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REVIEW ARTICLE

Ascus dysgenesis in hybrid crosses of Neurospora and Sordaria (Sordariaceae)

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Abstract. When two lineages derived from a common ancestor become reproductively isolated (e.g. *Neurospora crassa* and *N. tetrasperma*), genes that have undergone mutation and adaptive evolution in one lineage can potentially become dysfunctional when transferred into the other, since other genes have undergone mutation and evolution in the second lineage, and the derived alleles were never 'tested' together before hybrid formation. Bateson (1909), Dobzhansky (1936), and Muller (1942) recognized that incompatibility between the derived alleles could potentially make the hybrid lethal, sterile, or display some other detriment. Alternatively, the detrimental effects seen in crosses with the hybrids may result from the silencing of ascus-development genes by meiotic silencing by unpaired DNA (MSUD). Aberrant transcripts from genes improperly paired in meiosis are processed into single-stranded MSUD-associated small interfering RNA (masiRNA), which is used to degrade complementary mRNA. Recently, backcrosses of *N. crassa* / *N. tetrasperma* hybrid translocation strains with wild-type *N. tetrasperma* were found to elicit novel ascus dysgenesis phenotypes. One was a transmission ratio distortion that apparently disfavoured the homokaryotic ascospores formed following alternate segregation. Another was the production of heterokaryotic ascospores in eight-spored asci. Lewis (1969) also had reported sighting rare eight-spored asci with heterokaryotic ascospores in interspecific crosses in Sordaria, a related genus. Ordinarily, in both Neurospora and Sordaria, the ascospores are partitioned at the eight-nucleus stage, and ascospores in eight-spored asci are initially uninucleate. Evidently, in hybrid crosses of the family Sordariaceae, ascospore partitioning can be delayed until after one or more mitoses following the postmeiotic mitosis.

Keywords. ascus dysgenesis; Bateson–Dobzhansky–Muller incompatibility; chromosome translocations; heterokaryons; introgression; meiotic silencing by unpaired DNA.

Bateson–Dobzhansky–Muller incompatibility, introgression of translocations across species, meiotic silencing, and ascus dysgenesis phenotypes in Neurospora

Theodosius Dobzhansky's 1936 article was described as 'the first concerted effort to work out the genetic changes producing a puzzling reproductive barrier: hybrid sterility' (Coyne 2016), and is an important landmark in the modern evolutionary synthesis which brings the findings of genetics together with those of natural history. Independently, Bateson (1909) and Muller (1942), also helped propel the acceptance of the idea that incompatibility between allelic changes at different gene loci in geographically isolated lineages descended from a common ancestor can produce lethality, sterility, or other defects when secondarily brought together in the hybrid (Orr 1996). Recently, we uncovered two novel 'ascus dysgenesis' phenotypes in backcrosses between wild-type *N. tetrasperma* and hybrid *N. crassa* / *N. tetrasperma* translocation strains constructed by introgressing *N. crassa* insertional translocations (*IT*) into *N. tetrasperma* (Giri *et al.* 2016). One phenotype was a transmission ratio distortion that appeared to disfavour the homokaryotic ascospores formed following alternate segregation and the other was the production of eight-spored asci bearing heterokaryotic ascospores. It is possible that the ascus dysgenesis phenotypes reflect Bateson–Dobzhansky–Muller incompatibility (BDMI) of *N. crassa* genes in the *N. tetrasperma* genetic background. Typically, the introgressions involved crossing the *N. crassa*

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IT strain with the *N. crassa* / *N. tetrasperma* hybrid strain C4T4 *a*, backcrossing the *IT* progeny with C4T4 *a*, followed by two or three backcrosses of the *IT* progeny with the *N. tetrasperma* standard strain 85. The unselected fraction of the *N. crassa* genome decreases by a factor of 0.75 in each cross with strain C4T4 *a*, and by a factor of 0.5 in each cross with strain 85. Therefore, an unselected *N. crassa* gene has a probability of 14% (or less) of being cointrogressed with the *IT* ($0.75 \times 0.75 \times 0.5 \times 0.5$).

