**How to measure mitotic instability of segmental duplications.**
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## Background

When Newmeyer and Taylor (1967) used a quasiterminal inversion (*In(IL→IR)H4250*) to produce partial diploid progeny that are heterozygous for the *mat A* and *mat a* mating-type genes, they found that growth and morphology of strains carrying the duplicated segment were very abnormal. Because the heterozygous duplications produce dark pigment on complete medium or on minimal medium containing tyrosine and phenylalanine, the abnormal progeny were called 'dark agars' (DAs). No dark pigment is produced on unsupplemented minimal medium, but even so, the duplication colonies are readily recognized by their distinct spidery morphology. The inhibited *mat A*/*mat a* duplication strains are mitotically unstable, sectoring to produce euploid single-mating-type derivatives that are phenotypically normal.

Abnormalities of the H4250 duplication strains were attributed to the fact that *N. crassa* strains of opposite mating type are heterokaryon-incompatible in the vegetative phase. This hypothesis was supported by the finding that when H4250 duplications are homozygous *mat A/mat A* or *mat a/mat.a*, they do not show the DA phenotype. Also, when other duplications are heterozygous for heterokaryon incompatibility genes ('*het'* genes) at loci other than mating type, the duplication strains are morphologically abnormal, although the morphology may differ, depending on what *het* gene is responsible.These duplications are also unstable, although to varying degrees (Perkins 1972, 1975; Mylyk 1975, Smith *et al.* 1996).

The normal-growing products of escape from inhibition result predominantly from deletion of one of the duplicated chromosome segments, which removes one of the heterozygous *het* alleles. With most partial diploids, the translocated segment appears to be deleted preferentially (Perkins and Barry 1977, Metzenberg *et al.* 1974, Smith *et al*. 1996).The timing and frequency of escape provide a quantitative measure of instability. Differences in stability are under genetic control (Newmeyer and Galeazzi 1977).

Stability can be measured by determining the time elapsed between ascospore germination and escape from inhibition. Extensive data have been obtained using *mat A/mat a* duplications (see Fig. 4 in Schroeder 1970, Fig. 2 in Newmeyer *et al.* 1978, and Fig. 2 in Schroeder 1986). The procedure with H4250, which is described below, can serve as a model for experiments that use duplications from other rearrangements such as *T(IL→IIR)39311* (Schroeder 1970) and *T(IIL→VR)NM149* (Schroeder 1986).

Effects on duplication stability have been determined using mutagen-sensitive mutants and mutants having meiotic abnormalities that result from defects in DNA repair, and by using certain chemical agents (Schroeder 1970, 1986; Newmeyer and Galeazzi 1978). It has been suggeted that reduced deoxyribonucleotide pools may be responsible for heightened instability, based on properties shared by strains with different mutations that increase instability, together with increases in the instability of duplication strains grown on hydroxyurea or histidine (Schroeder 1986)..

## Procedure

Inversion H4250 (FGSC 1563 A, or 1564 a) is crossed by the strain to be tested. Ascospores are isolated to minimal medium in 10 × 75 mm slants, heatshocked, and incubated at 25°C. One third of the progeny are expected to be *mat A/mat a* duplications. Duplication progeny are identified by their slow, compact growth. The DAs are clearly recognizable even though dark pigment is not produced on minimal medium. Beginning at 2 days after heatshock, the duplication slants are examined daily under 10 –20×

magnification to determine whether sectoring is detectable or overgrowth has occurred. Tubes are marked according to the day escaped.

The numbers of DAs escaped on successive days.are plotted in a cumulative graph showing percent escaped on successive days. If the effect of a specific gene is being investigated, escaped strains.are scored for that gene. If it affects escape time, and if it is unlinked, to the duplication, two separate curves will be found because half of the duplication progeny are expected not to carry the gene. These provide an internal control. (See Schroeder figures cited above.)

If desired, mating type can be determined using *mat A* and *mat a fluffy* testers, confirming that each escaped mycelium is now single-mating-type and revealing which segment of the duplication has been deleted.

An alternative procedure that might be worth exploring would be to spread ascospores on petri plates of sorbose medium, heatshock the plates, and transfer individual DA colonies to 10 × 75 mm slants as soon as they can be recognized. This would avoid the labor of isolating nonduplications.

**References**

Metzenberg, R. L. , M. K. Gleason, and B. S. Littlewood. 1974. Genetic control of alkaline phosphatase synthesis in Neurospora: The use of partial diploids in dominance studies. Genetics 77: 25-43.

Mylyk, O. M. 1975. Heterokaryon incompatibility genes in *Neurospora crassa* detected using duplication-producing chromosome rearrangements. Genetics 80: 107-124.

Newmeyer, D., and D. R. Galeazzi. 1977. The instability of Neurospora duplication *Dp(IL→IR)H4250* and its genetic control. Genetics 85: 461-487.

Newmeyer, D., and D. R. Galeazzi. 1978. A meiotic UV sensitive mutant that causes deletion of duplications in Neurospora. Genetics 89: 245-269.

Newmeyer, D., and C. W. Taylor. 1967. A pericentric inversion in Neurospora, with unstable duplication progeny. Genetics 56: 771-791.

Newmeyer, D., A. L. Schroeder, and D. R. Galeazzi. 1978. An apparent connection between histidine, recombination, and repair in Neurospora. Genetics 89: 271-279.

Perkins, D.D. 1972. An insertional translocation in Neurospora that generates duplications heterozygous for mating type. Genetics 71: 25-51.

Perkins, D. D. 1975. The use of duplication-generating rearrangements for studying heterokaryon incompatibility genes in Neurospora. Genetics 80: 87-105.

Perkins, D. D., and E. G. Barry. 1977. The cytogenetics of Neurospora. Advan Genet. 19: 133-285.

Schroeder, A. L. 1970. Ultraviolet-sensitive mutants of Neurospora. 1. Genetic basis and effect on recombination. Mol. Gen. Genet. 107: 291-304.

Schroeder, A. L. 1986. Chromosome instability in mutagen sensitive mutants of Neurospora. Curr. Genet. 10: 381-387.

Smith, M. L., C. J. Yang, R. L. Metzenberg, and N. L. Glass. 1996. Escape from *het-6* incompatibility in *Neurospora crassa* partial diploids involves preferential deletion within the ectopic segment. Genetics. 144: 523-531.

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