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Abstract

We developed a simple ascospore plating assay for aneuploidy, based on identifying disomic progeny that inherit two independently selectable *mtr* alleles. We validated the assay using a known meiotic mutant, *mei-2*. We used this assay to demonstrate that elevated frequencies of aneuploidy previously reported to be associated with reduced DNA methylation were not, in fact, due to the methylation deficiencies. A new allele of the *mei-1* gene was responsible for some of the high aneuploidy.

A simple plating assay for aneuploidy in sexual progeny of *Neurospora crassa*, and a new allele of *mei-1.*

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We developed a simple ascospore plating assay for aneuploidy, based on identifying disomic progeny that inherit two independently selectable *mtr* alleles. We validated the assay using a known meiotic mutant, *mei-2*. We used this assay to demonstrate that elevated frequencies of aneuploidy previously reported to be associated with reduced DNA methylation were not, in fact, due to the methylation deficiencies. A new allele of the *mei-1* gene was responsible for some of the high aneuploidy.

Aneuploid progeny were initially reported to have been generated at higher than normal frequencies in crosses of *Neurospora crassa* mutants deficient in DNA methylation (Foss *et al*. 1993 Science **262**: 1737-1741, Foss *et al*. 1995 Science **267**:316). This suggestion grew from anecdotal observations of aneuploidy at frequencies higher than was expected based on literature values. The mutant examined in most detail, *dim-2* (defective in methylation) has no detectable methylation, at least in vegetative tissue (Foss *et al*. 1993 Science **262**:1737-1741) and ascospores (E. Selker and E. Kuzminova, unpublished). In an attempt to validate the finding of enhanced aneuploidy, methylation was reduced in a *dim+ met-7* (methionine) auxotroph, by starving for methionine, and a high frequency of aneuploidy was observed. The criteria for identifying progeny as aneuploid were one or more of the following: 1) patchy coloration due to heterozygosity for a pigment gene; 2) the ability to self-fertilize, which requires the two mating-type idiomorphs; 3) the presence of restriction patterns indicating RFLPs from both parents at a given locus; 4) intermediate levels of methylation, reflecting heterozygosity for a *dim* gene. In an attempt to quantify better the effect of methylation on aneuploidy we developed a simple plate assay for aneuploid sexual progeny.

Our simple assay for aneuploidy is based on identifying progeny that are disomic for LGIV, inheriting two independently selectable alleles of the *mtr* gene. We cross a strain that has a functional, wild-type allele of *mtr* with a strain carrying an allele of *mtr* that is defective by virtue of an insertion of the *E. coli hph* gene, encoding hygromycin resistance. An aneuploid carrying one of each *mtr* allele will plate under conditions that select simultaneously for both alleles, whereas neither euploid strain will. All strains carry *trp-2*, which allows for selection of *mtr*+ strains. In many of our tests one parent carried a mutation (*am33*; A. J. Griffiths)

that eliminates the vegetative incompatibility (and death) that would otherwise result from having two different mating types in a single cytoplasm. This mutation was included because there is evidence that aneuploids produced in wild-type crosses are frequently multiply disomic (Smith 1974 Genetics **76**:1-17). As it turns out, this mutation did not significantly influence the frequency of aneuploidy detected by our assay (Table 2). Plating assays employed in past studies to detect aneuploids involved crossing two strains, each carrying a different set of auxotrophic

markers, and selecting for prototrophs (Smith 1974 Genetics **76**:1-17). Only strains carrying both chromosomes, and rare recombinants, were able to plate. Our method is an improvement over this since homologous recombination cannot produce false positives in our assay, and our assay requires introduction of only two markers (*trp-2* and *mtr::hph*).

Neurospora strains and genotypes are listed in Table 1. Culture and crossing were carried out as per Davis and DeSerres (1970 Meth. Enzymol. **17**:47-143) using the crossing medium of Russo *et al.* (1985 Neurospora Newsl. **32**:10-11). Crosses involving *met-7* strains were done on medium containing 0.01 mg/ml methionine. Ascospore plating was on Vogel's agar with 2% sorbose and 0.1% dextrose. Permissive plating was on medium supplemented with 0.05 mg/ml anthranilic acid (and other supplements when needed). Selection for aneuploids was on medium containing 0.01 mg/ml tryptophan, 0.6 mg/ml arginine, (selection for *mtr*+; Stadler *et al*. 1991 Genetics **129**:39-45) and 0.1 mg/ml hygromycin B (Calbiochem).

We tested our assay using *mei-2* strains, which are defective in meiotic pairing and are known to generate aneuploids at enhanced frequencies (Smith 1975 Genetics **80**:125-133, Shroeder and Raju 1991 Mol. Gen. Genet. **231**:41-48). The data shown in Table 2 indicate that roughly one-third of the ascospores from crosses homozygous for *mei-2* produced colonies on the aneuploid selection medium, compared to about 2% for normal strains. Spores from the *dim-2* and *met-7* mutant crosses, which were either heterozygous or homozygous for the methylation defect, did not display enhanced frequencies of plating under the selection for aneuploids. Crosses heterozygous for *dim-3*, another gene required for normal methylation and previously implicated in inducing aneuploidy when heterozygous, also showed normal levels of aneuploidy in our assay. We conclude that reduced methylation does not induce aneuploid production.