Alternatively, the dysgenic phenotypes could have arisen from MSUD (Shiu *et al.* 2001). Most of the genetic studies in Neurospora use the isogenic *N. crassa* Oak Ridge background strains OR *A* and OR *a* (Perkins 2004). In this background MSUD, an RNAi-mediated process that eliminates transcripts from misaligned genes, is quite efficient (Ramakrishnan *et al.* 2011) and can be triggered by a relatively small (4.1 kb) misalignment engineered between allelic sequences during meiotic chromosome pairing (Samarajeewa et al. 2014). The improperly paired genes are transcribed into 'aberrant RNA', which is made double-stranded, and then processed into single-stranded MSUD-associated small interfering RNA (masiRNA) that is used by a silencing complex to identify and degrade complementary mRNA (Hammond *et al.* 2013b). Crosses homozygous for the engineered sequence or heterozygous for a semi-dominant MSUD suppressor do not exhibit MSUD. Conceivably, one or more ascus-development gene or any sufficiently long DNA sequence might be misaligned in crosses of *N. tetrasperma* with the hybrid translocation strains, and thus, give rise to the ascus dysgenesis phenotypes. In this article I summarize these phenotypes, revisit the report of Lewis (1969) of a similar phenotype in interspecific crosses in the related genus Sordaria, and discuss an approach to determine whether these phenotypes are caused by MSUD.

Hybrid *IT* ^{Nt} strains made by introgressing *N. crassa IT* into *N. tetrasperma*

The transfer of genes and genome segments from one species into another through hybridization and backcrosses is called introgression. We (Giri *et al.* 2015) had introgressed four *N. crassa IT*s (namely *T*(*EB4*), *T*(*IBj5*), *T*(*UK14-1*) and *T*(*B362i*) (Perkins 1997)) into *N. tetrasperma*. The introgressions enabled us to create the first heterokaryotic strains whose constituent nuclei contained complementary duplications and deficiencies, i.e., the heterokaryons had the genotype [*Dp* + *Df*] (Giri *et al.* 2015). The rationale for creating the [*Dp* + *Df*] heterokaryons was outlined in Kasbekar (2014).

Ascus development in *N. crassa* and *N. tetrasperma* share many similarities, but the differences are also striking (Raju 1992; Raju and Perkins 1994; Grognet and Silar 2015). In both species, the haploid *mat A* and *mat a* parental nuclei fuse to produce a diploid zygote nucleus that immediately undergoes meiosis, in which the *mat A* and *mat a* idiomorphs undergo first division segregation. Thereafter, a postmeiotic mitosis generates eight haploid nuclei (4 *mat A* + 4 *mat a*). In *N. crassa*, these nuclei are partitioned into the eight initially uninucleate ascospores, whereas in *N. tetrasperma* they go into four initially binucleate (dikaryotic) ascospores, each ascospore receiving a pair of nonsister nuclei (1 *mat A* and 1 *mat a*). Occasionally, a pair of smaller homokaryotic ascospores (1 *mat A* + 1 *mat a*) replaces a dikaryotic ascospore. The dominant *Eight-spore* (*E*) mutant increases the frequency of such replacement and can even generate asci with eight homokaryotic ascospores, resembling those of *N. crassa* (Dodge 1939; Calhoun and Howe 1968). Thus, *N. crassa* is a heterothallic species, produces only homokaryotic ascospores, that upon germination generate self-sterile mycelia, of *mat A* or *mat a* mating type, which can mate with mycelium of the opposite mating type derived from another ascospore. In contrast, *N. tetrasperma* produces dikaryotic [*mat A* + *mat a*] ascospores, that upon germination generate mycelia that can undergo a selfcross, as well as homokaryotic *mat A* or *mat a* ascospores that generate self-sterile mycelia. A subset of conidia (vegetative spores) produced by the dikaryotic *N. tetrasperma* mycelia by chance could be homokaryotic, and hence produce self-sterile mycelia. The mycelia produced from homokaryotic ascospores and conidia can out-cross with like mycelia of the opposite mating type (Raju and Perkins 1994; Bistis 1996). Thus, *N. tetrasperma* is a pseudohomothallic species.

