

New round spore mutations in *Neurospora crassa* accompanying changes in a duplication closely linked to the *R* locus.

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Various genotypes can result in round ascospores in *N. crassa* (Mitchell 1966 *Neurospora* Newsl. 10:6; Barry et al. 1972 *Neurospora* Newsl. 19:17). I have observed that round ascospores can also originate in crosses where one parent is the apparent breakdown of a partial diploid heterozygous at the heterokaryon incompatibility locus *het-5*. The breakdown may remove one of the duplicated chromosome segments carrying a *het-5* allele and possibly extend past the duplication and affect the nearby Round spore locus (*R*).

Crosses of normal chromosome sequence with the insertional translocation *T(I->II)MD2* result in a class of progeny which duplicates the far distal portion of linkage group IR (Figure 1). The duplication covers loci *un-18* and *het-5* but not *R* (Perkins and Jacobson, unpublished). Mutations at *R* are ascus dominant; in asci from *R* X *R*⁺, all eight ascospores are round. *R* cultures also show abnormal, peach-like vegetative morphology that is recessive in heterokaryons. Noncoverage is inferred from the cross normal sequence *R* X *T(I->II)MD2* *R*⁺; the ratio of *R* to *R*⁺ progeny (as judged by vegetative morphology) is 2:1 indicating that the duplication progeny, *Dp(I->II)MD2*, are *R* and the locus is outside the duplication (Figure 1).

