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Brief Communication

A transmission ratio distortion and the 'max-4' ascus phenotype: Do both reflect the same Bateson-Dobzhansky-Muller Incompatibility emerging during trans-species introgression of translocations in *Neurospora*?

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The $T(EB4)^{Nt}a$, $T(IBj5)^{Nt}a$, and $T(B362i)^{Nt}A$ strains were constructed by introgressing the insertional translocations EB4, IBj5, and B362i from *Neurospora crassa* into the related species *N. tetrasperma*. The progeny from crosses of $T(IBj5)^{Nt}a$ and $T(B362i)^{Nt}A$ with opposite mating-type derivatives of the standard *N. tetrasperma* strain 85 exhibited a unique and unprecedented transmission ratio distortion (TRD) that disfavored homokaryons produced following alternate segregation relative to those produced following adjacent-1 segregation. The TRD was not evident among the [*mat A* + *mat a*] dikaryons produced following either segregation. Further, crosses of the $T(IBj5)^{Nt}a$ and $T(B362i)^{Nt}A$ strains with the *Eight spore* (*E*) mutant showed an unusual ascus phenotype called 'max-4'. We propose that the TRD and the max-4 phenotype are manifestations of the same Bateson-Dobzhansky-Muller incompatibility (BDMI). Since the TRD selects against 2/3 of the homokaryotic progeny from each introgression cross, the BDMI would have enriched for the dikaryotic progeny in the viable ascospores, and thus, paradoxically, facilitated the introgressions.

Keywords. Adjacent-1 segregation; alternate segregation; dikaryon; homokaryon; introgression; translocations; transmission ratio distortion

1. Introduction

In sexual crosses in *Neurospora crassa* and *N. tetrasperma*, the parental *mat A* and *mat a* haploid nuclei fuse to produce the diploid zygote nucleus that undergoes meiosis and a post-meiotic mitosis to generate eight haploid progeny nuclei in a sac-like cell called the ascus. In *N. crassa*, the progeny nuclei are partitioned into eight initially uninucleate ascospores, each ascospore receiving a single *mat A* or *mat a* nucleus, whereas in *N. tetrasperma* they are partitioned into four initially binucleate ascospores, each ascospore receiving a non-sister *mat A* and *mat a* pair. Occasionally, a dikaryotic *N. tetrasperma* ascospore can be replaced by a pair of homokaryotic ones, thus producing smaller numbers of asci with more than four, and up to eight homokaryotic ascospores (4 *mat A* type and 4 *mat a* type). Dikaryotic ascospores germinate to produce mycelium containing nuclei of both mating types and, consequently, the mycelium can undergo a self-cross. In contrast, the self-sterile mycelia produced from the smaller fraction of homokaryotic ascospores, and from the homokaryotic conidia (vegetative spores) isolated from the dikaryotic mycelia, can out-cross with like mycelia of the opposite mating type. *N. crassa* ascospores generate mycelium that can only out-cross with opposite mating-type mycelia derived from another ascospore.

The *N. crassa* life-cycle facilitated the isolation and characterization >350 chromosome rearrangements, whereas none were isolated in *N. tetrasperma* (Perkins 1997). Nevertheless, by introgressing four *N. crassa* insertional translocations (*EB4*, *IBj5*, *UK14-1*, and

