenesis' phenotypes. One was a transmission ratio distortion that appeared to disfavour the homokaryotic ascospores formed following ALT segregation, and the other was the production of heterokaryotic ascospores in eight-spored asci (Giri *et al.* 2016). Heterokaryotic ascospores were never previously reported in eight-spored asci in any Neurospora species, although Lewis (1969) had reported sighting them in the rare 8B:0W asci (~1 such ascus per 30 perithecia) that
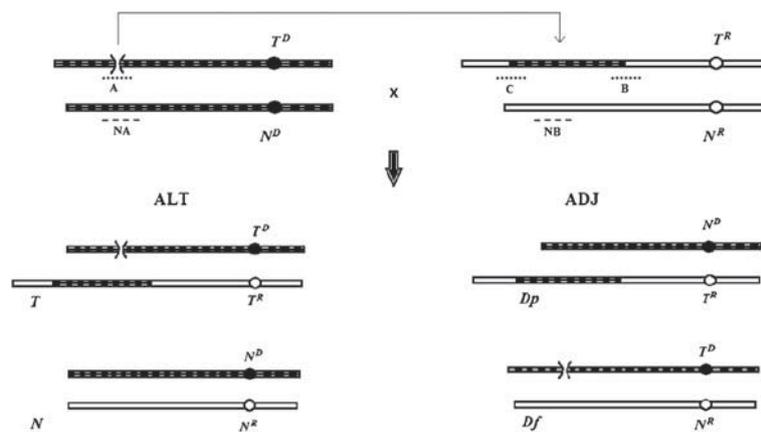

Figure 1. Alternate (ALT) and adjacent-1 (ADJ) segregation. T^D and T^R are the donor and recipient chromosomes of the *T* strain, and N^D and N^R are their *N*-derived homologues. Dotted lines indicate the A, B, and C breakpoint junctions, and dashed lines the NA and NB segments in the normal sequence homologues which are disrupted by the translocation. In ALT, T^D and T^R segregate to one spindle pole, and N^D and N^R to the other. Subsequently, meiosis II and post-meiotic mitosis generate eight parental-type nuclei, viz. 4 *T* + 4 *N*. In ADJ, N^D and T^R segregate to one pole and T^D and N^R to the other, to eventually produce eight non-parental nuclei, 4 *Dp* + 4 *Df*. The *T*, *N*, and *Dp* types are viable, whereas the *Df* type is inviable.

formed in interspecific crosses in Sordaria (a sibling genus in the family Sordariaceae). Now, we have repeated the $T(IBj5)^{Nt}a \times 85A$ and $T(B362i)^{Nt}A \times 85a$ crosses and re-confirmed the earlier findings. We have also performed the $T(EB4)^{Nt}a \times 85A$ and $T(UK14-1)^{Nt}A \times 85a$ crosses and found heterokaryotic ascospores in the rare eight-spored asci from them. Obtaining heterokaryotic ascospores in eight-spored asci suggests that occasionally one or more additional mitoses is interposed between the post-meiotic mitosis and nuclear partitioning. We suggest that the rare eight-spored asci-bearing heterokaryotic ascospores reflect a previously unrecognized background rate of uncoupling of ascospore partitioning from the post-meiotic mitosis during Neurospora ascus development.

2. Materials and methods

2.1 *Strains and genetic crosses*

The $T(EB4)^{Nt}a$, $T(IBj5)^{Nt}a$, $T(UK14-1)^{Nt}A$, and $T(B362i)^{Nt}A$ strains are available from the Fungal Genetics Stock Center, Kansas State University, Manhattan, Kansas, USA, with accession numbers FGSC 25288 to 25291, as are the *N. tetrasperma* standard strains 85 *A* (FGSC 1270) and 85 *a* (FGSC 1271). Neurospora genetic analysis was done essentially as previously described (Giri *et al*. 2016). Metzenberg's (2003) alternative recipe was used for making Medium N.