An *IT* transfers a donor chromosome segment into a recipient chromosome (Perkins 1997) and creates three breakpoint junctions, 'A' on the donor chromosome and 'B' and 'C' (proximal and distal) on the recipient chromosome (Singh *et al.* 2010). My students had defined the breakpoint junctions of several *N. crassa IT*s (Singh 2010; Singh *et al.* 2010; Giri *et al.* 2016), which enabled us to use PCR with breakpoint junction-specific primers to distinguish the *IT* progeny from their *N* and *Dp* siblings (*N*, normal sequence strain; *Dp*, duplication strain; see figure 1). This, in turn, made it possible to recurrently backcross normal sequence *N. tetrasperma* with the *IT* progeny from a previous backcross. The introgression crosses were continued until we obtained self-fertile (heterokaryotic) [*IT* + *N*] progeny (see below). From the [*IT* + *N*] mycelia, we isolated self-sterile (homokaryotic) conidial derivatives containing the introgressed translocation, now designated as IT^{Nt}, since nominally its genome is from *N. tetrasperma* except at the *N. crassa*-derived translocation breakpoint junctions. Of course, one could have used the conidially-derived IT^{Nt} homokaryons for additional introgression crosses and thereby eliminated any residual *N. crassa* genes by recombination, but the [IT^{Nt} + *N*] heterokaryon was a reasonable endpoint for the introgression crosses,

thus, strictly speaking, the IT^{Nt} strains are *N. crassa* / *N. tetrasperma* hybrids.

The IT^{Nt} strains are available from the Fungal Genetics Stock Center, Kansas State University, Manhattan, Kansas, USA, with the following strain numbers: FGSC 25288 *T(VR>VII) EB4 a*; FGSC 25289 *T(VIL>IR) IBj5 a*; FGSC 25290 *T(VIR>VL) UK14-1 A*; and FGSC 25291 *T(IVR>I) B362i A* (there was an error in assigning the strain numbers in Giri *et al.* (2015) and these numbers correct that error).

In $IT \times N$ crosses the chromosomes can segregate either through alternate (ALT) or adjacent-1 (ADJ) segregation (Perkins 1997; figure 1). In *N. crassa*, ALT produces eight viable parental-type progeny ascospores per ascus (4 IT + 4 N), whereas ADJ produces four viable duplication progeny, and four inviable ones with the complementary deficiency (4 Dp + 4 Df). Viable ascospores become pigmented (black, B) whereas inviable ones remain unpigmented (white, W). Since ALT and ADJ are equally likely, equal numbers of the two ascus types are produced (i.e., 8B : 0W = 4B : 4W), that together yield equal numbers of viable IT, N and Dp progeny. In contrast, ALT and ADJ in backcrosses of the IT^{Nt} strains with N strains of *N. tetrasperma* generate four heterokaryotic ascospores per ascus, respectively, of [IT^{Nt} + N] and [$Dp + Df$] genotypes. The [IT^{Nt} + N] heterokaryotic mycelia can produce homokaryotic (self-sterile) conidial derivatives of either mating types. If one mating type has the genotype IT^{Nt} then the other has the genotype N. The [Dp + Df] heterokaryons produce viable conidial homokaryotic derivatives of only the mating type of the Dp nucleus, since Df homokaryons are inviable. Selfcross of either heterokaryon can again generate [IT^{Nt} + N] and [Dp + Df] heterokaryotic progeny. Additionally, as a result of the occasional replacement of heterokaryotic ascospores by pairs of homokaryotic ones, the $IT^{Nt} \times N$ crosses also produce some self-sterile IT^{Nt}, N and Dp homokaryotic progeny. The fraction of homokaryotic progeny is increased when an E mutant strain is used as the N type strain and in $IT^{Nt} \times E$ crosses, ALT and ADJ segregations are expected to produce equal numbers of 8B : 0W and 4B : 4W asci, whereas 6B : 2W asci are generated by crossover between the centromere and a breakpoint, followed by ALT or ADJ segregation. In sum, ALT yields the [IT^{Nt} + N], IT^{Nt} and N progeny types, whereas ADJ yields [Dp + Df] and Dp types.