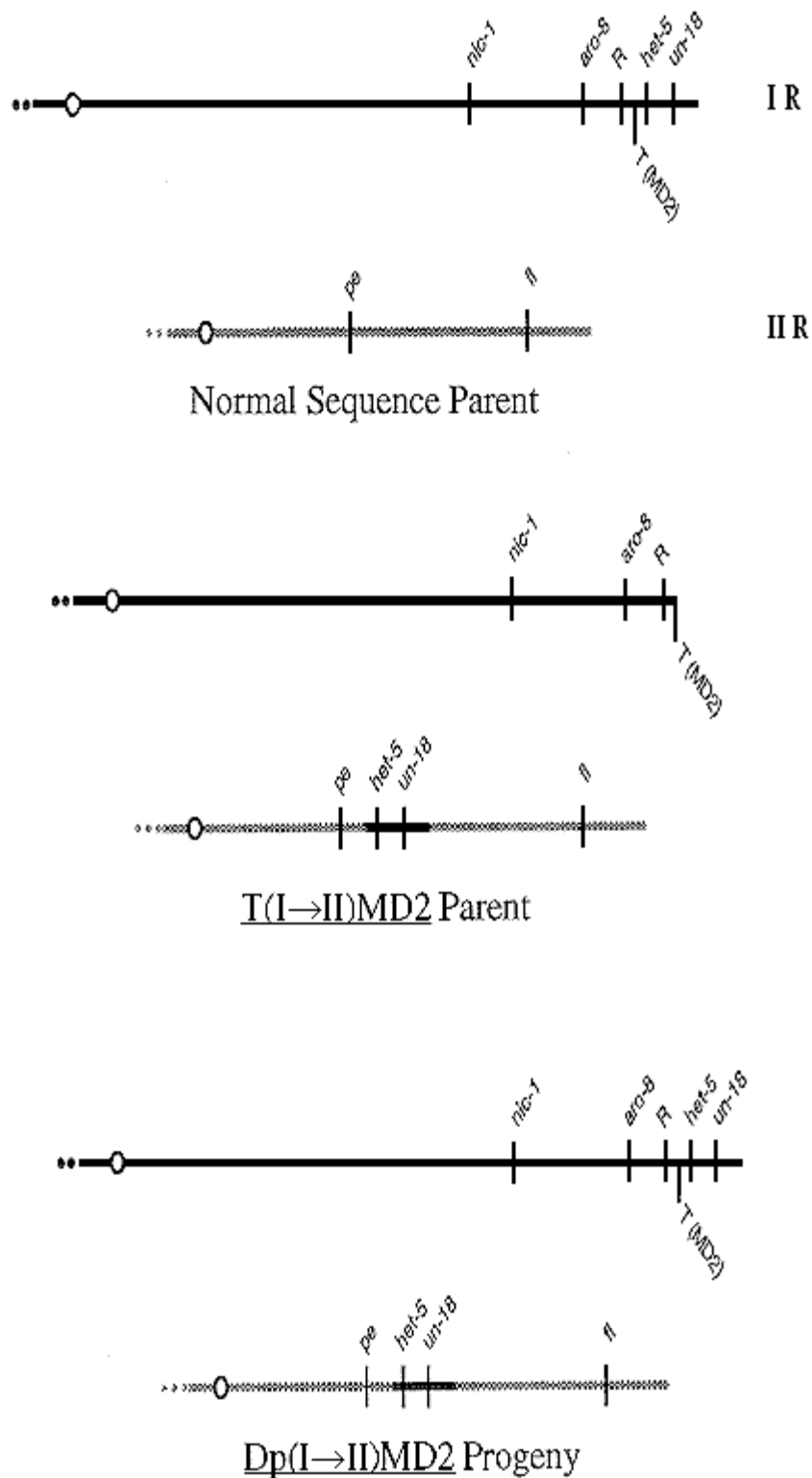


Figure 1. Partial genetic map of linkage groups I and II of parental normal sequence and *T(I→II)MD2* and *Dp(I→II)MD2* progeny. *het-5* and *un-18* are covered, but *R* is not. Insertion point

and orientation in linkage group II have not been determined exactly, although translocation breakpoints have shown linkage to *pe* (Perkins, unpublished).

If normal sequence and *T(I->II)MD2* parents in such crosses have different alleles at *het-5*, *Dp(I->II)MD2* progeny will be heterozygous for *het-5* and will exhibit abnormal restricted colony morphology. After prolonged incubation the colony will escape inhibition and resume growth. (Such escape is common in duplications that are heterozygous for a *het* gene and it can result from any process that eliminates the heterozygosity, e.g. deletion or mitotic crossovers that make it homozygous.) The morphology of the culture after escape resembles conidial separation mutants in a tap test and is characteristic of *T(I->II)MD2* and of *Dp(I->II)MD2* where *het-5* is homozygous. A proportion of these escaped progeny produced round ascospores in subsequent crosses even though *R* was not present in either parent.

The following set of crosses illustrates this in detail (Table 1). *het-5PA* was introgressed by backcrossing from strain Panama CZ30.6 (FGSC 1311) into Oak Ridge wild type (FGSC 2489 and 4200) which carries *het-5OR*. After the third backcross a *het-5PA* progeny was crossed to *T(I->)MD2 het-5OR*. Twenty-two of 69 progeny showed restricted colony morphology indicating heterozygous duplication of *het-5*. The inhibited *Dp(I->II)MD2* were not stable; all escaped and grew into *T(I->)MD2*-like morphology. All were then crossed to normal sequence. Only five were barren (the usual result of duplication X normal sequence), while the other 17 were fertile to some extent as judged by production of viable ascospores. Twelve of the fertile escaped *Dp(I->II)MD2* produced only normal shaped ascospores, three produced mixtures of normal and round, and two produced exclusively round ascospores. Each ascus contained either all normal or all round ascospores and each perithegium contained only one of the two types of asci. The three crosses producing both shapes contained a mixed population of normal and round-ascospore producing perithecia.

Progeny were analyzed from the two exclusively round ascospore crosses. No inhibited, presumably heterokaryon incompatible, progeny were recovered. This, combined with the *T(I->)MD2*-like morphology, suggests that the original *Dp(I->II)MD2* lost the *het-5PA* allele when it escaped and either remained a duplication or reverted to translocation sequence. In any case, they did not produce duplications heterozygous for *het-5* in subsequent crosses to normal sequence *het-5OR*.

Three vegetative morphologies were seen among the second generation progeny: wild type (14), *T(I->)MD2*-like (18), and a peach-like morphology which has been associated with *R* (Barry et al. 1972 *Neurospora* Newsl. 19:17). Morphology was correlated with the shape of the ascospores produced when these progeny were crossed to normal sequence. If they were fertile, cultures with wild type morphology and *T(I->)MD2*-like cultures both produced only normal ascospores. However, the majority of the *T(I->)MD2*-like cultures were barren and this would suggest that they were newly generated duplications. The peach-like cultures produced only round ascospores. The round ascospore trait was heritable through another cross to normal sequence. Yet another morphological class was apparent among the progeny of this cross. This class exhibited dense mycelium appressed to the agar and produced exclusively round ascospores in crosses to normal sequence.