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B362i) into *N. tetrasperma*, we created T^{Nt} strains whose genome was nominally from *N. tetrasperma*, except at the *N. crassa*-derived translocation breakpoint junctions. Crosses of the T^{Nt} strains with opposite mating-type derivatives of the *N. tetrasperma* standard strain 85 (viz., 85*A* or 85*a*) yielded self-fertile [*Dp* + *Df*] dikaryotic progeny containing complementary duplications and deficiencies in their constituent nuclei, a first in any system (Giri *et al*. 2015). They also produced self-fertile [T^{Nt} + *N*] progeny whose constituent nuclei of opposite mating types had the translocation and normal sequence genotypes. The [T^{Nt} + *N*] and [*Dp* + *Df*] types resulted, respectively, from alternate (ALT) and adjacent-1 (ADJ) segregation, and were distinguishable via their homokaryotic conidia; the [T^{Nt} + *N*] dikaryons produced homokaryotic conidia of either mating type, whereas the [*Dp* + *Df*] dikaryons produced them of only the mating type of the *Dp* nucleus, since the *Df* homokaryons were inviable. Either heterokaryon, when self-crossed, again yielded [T^{Nt} + *N*] and [*Dp* + *Df*] progeny (Giri *et al*. 2015). The homokaryotic *T*- and *N*-type ascospores also result from ALT, whereas ADJ produces the *Dp*- or *Df*-type homokaryons. Homokaryotic *T*, *N*, and *Dp* ascospores are viable and upon maturation become pigmented (black, B), whereas the *Df* type are inviable and remain unpigmented (white, W). ALT and ADJ are equally likely, therefore the rare eight-spored asci from *N. tetrasperma* T^{Nt} x *N* crosses are expected to include equal numbers of 8B:0W and 4B:4W ascus types, as in the more numerous eight-spored asci from the corresponding *N. crassa T* x *N* crosses. In contrast, four-spored asci are 4B:0W type regardless of ALT or ADJ.

The *T(IBj5)*^{Nt}*a* x 85*A* and *T(B362i)*^{Nt}*A* x 85*a* crosses displayed a transmission ratio distortion (TRD) that disfavored the homokaryotic progeny formed following ALT, without apparently affecting those from ADJ or the dikaryotic progeny (Giri *et al*. 2016). Such TRD was never seen in the corresponding *N. crassa T* x *N* crosses (Perkins 1997; Giri *et al*. 2015). We hypothesized that the presence of a *N. crassa*-derived gene in the T^{Nt} strains might have triggered a Bateson-Dobzhansky-Muller incompatibility (BDMI) in the *N. tetrasperma* genetic background to create an inadequacy for an ascospore maturation factor. Inadequate amount of an ascospore maturation factor might affect asci with more than four viable ascospores more severely than asci with four viable ascospores, thus effectively disfavoring the homokaryotic progeny formed following ALT, since ADJ never leads to production of more than four viable ascospores (Giri *et al*. 2016). The *T(EB4)*^{Nt}*a* x 85*A* cross did not show TRD, presumably because the BDMI-inducing *N. crassa* gene was absent from the *T(EB4)*^{Nt}*a* strain. Since this was the first putative BDMI reported in *Neurospora* we repeated the T^{Nt} x 85 crosses afresh to reconfirm these findings. This paper reports the re-confirmation.

The *N. tetrasperma Eight spore* (*E*) mutant increases the replacement of dikaryotic ascospores by homokaryotic pairs, and *E* x *WT* crosses produce many eight-spored asci, as well as asci with 7-5 ascospores (Dodge 1939). Crosses of the *T(IBj5)*^{Nt} *a* and *T(B362i)*^{Nt} *A* strains with *E* strains showed a novel and unprecedented phenotype, called max-4, wherein no asci contain more than four black ascospores. Although Giri *et al*. (2015) had reported the phenotype, images documenting it were not previously published, and we do so here. Finally, we put forth the novel hypothesis that the TRD and the max-4 phenotype are different manifestations of the same BDMI. Selection against 2/3 of the homokaryotic progeny in each introgression cross enriches for the dikaryotic progeny, and thus might have facilitated the introgressions.

2. Materials and methods

Metzenberg's (2003) alternative recipe was used to make Medium N. *Neurospora* genetic analysis was done essentially as described by Davis and De Serres (1970). The T^{Nt} strains used are available from the Fungal Genetics Stock Center (FGSC, Department of Plant Pathology, Kansas State University) with accession numbers FGSC 25288 to 25291 [Table S1 of Giri *et al*. (2016) incorrectly cited them as FGSC 25016 to 25019], as are the *N. tetrasperma* strains 85 *A* (FGSC

