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Different frequencies of RIP among early vs. late ascospores of Neurospora crassa

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Different frequencies of RIP among early vs. late ascospores of Neurospora crassa

Abstract

We have noticed that the frequency of RIP can be quite variable, even in crosses of the same strains. One possible source of variability is the time at which ascospores are harvested. We reasoned that the earliest ascospores shot from a perithecium might contain DNA that went through relatively few mitotic divisions in pre-meiosis. RIP occurs between fertilization and premeiotic DNA synthesis (Selker *et al.* 1987 Cell 51:741-752). Thus, early spores might have less exposure to RIP than late spores. Since all ascospores from a perithecium are thought to arise from a single fertilization event, a minimum of 7- 10 divisions are required to account for the number of ascospores normally produced (Perkins and Barry, 1977 Adv. Genet. 211:541-544). It is likely, however, that some ascospore lineages contain fewer divisions than others.

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Different frequencies of RIP among early vs. late ascospores of *Neurospora crassa*

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We have noticed that the frequency of RIP can be quite variable, even in crosses of the same strains. One possible source of variability is the time at which ascospores are harvested. We reasoned that the earliest ascospores shot from a perithecium might contain DNA that went through relatively few mitotic divisions in pre-meiosis. RIP occurs between fertilization and premeiotic DNA synthesis (Selker *et al*. 1987 Cell **51**:741-752). Thus, early spores might have less exposure to RIP than late spores. Since all ascospores from a perithecium are thought to arise from a single fertilization event, a minimum of 7- 10 divisions are required to account for the number of ascospores normally produced (Perkins and Barry, 1977 Adv. Genet. **211**:541-544). It is likely, however, that some ascospore lineages contain fewer divisions than others.

We investigated the possibility that the frequency of gene inactivation by RIP might differ in populations of ascospores shot early or late from a cross. Srains containing an unlinked duplication of *am* (NADPH-specific glutamate dehydrogenase) were crossed with single-copy strains (Table 1). We collected ascospores at different times and assayed for inactivation of *am* in these progeny. Crosses were carried out on Vogel's minimal medium (Vogel, 1956 Microbiol. Genet. Bull. 13:42-43) modified by reducing the ammonium nitrate concentration 10-fold and containing 0.1% sucrose and supplements (Russo et al. 1985 Neurospora Newslet. **32**:10-11). The female parent was inoculated 3 days before the male and the plates were incubated at 25°C. The first ascospores were found 9-10 days after fertilization. Lids containing ascospores were replaced with clean lids daily until the 12th day after fertilization. The last lids were left on for 3 days. Ascospores were harvested from the lids with sterile water, plated, heat-shocked and incubated overnight at 33°C. Progeny were picked to agar slants, and their conidia were spotted on plate medium supplemented with glycine, inositol and lysine to score for *am*. We compared inactivation of *am* among ascospores harvested at the various times. The results of four crosses are shown in Table 2. The frequency of am inactivation was significantly lower in the early ascospores. Overall, only about 2% of the spores shot by 10 days after fertilization were Am- (frequency of RIP >5%), whereas about 28% of spores shot after 12 days were Am- (frequency of RIP ~ 56%).

We wished to check a second duplicated sequence for variation in the frequency of RIP as a function of when the ascospores were shot. We therefore crossed, as described above, an mtr+ strain carrying a duplication resulting from insertion of mtr sequences at the am locus, unlinked to the native mtr gene (Irelan, Hagemann and Selker, 1994 Genetics, **138**:1093-1103). Ascospores shot before day 13 post fertilization and between 13-15 days or 15-17 days post fertilization were collected, heat-shocked, and plated on nonselective medium (Vogel's supplemented with 0.2 mg/ml alanine, 0.05 mg/ml anthranilic acid, 2% sorbose, 0.5% fructose and 0.5% glucose). More than 100 progeny from each sample were then scored for mtr on medium with 0.05 mg/ml p-fluorophenylalanine. The results, shown in Table 3, were consistent with those obtained with the duplication of am: the early spores showed significantly less RIP than those shot later. To expand the data set further, we compared results of plating equal

aliquots of activated ascospores (stored in water 20 days at 4°C prior to heat shock) directly on nonselective and selective media. In this experiment, thirteen day ascospores produced 7.3% (31/420) mtr progeny (RIP frequency ~ 15%), while 15-17 day spores produced 35% (39/112) mtr progeny (RIP frequency ~ 70%). We conclude that the time at which ascospores are harvested can affect the apparent frequency of RIP. This finding could be of practical use to Neurospora researchers. Those desiring to obtain mutants by induction of RIP may be wise to avoid the first ascospores shot from a cross. Conversely, researchers should be able to enrich for RIP escapees, or possibly lightly mutated alleles, by collecting only the earliest ascospores.

TABLE 1
Neurospora crassa strains

Strain	Ectopic gene	Other markers	Source
N261	none	A; lys-1	J. Kinsey
N262	none	a; lys-1	J. Kinsey
N276	am+	a	lab collection
N277	am+	A	lab collection
N1100	am::mtrP-SwaI	A; trp-2	lab collection
N1101	none	am33; trp-2	lab collection

The ectopic *am*+ gene was from T-510 5.6 (Selker and Garrett, 1988 Proc. Natl. Acad. Sci. USA, **85**:6870-6874) and the ectopic *mtr*- gene was from N648 (Irelan, Hagemann and Selker, 1994 Genetics, **138**:1093-1103).

TABLE 2
Frequency of am inactivation by RIP in ascospores shot at various times after fertilization

				Am-/Total	
Cross	Female	Male	9-10 days	11 day	12-15 days
1	N261	N276	0/11		5/11
2	N262	N277	1/77	4/54	26/71
3	N277	N262	0/3	2/57	5/58
4	N277	N262	3/79	6/80	26/80
Total			4/170(2%)	12/191(6%)	62/220(28%)

TABLE 3
Frequency of mtr inactivation by RIP in ascospores shot at various times after fertilization

			Mtr-/Total	
Female	Male	10-13 days	13-15 day	15-17 days
N1100	N1101	10/117 (9%)	76/237 (32%)	34/117 (29%)

Ascospores were collected at the indicated times after fertilization and stored in water at 4°C for 37 days before plating.

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