2.2 *Determination of ascospore genotype*

*T*s are defined by breakpoint junction A on the donor chromosome, and junctions B and C (proximal and distal) on the recipient chromosome. Additionally, PCR amplification of genome segments from the *N* strain homologues of the translocation donor and recipient chromosome (designated as N^D and N^R) were used as positive controls. The primer sequences for the PCR were previously described (Giri *et al*. 2016). *T* progeny contain all the three translocation breakpoints, *Dp* progeny contain the B and C breakpoints but not A, whereas the *N* progeny contain none of these breakpoints. Additionally, one can test for the normal sequence homologs of the translocation donor and recipient chromosomes by PCR with primers diagnostic for 'normal A' and 'normal B' chromosomes. Genomic DNA from *N* progeny can amplify with both the normal A and normal B primers, *Dp* DNA can amplify with normal A but not normal B, and *T* DNA cannot amplify with either normal A or normal B.

PCR was also used to establish the *mat A* and *mat a* idiomorphs (i.e. *mat* locus allelic variants having highly dissimilar DNA sequences).

3. Results

The four T^{Nt} strains were crossed with opposite mating-type derivatives of *N. tetrasperma* strain 85, and the progeny ascospores from these crosses were harvested on water agar as well-separated clumps of 4–8 ascospores, each clump representing an individual ascus. We examined more than 800 asci from each cross, and the results summarized in table 1 show that for each cross a majority of asci were four-spored, but we also obtained decreasing fractions of five-, six-, seven-, and eight-spored asci. Moreover, mycelia from ascospores from the four-spored asci could undergo a self-cross (data not shown). Thus, the $T^{Nt} \times 85$ crosses behaved largely as one would expect for crosses in the *N. tetrasperma* strain 85 genetic background, although in 85 *A* × 85 *a* crosses the frequency of asci with more than 4 ascospores is typically less than 3%.

About 1–2% of the asci from each cross were eight-spored (table 1). The eight-spored asci were further classified as 8B:0W, 6B:2W, or 4B:4W type based on the number of black : white ascospores. Ascospores from these different ascus types were carefully picked from the water agar to 100 μL sterile water in separate tubes, they were germinated, and from the resulting mycelia we prepared genomic DNA and used it to determine the genotype by PCR as described in the 'Materials and methods' section. For example, from the cross $T(EB4)^{Nt}a \times 85 A$, we collected separate pools of 56, 12, and 42 black ascospores from the seven 8:0 asci, three 4:4 asci, and seven 6:2 asci. The ascospore pools were germinated, progeny mycelia were recovered from, respectively, 8, 2, and 9 germlings, and their genotype was determined, and the results are summarized in table 2.

Ordinarily, ascospores from the 8:0 asci are expected to be *T* or *N* homokaryons, whereas those from the 4:4 asci are expected to be *Dp* homokaryons, and those from 6:2 asci to be *T*, *N*, or *Dp* homokaryons. These expectations were fulfilled by 0 of 8, 2 of 2, and 7 of 9 progeny tested from the 8:0, 6:2, and 4:4 asci from $T(EB4)^{Nt}a \times 85 A$, while the remaining 10 progeny had genotypes that were not consistent with these expectations (table 2). Five progeny (three from the 8:0 asci plus two from the 6:2 asci) were either [*T* + *N*] or [*Dp* + *Df*] heterokaryons (designated as 'Het1' type in table 2) and their constituent nuclei had *mat A* and *mat a* mating type, and the other 5 progeny (from the 8:0 asci) were *Dp* homokaryons (table 2).