These new round ascospore strains resemble the known *R* mutant by being ascus dominant, female sterile in heterozygous crosses and completely sterile in homozygous crosses. Crosses between these strains and *R* are also sterile.

Table 1. Analysis of the novel round ascospore progeny originating from *Dp(I->II)MD2* X normal sequence.

Cross	Number of Progeny Tested*	Vegetative Morphology	Ascospore Shape
1. <i>het-5PA</i> X <i>T(I->II)MD2</i> <i>het-5OR</i>	5	MD2-like	--
	12	MD2-like	wild type
	3	MD2-like	wild type
	2	MD2-like	and round
2.& First generation Round X normal sequence	14	wild type	wild type
	4	MD2-like	wild type
	14	MD2-like	--
	4	peach-like	round
3.& Second generation Round X normal sequence	36	wild type	wild type
	2	wild type	--
	3	MD2-like	wild type
	15	MD2-like	--
	1	appressed mycelium	wild type
	11	appressed mycelium	round

* Only duplication progeny are listed for cross 1. All originally exhibited inhibited morphology, but escaped after prolonged incubation. Total progeny tested listed for crosses 2 and 3.

Morphology of the MD2-like culture resembles conidial separation mutants in a tap test and is characteristic of *T(I->II)MD2* and of *Dp(I->II)MD2* where *het-5* is homozygous. The morphology of the appressed mycelium class in cross 3 consisted of dense mycelium appressed to the agar.

Ascospore shape produced in crosses of progeny X normal sequence. Wild type is spindle shape; round is spherical similar to *R* mutant; (--) is barren in crosses with no ascospores produced.

het-5PA had been backcrossed three generations to normal sequence Oak Ridge wild type (FGSC 2489 and 4200).

& Progeny from the previous generation which produced exclusively round ascospores were used as male parents and individually crossed to normal sequence (FGSC 2489 and 4200). The number of progeny were pooled from all crosses.

The escaped *Dp(I->II)MD2*, or derived progeny, that produced round ascospores could not be classified as either normal or parental *T(I->)MD2* sequence. In each cross, white inviable ascospores were produced in varying proportions. This was true in each of the crosses described above and when the round ascospore strains were crossed to *T(I->)MD2*. Distribution of white ascospores in unordered asci shot from perithecia was inconclusive in determining the nature of chromosome sequence. Direct examination of rosettes also did not reveal patterns characteristic of heterozygous *T(I->)MD2* or known simple rearrangements. Moreover, a portion of progeny in

each generation were barren in subsequent crosses. This suggests possible novel chromosome aberrations which may be producing duplication progeny.

A proportion of escaped *Dp(I->II)MD2* from a seventh backcross *het-5* X *T(I->)MD2* also produced round ascospores, confirming the correlation with the escape of heterozygous *het-5* duplication rather than other interactions of Panama and Oak Ridge backgrounds. *Dp(I->II)MD2* homozygous for *het-5OR* was stably barren, thus supporting the conclusion.

Somatic instability of duplications is common when they are placed at selective disadvantage, for example when heterozygous for *het* genes (Perkins and Barry 1977 Adv. Genet. 19:133-285). The unusual aspect of this study is that a gene outside the duplicated area is apparently affected during breakdown of the duplication. A possible explanation is that the distal portion of the normal linkage group IR is lost during escape resulting in quasi-translocation sequence. This is also unusual since most studied duplication breakdowns yield normal sequence (Perkins and Barry 1977 Adv. Genet. 19:133-285). The lost portion of IR may occasionally extend proximally beyond the *T(I->)MD2* breakpoint and thus affect the *R* locus. Some *Dp(I->II)MD2* which have escaped appear to be mixtures of round and normal ascospore genotypes, suggesting that either loss of distal IR occurs repeatedly in a colony or the chromosome sequence after deletion is itself unstable.

The round ascospore trait became stable after the first cross. It is unclear, however, if the chromosome sequence stabilized after meiosis. The varying proportion of white ascospores, differing colony morphologies, and barren progeny cannot be simply explained and requires further investigation.

These observations were incidental to a study carried out at Stanford University to localize *het-5* right of the *T(I->)MD2* breakpoint. Support from PHS Grant AI-01462 and helpful suggestions from members of David D. Perkins laboratory are gratefully acknowledged.