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Abstract

A system for studying aneuploid production in *Neurospora crassa*

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A system for studying aneuploid production in *Neurospora crassa*.

Crosses between the complementing histidine-5 allele K78 and K746 yield two kinds of his^+ progeny. One results from recombination between the alleles and the other primarily from nondisjunction of chromosome IV giving disomic ascospores. Less frequently, disomic progeny may be produced as a result of extra chromosome replication or chromosome non-conjunction (pairing failure). The disomic spores form pseudowild colonies on minimal medium that are distinguishable from the much rarer his^+ recombinants by their slower growth rate.

The inclusion in the parent strains of linked auxotrophic markers, flanking the his-5 locus, permits easy recognition of parental and pseudowild types among conidia formed by pseudowilds. In contrast, conidia from his^+ recombinants are homokaryotic and do not show marker segregations.

Genotypes of parent strains.

Parent A. pyr-3 (1298), his-5 (K78), a

Parent B. his-5 (K746), leu-2 (37501), A

Reciprocal crosses were prepared, some of which were treated with p-fluorophenylalanine. The progeny were then screened on selective media to estimate the frequency of pseudowilds. The amino acid analogue p-fluorophenylalanine is known to increase meiotic non-disjunction of chromosome I of Neurospora (Griffiths and DeLange. Mutat. Res. 1977, 46: 345) and should significantly increase frequencies of pseudowilds in these crosses.

Preparation and treatment of crosses

Petri dishes (90 mm diameter) containing 20 ml of Westergaard's crossing medium supplemented with uracil, histidine and leucine, were inoculated with drops of conidial suspension of one parent. To increase the fertility of the crosses, macerated Whatman's No. 1 filter paper was added to the crossing medium at the rate of 270 $cm^2/1$. Petri plates were incubated for five days to allow protoperithecia to form. During this period, the petri dish walls were wiped with alcohol twice daily to prevent the mycelium spreading over the sides of the plates. Fertilization was then effected by the addition of a dense suspension of conidia. Excess water was removed after 30 min and $4_{1/2}$ h later, 5 ml of water or 5 ml of p-fluorophenylalanine solution (0.05 mg/ml) was added to each petri plate. The water or p-fluorophenylalanine solution

was drained off after 16 h incubation and the plates were then incubated in an inverted position for 21 days. Ascospores were subsequently collected in sterile water from the petri dish lids.

Crosses treated with p-fluorophenylalanine show dramatically increased frequencies of pseudowild type progeny - 3.5 times the control value in the cross A♀ x B♂, and 7.1 times in the cross B♀ x A♂. These increases are of the same order as those observed by Griffiths and DeLange.

Table 1

Analysis of Crosses

Cross ♀ x ♂	No. of viable ascospores	No. of pseudo-wilds	% pseudo-wilds	Probability that results do not differ	% Recombination between pyr-3 and *leu-2*	
A x B	100,000	189	0.19	< 0.001	22.5	
A x B + p-f	12,600	85	0.67		22.7	21.4
B x A	52,800	167	0.32	< 0.001	20.2	
B x A + p-f	3,040	69	2.27		21.1	

Overall frequency of *his*$^+$ allelic recombinants = 9.5 per 10^5 ascospores

p-f - p-fluorophenylalanine treated,

The final column in Table 1 shows estimates for recombination between pyrimidine-3 and leucine-2 in the crosses (The estimates are based on frequencies of *pyr*$^+$ *leu*$^+$ colonies detected on histidine supplemented medium (values were corrected by substracting pseudowild type frequencies). These frequencies do not differ significantly (heterogeneity $\chi^2 = 0.862$ p = 0.08-0.09) indicating the p-fluorophenylalanine does not influence crossing-over.

This simple test system seems ideal for studying,aneuploid production, and for detecting chemical agents that might influence formation of aneuploid products during meiosis. - - - - Department of Genetics, University of Aberdeen, Aberdeen, Scotland, United Kingdom.