Table 2 also shows that from the two 8:0 and six 4:4 asci collected from $T(IBj5)^{Nt}a \times 85A$, only 0 of 7, and 2 of 3 progeny tested showed the expected genotypes, while the remaining 8 progeny had unexpected genotypes. Of the 7 progeny examined from the 8:0 asci, 5 were *Dp* homokaryons and 2 were Het1 type, and 1 progeny

Table 1. Ascus types from $T^{Nt} \times 85$ crosses

		[1]Ascus type (%)						
Cross	N	4	5	6	7	8:0	6:2	4:4
$T(EB4)^{Nt}$ a × 85 A	849	56	24	13	5	1	1	0.3
$T(IBj5)^{Nt}$ a × 85 A	986	75	15	6	3	0.2	0.1	1
$T(UK14-1)^{Nt}$ A × 85 a	626	78	15	5	2	1	-	-
$T(B362i)^{Nt}$ A × 85 a	996	74	17	7	1	0.1	0.3	0.2

[1]Fraction of 4-, 5-, 6-, 7- and 8- spored asci. The 8-spored asci are further identified as 8B:0W, 6B:2W, and 4B:4W types (see text).
N = number of asci examined on water agar.

examined from the 4:4 asci was Het1 type. Of the 3 Het1-type progeny examined, 2 were [*mat A + mat a*] heterokaryons, but 1 was homoallelic for mating type. Unexpected genotypes were also found in a significant fraction of the progeny from the $T(UK14-1)^{Nt}A \times 85a$ and $T(B362i)^{Nt}A \times 85a$ crosses (table 2). We obtained 6 Het1-type heterokaryons, including 2 homoallelic for mating type, plus 4 possible *Dp* homokaryons from the 8:0 asci. The B breakpoint junction of $T(UK14-1)^{Nt}$ is not known, and therefore we were unable to distinguish between the *N* and *Dp* genotypes; hence, we cannot rule out the possibility that the 8:0 asci from this cross produced up to four *Dp*-type progeny.

4. Discussion

Each of the four $T^{Nt} \times 85$ crosses examined yielded rare eight-spored asci containing ascospores with unexpected genotypes, including [$T^{Nt} + N$] or [$Dp + Df$] heterokaryons. Obtaining heterokaryotic ascospores from an eight-spored ascus is incommensurate with the supposition that ascospore partitioning occurs only at the eight-nucleus stage with each of the eight ascospores receiving one of the eight nuclei made following the post-meiotic mitosis. Our results reveal that in a small subset (~1–2%) of asci one or more nuclei from the post-meiotic mitosis can undergo additional mitoses before nuclear partitioning, and thus produce [$T^{Nt} + N$] and

Table 2. Genotype of ascospores from eight-spored asci

		Ascospore genotype by PCR	
Translocation strain[1]	Asci[2]	Expected[3]	Unexpected[4]
$T(EB4)^{Nt}$ a	8B:0W (7)	-	Het1 (3) + *Dpa* (4) + *DpA* (1)
	4B:4W (3)	*DpA* (1) + *Dpa* (1)	-
	6B:2W (7)	*Na* (3) + *DpA* (4)	Het1 (2)
$T(IBj5)^{Nt}$ a	8B:0W (2)	-	Het1 (2)[6] + *Dpa* (5)
	4B:4W (6)	*Dpa* (2)	Het1 (1)
$T(UK14-1)^{Nt}$ A	8B:0W (4)	*TA* (2)	Het2 (3) + *N* or *Dp*[5] (2A, 2a)
$T(B362i)^{Nt}$ A	8B:0W (1)	*Na* (2)	Het1 (2)[6]
	4B:4W (2)	*DpA* (2)	-
	6B:2W (3)	*Na* (1)	Het1 (1)[6]