Both [IT^{Nt} + N] and [Dp + Df] heterokaryons contain the same genes and therefore are expected to share the same phenotype. However, if the Df nucleus were

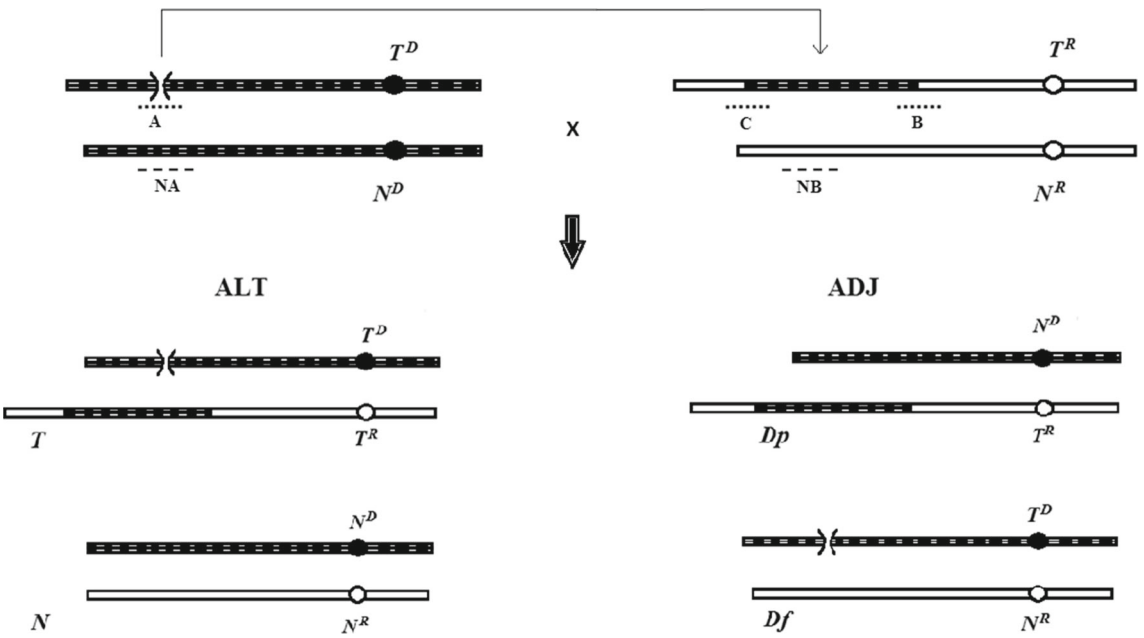

Figure 1. Alternate (ALT) and adjacent-1 (ADJ) segregation. T^D and T^R are the donor and recipient chromosomes of the IT strain, and N^D and N^R are their N-derived homologues. Dotted lines indicate the A, B and C breakpoint junctions, and dashed lines indicate the NA and NB segments in the normal sequence homologues 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 postmeiotic mitosis generate eight parental-type nuclei, namely 4 T + 4 N. In ADJ, N^D and T^R segregate to one pole and T^D and N^R to the other, eventually to produce eight non-parental nuclei, 4 Dp + 4 Df. The T, N and Dp types are viable, whereas the Df type is inviable. IT progeny contain A, B and C breakpoints, Dp progeny contains B and C breakpoints but not A, and N progeny contains none of these breakpoints.

deleted for a nucleus-limited gene (Kasbekar 2014), then it would not be complemented by the *Dp* nuclei sharing the same cytoplasm and [*Dp* + *Df*] and [*IT* + *N*] heterokaryons could potentially have different phenotypes.

