A simple explanation for 66.7% limiting values in tetrad analysis

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Abstract
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Selitrennikoff, C. P. Use of conidial separation defective strains.

The problem of contamination of laboratories by Neurospora macroconidia is well known and has prompted the use of morphological mutants or even other fungi (e.g., Sordaria) for class experiments (Barratt 1965, NN#10:33; Gardner and Mertens 1970, Genetics Laboratory, Investigations, Burgess). I suggest the use of conidial separation defective strains (csp-1 and csp-2). These mutants produce chains of conjoined conidia, yet are comparable in macroscopic appearance to normal cultures. The virtual absence of free conidio formed by these strains permits their experimental manipulation (even student loop transfers) without the fear of aerial dispersal of conidia.


The utility of Neurospora in an undergraduate genetics laboratory course for demonstrating basic genetic principles has been previously described (Neurospora in teaching 1966, NN#10:15). A reliable, visual method for demonstrating intergenic complementation is presented here. This procedure exploits the observation that csp-1 and csp-2 strains produce conjoined conidio so that when agar slant cultures are inverted and sharply tapped, no free conidio are observed falling = the "tap test" (Selitrennikoff and Nelson 1973, NN#20:34). However, when either wild-type or auxotrophic cultures containing the csp-1 and csp-2 mutations are available from the Fungal Genetics Stock Center.


The students were asked to design their own experimental protocol in order to obtain data which would allow them to answer these questions: 1) Is each morphological mutant gene recessive to its wild-type allele? 2) How many loci do the 4 mutant genes (csp-1, csp-2, nit-2, nit-3) and heterokaryon compatible double mutant cultures isolated (Selitrennikoff, in preparation). These compatible strains can be used in a classical "cis-trans" test requiring only the tap test to detect the presence or absence of complementation. To illustrate, we present an experiment which was performed by the Fall 1973 Genetics Laboratory students at California State University at Fullerton. Each group of 3 students was given agar slant cultures (Vogel's medium N + 1.5% sucrose + 50 μg/ml nicotinamide) of the following strains (each tube was labeled with only the allele number and the appropriate auxotrophic locus designation) and 20 sterile slants of Vogel's N + 1.5% sucrose minimal medium.

Table 1. Heterokaryotic cultures were formed by co-inoculation of aerial hyphae onto minimal medium. After 7 days' growth at 25°C, tubes were scored by the tap test. 

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>nic-2</th>
<th>UCLA37</th>
<th>FS590</th>
<th>FS591</th>
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</table>

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Millington-Ward, A. M. A simple explanation for the 66.7% limiting values in tetrad analysis.