[1]The T^{Nt} strains were crossed with *N. tetrasperma* strain 85 derivatives of the opposite mating type.
[2]Number of asci is indicated in parenthesis.
[3]Expected ascospore genotypes in 8B:0W asci are *T* or *N*, in 4B:4W asci *Dp*, and in 6B:2W asci *T*, *N*, or *Dp*. Number of ascospores with the expected genotypes is indicated in parenthesis.
[4]These genotypes are unexpected in ascospores of from the ascus type. Het1 = [*T* + *N*] or [*Dp* + *Df*] heterokaryon, Het2 = [*Dp* + *N*]/[*Dp* + *Dp*] or [*N* + *N*] heterokaryon (see footnote 5). Numbers of ascospores with this genotype is indicated in parenthesis.
[5]Since the $T(UK14-1)^{Nt}$ junction B is not known the *N* and *Dp* genotypes are indistinguishable therefore we cannot rule out the possibility of the *Dp* type.
[6]From the cross with $T(IBj5)^{Nt}$ one of the two Het1 ascospores was homokaryotic for *mat A*. From the cross with $T(B362i)^{Nt}$ one of the two Het1 ascospores from the 8B:0W ascus and the only Het 1 ascospore from the 6B:2W ascus was homokaryotic for *mat a*.

[*Dp* + *Df*] (i.e. Het1)-type progeny, whose constituent nuclei can either be of opposite mating types (i.e. [*mat A* + *mat a*]) or of the same mating type (figure 2). Het1-type progeny with nuclei of the same mating type can be generated by a crossover proximal to a translocation breakpoint, such that the *mat* locus undergoes first-division segregation, whereas the breakpoint undergoes second-division segregation (figure 2). Figure 2 shows that ascospores that receive a pair of 'first-cousin' nuclei can become homoallelic for markers that underwent first-division segregation and heteroallelic for markers that underwent second-division segregation, whereas ascospores that receive a pair of 'second-cousin' nuclei can become homoallelic for markers that underwent second-division segregation and heteroallelic for markers that underwent first-division segregation. Nuclei that are related only through mitosis (i.e. sister nuclei and their mitotic progeny) are genetically identical, therefore ascospores that receive them are homokaryotic. The production of supernumerary mitoses can also account for the generation of *Dp*-type homokaryons in the 8:0 asci, whereas normally one expects the *Dp*-type progeny only from the 4:4 and 6:2 asci.

Lewis (1969) had reported sighting rare 8B:0W asci from interspecific crosses between the heterothallic species *Sordaria heterothallis* and *S. thermophila*, and at least two ascospores produced by such asci were heterokaryotic. This suggested that interposition of additional mitoses between the post-meiotic mitosis and the partitioning of ascospores can also occur in interspecies crosses in Sordaria. On the other hand, all eight ascospores in the Sordaria 8B:0W asci were of the same mating type and had either the *S. heterothallis* or *S. thermophila* parental genotype. It was proposed that interaction between the opposite mating-type nuclei enabled intraspecific nuclear fusions to occur in the ascogenous hyphae and that the resulting diploid nucleus then underwent meiosis.

Two different models can be put forth to account for the occurrence of additional mitoses prior to nuclear partitioning. In model 1, the presence of an *N. crassa*-derived gene in the *N. tetrasperma* genetic background triggers a Bateson–Dobzhansky–Muller incompatibility (BDMI) that induces the additional mitoses. It is well known that when two lineages derived from a common ancestor become reproductively isolated (e.g. *N. crassa* and *N. tetrasperma*), genes that have undergone mutation and adaptive evolution in one lineage can potentially become dysfunctional when transferred into the other lineage, since other genes have undergone mutation and evolution in the second lineage and the sets of derived alleles were never 'tested' together prior to hybrid formation (Coyne 2016). Such BDMI can produce lethality, sterility, or other some other detriment when the incompatible genes are secondarily brought together in the hybrid. 'Occam's razor' forces us to assume no more than
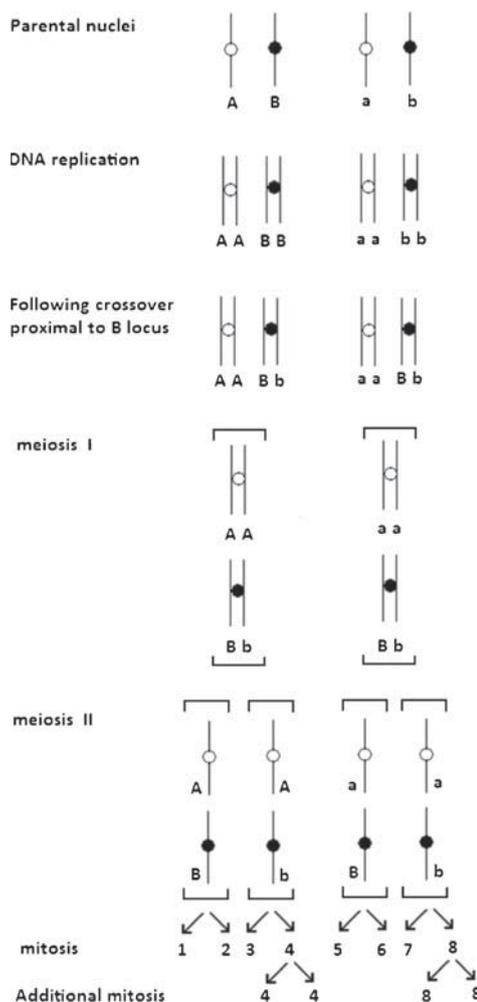