One strain, N1472, when crossed with any other strain that we tested, gave the lowest frequencies of aneuploidy. A likely explanation for this is that vegetative incompatibility killed off most of the aneuploids produced, since N1472 is not completely compatible with the other strains used here, which are Oak Ridge-compatible. Control experiments to test this hypothesis, using incompatible strains that were either *mei-1*, *dim-2* or *dim*+, showed up to 10-fold reductions in frequencies of aneuploidy by our assay when compared to crosses of identical genotype involving Oak Ridge-compatible strains (data not shown). Thus, it is important to use compatible strains in order to maximize detection of aneuploids; this probably holds for any assay method.

In contrast to results from our new assay for aneuploidy, generation of aneuploids in *met-7* crosses reported previously (Foss *et al* 1993 Science **262**:1737-1741) was strikingly elevated. Because we had to construct new *met-7* strains carrying appropriate genetic markers for our plating assay for aneuploids, we tested these new strains for the ability to generate *al-2*/*al*+ (white/orange) mosaic aneuploids, one of the tests for aneuploidy in the previous study, and these strains failed to generate mosaics. Reasoning that we must have lost the "aneuploidy factor" in our strain construction, we therefore crossed a *met-7* strain used in the previous study with a multiply marked strain (FGSC 4453) in an attempt to map the defect causing the aneuploidy. In our mapping cross we did not follow the defect using our plating assay for aneuploidy, since this requires the introduction of additional markers. Instead, we followed another sexual-cycle phenotype that is associated with the ability to generate aneuploids;

misshapen spores and a high proportion of white spores (dead, presumably due to a chromosomal deficiency). We established a linkage distance of 13 map units (200 progeny analyzed) between the defect associated with aneuploid production from the *met-7* strain, and *pyr-1*, on LG IV.

One gene known to affect meiotic chromosome segregation, *mei-1,* is known to map near *pyr-1*. To determine whether the new defect was allelic with *mei-1*, we crossed *mei-1* strains (FGSC 2919 and 2920) with our mutant strains. Although both *mei-1* and the new defect are recessive (they appear normal in heterozygous crosses), we failed to see complementation of the defect; crosses between our mutant and *mei-1* strains generated as many white spores (~80%) as crosses homozygous for our mutant or for *mei-1*. We conclude that the new defect is an allele of *mei-1*, which we name *mei-1(AH)*. The *met-7* strain FGSC 3915 was the source of *mei-1(AH)*.

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Table 1. Neurospora Strains

Name	Genotype
N568 (FGSC 8288)	*mtr::hph trp-2 A*
N569 (FGSC 8289)	*mtr::hph trp-2 a*
N1465	*mtr::hph trp-2 al-2 A*
N1466 (FGSC 8290)	*mtr::hph trp-2 al-2 A*
N1467	*trp-2 arg-3 am33*
N1468 (FGSC 8291)	*trp-2 arg-3 am33*
N1469	*trp-2 arg-3 am33*
N1470	*dim-2 mtr::hph trp-2 al-2 A*
N1471	*dim-2 mtr::hph trp-2 al-2 A*
N1472	*dim-2 trp-2 arg-3 inl am33*
N1473	*dim-3 trp-2 a*
N1474	*dim-3 trp-2 A*
N1475	*trp-2 a*
N1476	*trp-2 A*
N1479	*mei-2 mtr::hph trp-2 al-2 A*
N1480	*mei-2 trp-2 arg-3 am33*
N1481	*mei-2 trp-2 arg-3 am33*
N1482	*trp-2 mei-1(AH) a*
N1483	*mtr::hph trp-2 al-2 A*
N1484	*met-7 thi-3 mtr::hph trp-2 al-2 A*
N1485	*met-7 thi-3 trp-2 mei-1(AH) a*
FGSC2919	*mei-1 A*
FGSC2920	*mei-1 a*
FGSC3915	*met-7 thi-3 mei-1(AH) a*
FGSC4453	*arg-5 thi-4 pyr-1 lys-1 inl nic ars A*

Allele numbers: *al-2(R401 or Y122M38), arg-3(30300), dim-2(KC), dim-3(KC), mei-2(ALS181), met-7(NM251), thi-3(18558).*

Table 2. Generation of aneuploids in crosses of *Neurospora crassa*

Cross Type	Parent Strains[1]	Aneuploid Frequency (%)[2]
dim+ x *dim+*	N1465, N1467	1.77 ± 0.31 (3)
	N1466, N1467	2.0

	N1466, N1468	2.7
	N1466, N1469	2.8
	N1475, N568	4.7
	N1476, N569	1.3
mei-2 x *mei-2*	N1479, N1480	40, 27
	N1479, N1481	35, 26
dim-2 x *dim-2*	N1470, N1472	0.17 ± 0.06 (3)
	N1471, N1472	0.2
dim+ x *dim-2*	N1465, N1472	0.6
	N1466, N1472	0.7, 0.1
dim-2 x *dim+*	N1470, N1467	5.24 ± 2.04 (5)
	N1471, N1467	1.7, 2.4
dim-3 x *dim+*	N1473, N568	1.7, 1.3
	N1474, N569	2.5, 1.1
met-7 x *met-7*	N1484, N1485	3.8, 3.1*
met-7 x *met+*	N1484, N1482	1.6, 1.2*
met+ x *met-7*	N1483, N1485	2.8, 2.6*

[1] See Table 1 for strain genotypes.

[2] Values listed represent the efficiency of plating on the aneuploidy selection medium relative to the permissive medium. Each number is for an independent cross, except as indicated by *, which denotes two platings from the same cross. For crosses performed more than twice, values are means ± standard deviations, where numbers in parentheses indicate number of repetitions of the cross.

Return to the FGN 44 Table of Contents

Return to the FGSC home page