Transmission ratio distortion in backcrosses of some *IT* ^{Nt} strains with *N. tetrasperma*

It is time-consuming to identify and genotype heterokaryotic progeny of *N. tetrasperma* $IT^{Nt} \times N$ crosses. Self-fertility provides the first evidence that a strain is heterokaryotic and it takes ~10–20 days to establish that a mycelium is self-fertile. Then the conidia from the heterokaryotic mycelium must be streaked on sorbose-supplemented medium to obtain well-isolated derivative colonies, and typically, 60 or more colonies must be picked to crossing medium and scored for self-sterility (another 10–20 days). Finally, the ~10% of conidial derivatives expected to be self-sterile (and hence homokaryotic) can be tested by PCR to establish their genotype. The inference that backcrosses of $T(EB4)^{Nt}a$, $T(IBj5)^{Nt}a$, $T(UK14\text{-}1)^{Nt}A$ and $T(B362i)^{Nt}A$, with opposite mating type derivative of the *N. tetrasperma* standard laboratory strain 85, produced equal numbers of [IT^{Nt} + *N*] and [*Dp* + *Df*] progeny was based on analysis of admittedly small numbers of heterokaryons, namely 8, 6, 6 and 10 (Giri *et al.* 2015). In contrast, the homokaryotic progeny are more quickly identified and genotyped; they fail to selfcross even after 20 days, thus confirming their status as mating type homokaryons, thereafter their genotype can be established by PCR. Among the self-sterile progeny from the crosses $T(EB4)^{Nt}a \times 85\ A$ and $T(EB4)^{Nt}a \times E\ A$, we obtained, 11 IT^{Nt}, 11 *N* and 9 *Dp*, and 7 IT^{Nt}, 15 *N* and 17 *Dp* types. We abbreviate this phenotype to *Dp* = *N* = *T*. However, unexpectedly, the self-sterile progeny from crosses $T(IBj5)^{Nt}a$ and $T(B362i)^{Nt}A$ with *E* and 85 derivatives displayed the *Dp* >> *N*, *T*, or *Dp*, *N* >> *T* phenotype (Giri *et al.* 2016). The $T(IBj5)^{Nt}a \times 85\ A$ cross produced 1 IT^{Nt}, 1 *N* and 23 *Dp* progeny; $T(IBj5)^{Nt}a \times E\ A$ produced 0 IT^{Nt}, 2 *N* and 28 *Dp*; and $T(B362i)^{Nt}A \times 85\ a$ produced 0 IT^{Nt}, 3 *N* and 48 *Dp*. These ratios were designated as the *Dp* >> *N*, *T* phenotype. The cross $T(B362i)^{Nt}A \times E\ a$ produced 0 IT^{Nt}, 27 *N* and 14 *Dp* and this ratio was defined as the *Dp*, *N* >> *T* phenotype.

The following model was proposed to explain these results. If the chromosomes undergo alternate segregation (ALT), then the number of viable ascospores that can form in an ascus can range from four to eight, depending on whether all the ascospores are [T^{Nt} + *N*] heterokaryons (four), to whether all the ascospores are homokaryons (eight, $4T^{Nt}$+ 4*N*) formed by the replacement of heterokaryons by homokaryotic pairs. In contrast, not more than four viable ascospores are possible following adjacent-1 segregation (ADJ), regardless of whether or not replacement occurs. If there is no replacement, then four heterokaryotic [*Dp* + *Df*] ascospores are formed, and if replacement is total even then we get four viable *Dp* ascospores, and the other four are inviable *Df* type. BDMI, triggered by the action of *N. crassa* genes in an otherwise *N. tetrasperma* cross, or MSUD, triggered by misalignment between the *N. crassa* and *N. tetrasperma* homologues in meiosis, might create an insufficiency for an ascospore maturation factor whose quantity is just adequate to promote the maturation of four ascospores, but when 5 (or more) otherwise viable ascospores compete for this limited resource none gets enough. In asci bearing >4 viable ascospores this produces a situation akin to a 'tragedy of the commons', consequently, fewer homokaryons mature properly following ALT, than following ADJ, and the *Dp* >> *N*, IT^{Nt} phenotype is produced. The idea is that the more viable ascospores are, the more is the competition among them for the maturation factor, and few of them obtain enough factor to mature properly. However, the factor deficiency does not affect the [*Dp* + *Df*] / [IT^{Nt} + *N*] heterokaryon ratio. Although, Jacobson *et al.* (2008) reported that MSUD is reduced or absent in *N. tetrasperma*, we had found that MSUD can occur at least early in a selfcross, although its strength subsequently declines (Ramakrishnan *et al.* 2011).