Table 1. Genotype of self-sterile progeny from T^{Nt} x 85

Cross T^{Nt} x 85	N (self-sterile)	Homokaryons			Alt : Adj	Phenotype
		T	*N*	*Dp*		
T(EB4) a	60 (19)[†]	1	8	8	9:16	ALT = ADJ
T(IBj5) a	76 (27)	3	6	18	9:36	ALT < ADJ
T(UK14-1) A	18 (16)	5	11		–	–
T(B362i) A	36 (21)	0	2	19	2:38	ALT < ADJ

N = number of progeny examined from cross, number of self-sterile progeny is given in parentheses. *T* and *N* are derived from alternate segregation (ALT); *Dp* and *Df* are derived from adjacent-1 segregation (ADJ). Since *Df* ascospores are inviable, the number of *Dp* progeny was doubled to estimate the number of ADJ-derived homokaryons. The chi-square table of Perkins (1994) was used to establish whether the deviation of ALT:ADJ ratio in one direction from 1:1 was significant ($p < 0.01$).

[†] One self-sterile progeny was [*TA* + *NA*] or [*DpA* + *DfA*], another was [*DpA* + *NA*].

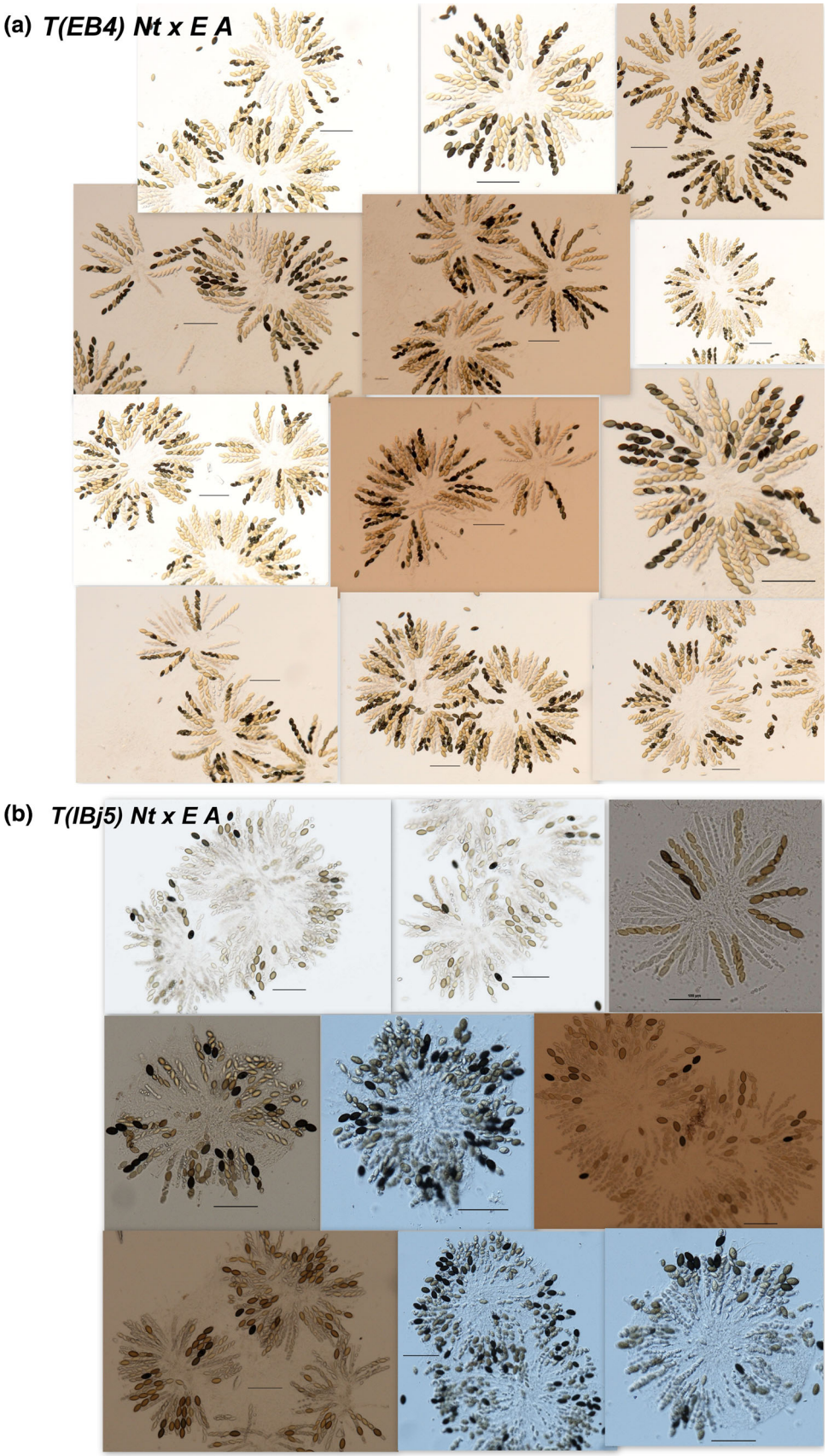

Figure 1. Rosettes of asci from the crosses (**A**) *T(EB4)*^{Nt}*a* x *E A*; (**B**) *T(IBj5)*^{Nt}*a* x *E A*; (**C**) *T(B362i)*^{Nt}*A* x *E a*. Note that asci with five or more black ascospores can be seen in (**A**), but not in (**B**) or (**C**). Thus, (**B**) and (**C**) display the max-4 phenotype.

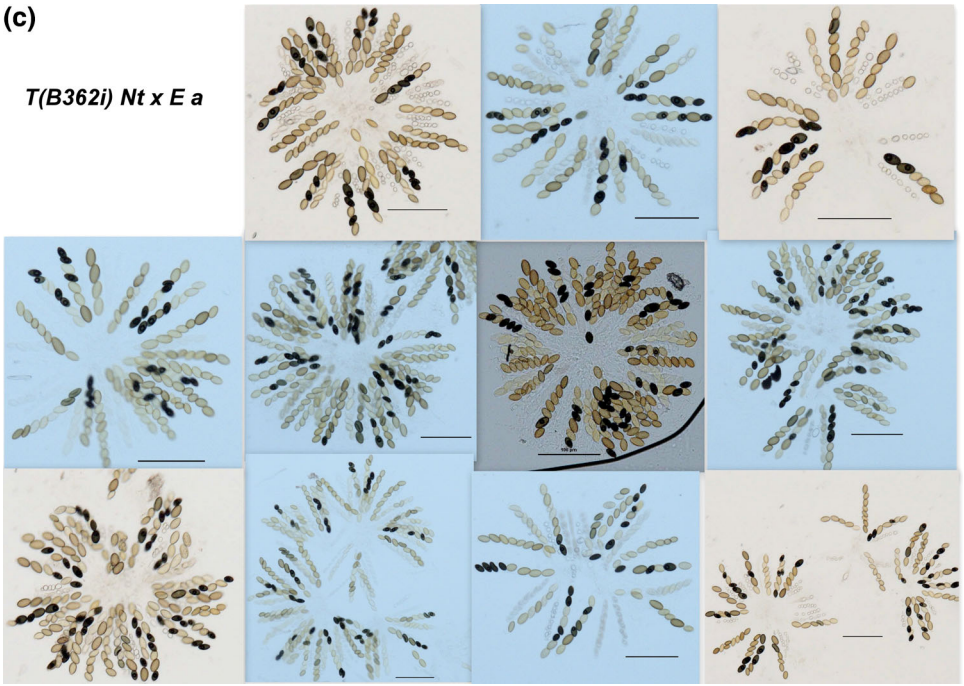

Figure 1. continued

1270), 85 *a* (FGSC 1271), the *E* mutant strains *lwn; al(102), E A* (FGSC 2783) and *lwn; al(102), E a* (FGSC 2784).

