A simple classroom complementation experiment

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A simple classroom complementation experiment

**Abstract**
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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 Metens 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. Prototrophic and auxotrophic cultures containing the csp-1 and csp-2 mutations are available from the Fungal Genetics Stock Center.


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 cultures or forced heterokaryons of csp-1/csp-2 are tapped, they release an easily observed copious cloud of freed conidio.

The one allele of csp-1 (UCLA37) and the three alleles of csp-2 (FS591, FS590, UCLA 101) were each crossed to nic-2 (43002) and nit-3 (Y31881) 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 top 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.

1. nic-2 A
2. nic-3 A
3. csp-1 (UCLA37), nic-2 A
4. csp-1 (UCLA37); nic-3 A
5. csp-2 (FS590); nic-2 A
6. csp-2 (FS590); nic-3 A
7. csp-2 (FS591); nic-2 A
8. csp-2 (FS591); nic-3 A
9. csp-2 (UCLA101); nic-2 A
10. csp-2 (UCLA101); nic-3 A.

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 nic-2</th>
<th>FS590 nic-2</th>
<th>FS591 nic-2</th>
<th>UCLA10 nic-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>nic-3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>UCLA37 nic-3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FS590 nic-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FS591 nic-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UCLA10 nic-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 i.e., UCLA37, FS591, FS590 and UCLA 101, represent? All groups independently performed the heterokaryon analysis with the results indicated in Table 1 and concluded that FS590, FS591 and UCLA 101 were functional alleles, UCLA37 was a separate locus, and all were recessive. The above 10 strains are available from the Fungal Genetics Stock Center.

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

This note is in response to Griffiths and Penson (1973 NN#20: 37). Suppose a box contains two black and two white balls, which are to be transferred to a linear sock in turn and without looking (Fig. 1). At the first dip into the box, there is a 50:50 chance of taking out a black or a white ball. Suppose a black ball is transferred to the tube (Fig. 2). There are now two white and one black ball remaining in the box. Therefore, at the second transfer, there is twice as much chance of taking a white as there is of taking a block ball. There is, therefore, twice as much chance of forming a second-division segregation asci as there is a first. There are, therefore, under random segregation, twice as many second-division segregation asci (4/6) as there are first (2/6). Therefore, under random conditions, there are 4/6 (≈ 66.7%) second-division segregation asci.