The *Dp*, *N* >> IT^{Nt} phenotype might reflect a secondary loss of IT^{Nt} nuclei from the [IT^{Nt} + *N*] germlings thus producing *N* homokaryotic cultures (Giri *et al.* 2016). The IT^{Nt} nuclei, which contain more *N. crassa*-derived sequences than the *N* nuclei (which can contain some unselected *N. crassa* genome segments), might be susceptible to a second BDMI that acts during early germling growth. Alternatively, the second BDMI might affect the viability of IT^{Nt} nuclei in [IT^{Nt} + *N*] asci, effectively converting them into four-spored [T^{lethal} + *N*] asci. Presumably, the *N. crassa* gene(s) triggering such BDMIs (or MSUD) are not present in the $T(EB4)^{Nt}a$ strain. One way to test this model is to repeat the introgressions and ask whether any of the new $T(EB4)^{Nt}$ and $T(IBj5)^{Nt}$ strains, respectively, do or do not induce the transmission ratio distortion. The selected markers (here, the translocation breakpoints) are the same in both introgressions, but the unselected genome fractions can differ. If the TRD-causing gene is from the unselected fraction, then by repeating the introgressions, one might obtain a new T^{Nt} strain with a different TRD phenotype than the old T^{Nt} strain (Alfred D. Hershey's idea of heaven was 'to have one experiment that works, and keep doing it all the time' (Judson 1979)).

Heterokaryons tend not to outcross as females (Bistis 1996), and crosses involving *Dp* strains are barren, because in *Dp*-heterozygous crosses the *Dp*-borne genes are silenced by MSUD (Perkins 1997; Shiu *et al.*

2001), therefore, the transmission ratio distortion reinforces reproductive isolation between *N. crassa* and *N. tetrasperma*. Moreover, the distortion can potentially be triggered even before the introgression crosses have advanced sufficiently to produce self-fertile heterokaryons and the consequent precipitous drop in *T* progeny numbers can jeopardize further introgression efforts (Giri *et al.* 2016).