Figure 2. Progeny nuclei from a cross made between strains of genotypes A; B and a; b. A crossover is shown to occur on the chromosome whose centromere is shown with the filled circle and bearing the markers B and b. The A and a alleles segregate following the first meiotic division, whereas B and b segregate only after the second meiotic division, thus the A/a alleles undergo first-division segregation whereas B/b undergo second-division segregation. Meiosis produces four haploid nuclei indicated by the vertical brackets. A post-meiotic mitosis produces four pairs of sister nuclei, viz., (1, 2), (3, 4), (5, 6), and (7, 8). Ascus dysgenesis is represented by one or more of the eight nuclei undergoing an additional mitosis, shown here for '4' and '8'. Note that sister nuclei and their mitotic progeny have identical genotypes. First-cousin nuclei (e.g. 1 and 3) are homoallelic for markers A and a that underwent first-division segregation, but heteroallelic for markers B and b that underwent second-division segregation; in contrast, second-cousin nuclei (e.g. 1 and 5) are homoallelic for markers B and b that underwent second-division segregation, but heteroallelic for markers A and a that underwent first-division segregation. Het 1 progeny with *mat A* and *mat a* nuclei come from an ascospore that received second-cousin nuclei. Het 1 progeny with constituent nuclei of same mating type come from ascospore that received first-cousin nuclei.

one presumptive BDMI-inducing *N. crassa* gene, therefore all the four T^{Nt} strains must contain the same *N. crassa* gene, and one should be able to find it in the relatively small fraction of the *N. crassa* genome common to all the four T^{Nt} strains. However, to construct the T^{Nt} strains we had used only the *T* progeny in each backcross generation, i.e. those possessing the *T*-specific breakpoints, and these breakpoints were present on five of the seven Neurospora chromosomes. This renders it unlikely that all the four T^{Nt} strains could have inherited the same unselected *N. crassa* gene.