Insertional translocations (e.g. EB4, IBj5, and B362i) are defined by three breakpoint junctions: A on the donor chromosome, and B and C (proximal and distal) on the recipient chromosome, whereas quasiterminal translocations (e.g. UK14-1) have only two breakpoint junctions, A and B, on the participating chromosomes. The primer sequences for PCR amplification across the breakpoint junctions of the T^{Nt} strains and from the normal sequence homologues of the translocation donor and recipient chromosome (N^D and N^R) were reported by Giri *et al.* (2016). Genomic DNA from *T* progeny can PCR amplify across the A, B, and C breakpoints, but not with the N^D and N^R primers; DNA from *Dp* progeny can PCR amplify across B and C but not A, and also give products with the N^D (but not N^R) primers; and DNA from *N* does not amplify with primers for A, B, or C, but can give products with the N^D and N^R primers.

The chi-square Table provided by Perkins (1994) was used to establish whether the ALT:ADJ ratio deviated significantly in one direction ($p < 0.01$) from 1:1.

3. Results

We repeated the crosses of the four T^{Nt} strains with strain 85 derivatives of the opposite mating type, isolated progeny from random populations of shot ascospores collected from lids, identified the self-sterile progeny (i.e. mating-type homokaryons) based on the fact that they did not undergo self-cross on crossing medium, and used PCR to determine their *T*, *N*, or *Dp* genotype. The results, summarized in table 1, show that the $T(EB4)^{Nt}a$ x 85 *A* cross produced comparable numbers of homokaryotic progeny from ALT and ADJ (ALT = ADJ), whereas the $T(IBj5)^{Nt}a$ x 85 *A* and $T(B362i)^{Nt}A$ x 85 *a* crosses yielded fewer homokaryotic progeny from ALT than ADJ (ALT < ADJ). The B junction of $T(UK14\text{-}1)^{Nt}$, a *QT*, is not yet defined. Consequently, only the *T* progeny from $T(UK14\text{-}1)^{Nt}$ *A* x 85 *a* can be identified by their A breakpoint junction, whereas the *Dp* and *N* progeny are not distinguishable (table 1).

Figure 1A, B, and C present composites of perithecial rosettes from crosses of the $T(EB4)^{Nt}a$, $T(IBj5)^{Nt}a$ and $T(B362i)^{Nt}A$ strains with *E* strains of the opposite mating type. Note that the max-4 phenotype (i.e., no ascus contains more than 4 black ascospores) was shown only by the T^{Nt} strains that exhibited TRD in crosses with strain 85 derivatives.

4. Discussion

The results in table 1 recapitulate those of Giri *et al.*, (2016) and thus confirm that crosses of $T(IBj5)^{Nt}a$ and $T(B362i)^{Nt}A$, but not $T(EB4)^{Nt}a$, with opposite mating-type derivatives of strain 85 exhibit a TRD (i.e.,

deviation from ALT = ADJ) in their homokaryotic progeny. Further, the results presented in figure 1 are consistent with the hypothesis that the TRD and max-4 phenotype are different manifestions of the same BDMI. We hypothesize that the *N. crassa* gene that triggers BDMI is present in $T(IBj5)^{Nt}a$ and $T(B362i)^{Nt}A$, and is absent in $T(EB4)^{Nt}a$. The BDMI-inducing *N. crassa* gene is unlikely to be linked to both $T(IBj5)^{Nt}$ and $T(B362i)^{Nt}$. Therefore, if one were to do additional backcrosses of these translocations to 85 we might derive T^{Nt} strains that do not instigate the max-4 phenotype. By identifying the concomitantly lost *N. crassa* genome segments it should be possible to locate the BDMI-inducing gene.

By selecting against ALT–derived homokaryotic progeny in the viable ascospores, the BDMI-inducing gene would have enriched for progeny from 4-spored asci, and thus, paradoxically, facilitated the introgressions. This would result in the retention of more hitchhiking genes from *N. crassa* in the T^{Nt} strains, and might account for the ascus development defect seen in $T(IBj5)^{Nt}a$ x 85 *A* compared to $T(B362i)^{Nt}A$ x 85 *a*. Additionally, we have noted in Table 1 that one self-sterile progeny from the cross $T(EB4)^{Nt}a$ x 85 *A* had the genotype [*TA* + *NA*] or [*DpA* + *DfA*], and another was [*DpA* + *NA*]. The provenance of such self-sterile dikaryons was previously considered (Giri *et al*. 2016; Kasbekar and Rekha 2017).

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