Heterokaryotic ascospores in 8B : 0W asci

We were surprised that the crosses $T(IBj5)^{Nt}a \times 85\ A$ and $T(B362i)^{Nt}A \times 85\ a$ showed the $Dp >> N, IT$ phenotype, because these crosses also produced a few eight-spored asci, including 8B : 0W types (Giri *et al.* 2015), and one would have expected the 8B : 0W asci to yield *IT* and *N* ascospores. To address this anomaly, we harvested asci on water agar, carefully picked only the rare eight-spored ones, and used PCR to genotype the ascospores. We found that several ascospores from the eight-spored asci produced heterokaryotic mycelia (Giri *et al.* 2016). Ordinarily, one does not expect to see any heterokaryotic ascospores in the 8B : 0W asci since ascospores are partitioned at the eight-nucleus stage and each of the eight ascospores receives one of the eight nuclei. Moreover, eight-spored asci with heterokaryotic ascospores were not previously reported in either *N. crassa* or *N. tetrasperma*. We, interpreted our results to suggest that in $IT^{Nt} \times 85$ crosses the postmeiotic mitosis might be followed by one or more additional mitoses before nuclear partitioning into the ascospores (Giri *et al.* 2016). We have now found that even the rare 8B : 0W asci from the crosses $T(EB4)^{Nt}a \times 85\ A$ and $T(UK14\text{-}1)^{Nt}A \times 85\ a$ included some heterokaryotic ascospores (S. Rekha and D. P. Kasbekar, unpublished results), suggesting that anomalous heterokaryotic ascospores can form independently of the transmission ratio distortion. However, had we not seen the transmission ratio distortion in the $T(IBj5)^{Nt}a \times 85\ A$ and $T(B362i)^{Nt}A \times 85\ a$ crosses that produced 8B : 0W asci, we might not have examined the 8B : 0W asci from any of the $IT^{Nt} \times 85$ cross, instead we would have tacitly assumed that they were *T* or *N* type homokaryons.

Heterokaryotic ascospores from 8B : 0W asci in Sordaria

Only one other article in the literature, namely Lewis (1969), has reported the detection of heterokaryotic ascospores from 8B : 0W asci. Lewis had found that interspecific crosses between the heterothallic species *Sordaria heterothallis* and *S. thermophila* produced abundant perithecia that contained mostly aborted asci. However, occasionally they made some rare 8B : 0W asci (~1 such ascus per 30 perithecia). All eight ascospores in such asci were of the same mating type and had either the *S. heterothallis* or *S. thermophila* parental genotype. Such homozygous asci were never seen in intraspecific crosses. Lewis used *S. heterothallis* heterokaryotic strains with marked nuclei of the same mating type as one parent in the interspecific cross and showed that the homozygous asci arose from intraspecific nuclear fusion. He proposed that interaction between the opposite mating-type nuclei enabled intraspecific nuclear fusions to occur in the ascogenous hyphae, and the resulting diploid nucleus then underwent meiosis. He found at least two such ascospores produced heterokaryotic mycelia (see table 6 of Lewis (1969), ascospores 92_7 and 240_8), and suggested that these mycelia were derived either from heterokaryotic ascospores or from the breakdown of disomic ascospores. In either case, one would have expected some other ascospore in the ascus to either be anucleate or to have the complementary lethal nullosomy and such ascospores should have remained unpigmented. However, all the eight ascospores were black, therefore, I suggest that the heterokaryotic mycelia must have emanated from heterokaryotic ascospores that resulted from the occurrence of one or more additional mitoses following the postmeiotic mitosis but prior to nuclear partitioning, just as we have proposed for the Neurospora $IT^{Nt} \times 85$ crosses.

Does MSUD underlie the ascus dysgenesis phenotypes?

The genes *sad-1*, *sad-2*, *sad-3*, *sad-4*, *sad-5*, *sad-6*, *sms-2*, *dcl-1* and *qip* encode MSUD proteins (Shiu and Metzenberg 2002; Lee *et al.* 2003, 2010; Shiu *et al.* 2006; Alexander *et al.* 2008; Xiao *et al.* 2010; Hammond *et al.* 2011, 2013a; Samarajeewa et al. 2014). Several of these genes were identified as deletions or severely RIP-altered alleles that semidominantly suppressed MSUD in heterozygous crosses. Presumably in *Suppressor* × *wild type* crosses, the deletion allele prevented its wild-type homologue from pairing properly, induced its autogenous silencing, and decreased the encoded protein's level below the threshold required for generalized MSUD. The *sad-1Δ* and *sad-2Δ* mutants (i.e. *Sad-1* and *Sad-2*) were strong dominant suppressors of MSUD, whereas the *sad-3Δ*, *sad-4Δ*, *sad-5Δ*, *sad-6Δ*, *sms-2Δ* (i.e. *Sms*-2, *Suppressor of meiotic silencing-2*), *dcl-1Δ* and *qipΔ* deletions were not (Lee *et al.* 2003, 2010; Alexander *et al.* 2008; Xiao *et al.* 2010; Hammond *et al.* 2011, 2013a). Hammond *et al.* (2013a) suggested that higher expression levels or longer protein half-life might make the latter genes harder to self-silence. Gene expression or protein stability differences might likewise explain why MSUD in some genes (e.g. *act*, *asm-1* and *mei-3*) was suppressible even by the weak suppressors, whereas in others (e.g. Bml^r and *r*) MSUD was suppressible only by *Sad-1* and *Sad-2* (Ramakrishnan *et al.* 2011; Hammond *et al.* 2013a). *Sad-1* and *Sad-2*