In model 2, which we favour, we propose that the heterokaryotic ascospores from eight-spored asci represent a background error rate in Neurospora ascus development that were fortuitously uncovered in crosses deficient for the *tol* (*tolerant*) gene product. Ordinarily, this background would not be detectable in *N. crassa*, since any rare mating-type heterokaryons formed would suffer *tol*-mediated mating-type vegetative incompatibility (Newmeyer 1970). In wild-type *N. crassa*, a [*mat A* + *mat a*] heterokaryon is vegetatively incompatible and unstable. However, if the normal *N. crassa* tol^C gene on chromosome 4R is replaced by a recessive mutant allele, *tol*, then heterokaryons of constitution [*tol mat A* + *tol mat a*] become fully compatible and stable, provided that they are homokaryotic at other *het* loci (Perkins *et al.* 2001). When the tol^T allele of *N. tetrasperma* is substituted by tol^C the [*mat A* + *mat a*] heterokaryons become unstable, thus the tol^T allele resembles the mutant *N. crassa tol* allele (Jacobson 1992). Further, in *N. crassa* any rare heterokaryons that were homoallelic for mating type would be difficult to distinguish from a homokaryon, since the only detectable difference between the two genotypes is that the heterokaryons are heteroallelic for markers that underwent second-division segregation (figure 2). The background error also would not be easy to detect in routine *N. tetrasperma* crosses because the mating-type heterokaryons from eight-spored asci are vastly outnumbered by similar heterokaryons from the four- to seven-spored asci, whereas any rare heterokaryon that was homoallelic for mating type and heteroallelic for markers that underwent second-division segregation would be difficult to distinguish from the much larger numbers of homokaryons produced from the five- to seven-spored asci (table 1), because it entails searching for markers that became heteroallelic following second-division segregation. If indeed model 2 is correct, then any attempt to search for a BDMI-inducing *N. crassa*-derived gene common to the four T^{Nt} strains would be doomed to failure.

We suggest two experimental approaches that might distinguish between the two models. In one, new *T*s could be engineered directly in *N. tetrasperma* and their crosses with the strain 85 derivative of the opposite mating type could then be examined for the production of eight-spored asci with heterokaryotic ascospores, as would be predicted by model 2. In the other approach the *N. crassa* genome fraction

in the T^{Nt} strains can be further reduced by doing additional introgression crosses, in the expectation that at some stage the T^{Nt} × 85 crosses might fail to make eight-spored asci with heterokaryotic ascospores. If this expectation is borne out, it would support model 1.

References

Bistis GN 1996 Trichogynes and fertilization in uni- and bimating type colonies of *Neurospora tetrasperma*. *Fungal Genet. Biol.* **20** 93–98

Coyne JA 2016 Theodosius Dobzhansky on hybrid sterility and speciation. *Genetics* **202** 5–7

Giri DA, Rekha S and Kasbekar DP 2015 Neurospora heterokaryons with complementary duplications and deficiencies in their constituent nuclei provide an approach to identify nucleus-limited genes. *G3* **5** 1263–1272

Giri DA, Rekha S and Kasbekar DP 2016 Crosses heterozygous for hybrid Neurospora translocation strains show transmission ratio distortion disfavoring homokaryotic ascospores made following alternate segregation. *G3* **6** 2593–2600

Jacobson D 1992 Control of mating type heterokaryon incompatibility by the *tol* gene in *Neurospora crassa* and *N. tetrasperma*. *Genome* **35** 347–353

Lewis LA 1969 Genetics of an interspecific cross in the genus Sordaria. I. Analysis of 8-spored asci homozygous for the mating type factor. *Genetics* **62** 79–101

Metzenberg RL 2003 Vogel's medium N salts: avoiding the need for ammonium nitrate. *Fungal Genet. Newsl.* **50** 14

Newmeyer D 1970 A suppressor of the heterokaryon incompatibility associated with mating type in *Neurospora crassa*. *Can. J. Genet. Cytol.* **15** 577–585

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

Perkins DD, Radford A and Sachs MS 2001 *The Neurospora compendium chromosomal loci* (San Diego, California, USA: Academic Press)

Raju NB 1992 Functional heterothallism resulting from homokaryotic conidia and ascospores in *Neurospora tetrasperma*. *Mycol. Res.* **96** 103–116

Raju NB and Perkins DD 1994 Diverse programs of ascus development in pseudohomothallic strains of Neurospora, Gelasinospora, and Podospora. *Dev. Genet.* **15** 104–118

Singh PK 2010 *Genetic and molecular analysis of Neurospora duplications and duplication-generating translocations*. PhD Thesis, Jawaharlal Nehru University, New Delhi

Singh PK, Iyer SV, Sowjanya TN, Raj BK 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

Corresponding editor: Luis M Corrochano