also suppressed the barren phenotype of duplication-heterozygous crosses (i.e. $Dp \times N$), as well as the classical ascus-dominant mutations, which are known or suspected to be deletion alleles, that trigger MSUD in their wild-type homologues (e.g. ban^+, $dip\text{-}1^+$, pk^+ and r^+) (Shiu *et al.* 2001, 2006; Kasbekar 2013). No MSUD suppressor has yet been isolated in *N. tetrasperma*. A RIP-induced *sad-1* mutant obtained in my laboratory (Bhat *et al.* 2004) was not sufficiently altered in DNA sequence to exert a suppressor phenotype. Instead, we are introgressing the *N. crassa Sad-2* suppressor into *N. tetrasperma* (D. A. Giri and D. P. Kasbekar, unpublished results). Suppression of ascus dysgenesis in $IT^{Nt} \times Sad\text{-}2^{Nt}$ crosses would suggest that MSUD is involved in their generation. If MSUD is involved, then an RNA-seq-based analysis of masi-RNA produced by $IT^{Nt} \times N$ crosses might flag candidate misaligned genes, in an approach similar to that used by Wang *et al.* (2015), to discover a novel DNA transposon in the otherwise well-studied OR *A* strain. A $Sad\text{-}2^{Nt}$ strain will also allow us to examine $E \times Sad\text{-}2^{Nt}$ crosses.

Conclusions

Crosses of normal sequence *N. tetrasperma* strains with hybrid *N. crassa* / *N. tetrasperma* translocation strains ($N \times IT^{Nt}$) exhibited two different ascus dysgenic phenotypes: (i) a transmission ratio distortion in the homokaryotic progeny, resulting in $Dp >> N, T$ or $Dp, N >> T$ progeny ratios, and (ii) the production of eight-spored asci containing heterokaryotic ascospores. These phenotypes are attributable to either BDMI of *N. crassa* genes in crosses in the *N. tetrasperma* genetic background, or to the triggering of MSUD due to misalignment of genes on *N. tetrasperma*-derived and *N. crassa*-derived chromosome segments. The construction of $Sad\text{-}2^{Nt}$ MSUD suppressor strain would make it possible to ask whether these phenotypes are seen in $Sad\text{-}2^{Nt} \times IT^{Nt}$ crosses. Had we continued the introgressions crosses even after the recovery of the $[IT^{Nt} + N]$ heterokaryotic strains, then it is conceivable that recombination might have eliminated the BDMI-inducing *N. crassa* gene (or MSUD-inducing *N. crassa*-derived misaligned segment) and the ascus dysgenesis phenotypes also could have disappeared, unless perchance the gene was tightly linked with the translocation itself. This possibly happened during the construction of the $T(EB4)^{Nt}$ strain with respect to the transmission ratio distortion, but not *vis-à-vis* the production of heterokaryotic ascospores in eight-spored asci. Both Neurospora and Sordaria belong to the family Sordariaceae and in hybrid crosses of both the genera BDMI (and/or MSUD) appears to uncouple ascospore partitioning from the postmeiotic mitosis, and allows one or more additional mitoses to occur. The resulting supernumerary nuclei go into making the heterokaryotic ascospores. Future research will have to address whether the dysgenic phenotype in the two genera shares a common molecular basis.

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