Studies of the genetics and physiology of a nitrate non-utilizing strain of Neurospora

R. M. Blakely
A. M. Srb

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Abstract
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Ratio of inos-reversions to ad-reversions

<table>
<thead>
<tr>
<th>Exposure time (min.)</th>
<th>Experiment I</th>
<th>Experiment II</th>
<th>Experiment III</th>
<th>Mean</th>
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<td>.95</td>
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<td>3</td>
<td>1.1</td>
<td>1.1</td>
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At low temperatures the divergence between the dose-effect curves for the two loci becomes emphasized and the point of intersection shifts to higher values; this is due to the pronounced response of the ad-reversions and the very slight response of the inos-reversions to temperature. (see graph).

Possible interpretations take account of differences between the loci in (a) photo-repair during treatment (our UV source is not monochromatic), (b) dark repair after treatment, the period available for dark repair presumably increasing with time of treatment. These interpretations will now be tested. ---

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Blakely, Ruth M. and Adrian M. Srb.

Studies of the genetics and physiology of a nitrate non-utilizing strain of Neurospora.

Physiological examination of a strain of Neurospora isolated from forest soil from Bruner, Borneo, by J. H. Warcup showed it to be a naturally occurring nitrate non-utilizer. Nutritional experiments in liquid modified Fries' minimal media using a variety of nitrogen sources show that the Borneo isolate is unable to utilize either nitrate or nitrite at temperatures ranging from 18°C to 35°C and at pH's ranging from 5.5 to 7.5. The ammonium ion, amides, amino acids and adenine are good nitrogen sources. The requirement for reduced nitrogen is not alleviated by the addition of vitamin supplements or pyruvate.

The characteristic has been examined genetically by means of a backcrossing program into N. crassa 74 A using an intermediate strain for the initial cross, and it is apparently determined by a single gene. Well over 1000 backcross isolates were tested for utilization or non-utilization of both nitrate and nitrite. The response was always the same to both. Crosses to markers on all chromosome of N. crassa have shown that this gene, designated as nit-4+, is in linkage group IV, about 15 map units to the right of cot. Thus nit-4 is linked to nit-3 which is to the left of cot. Strains bearing the mutant gene nit-3 cannot utilize nitrate but respond well to nitrite. Crosses of nit-4 to standard markers 33 nit-2 and nit (2003) show independent assortment of these genes.

Revertants to nitrate utilization were obtained in low frequency when conidia of a colonial mutant of the Borneo isolate were plated on nitrate medium. The frequency of revertants could be increased by exposing conidia to B-propiolactone. These revertants have not yet been analyzed genetically. ---

Department of Plant Breeding, Cornell University, Ithaca, New York.
### Table

<table>
<thead>
<tr>
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</table>

Mean dry weights in mg. of 3 replicate mycelial pads of Borneo strain and N. crassa 74 A after 7 days growth in modified Fries' liquid medium with different nitrogen sources, buffered at 3 pH levels at 3 temperatures. A. No added nitrogen. B. NaNO₂ at 0.0185 g nitrogen per liter. C. NaNO₃ at 1.11 g nitrogen per liter. D. NH₄Cl at 1.11 g nitrogen per liter.

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**Case, M.E. and N.H. Giles.** The problem of mitotic recombination in *Neurospora.*

There is evidence that mitotic recombination (or reassortment) occurs in certain ascomycetes. One such case has been reported in *Neurospora* utilizing unlinked markers (Weir, J.A., *Genetics* **45**:1016, 1960). This phenomenon has been considered as one possible explanation for interallelic complementation and also for atypical segregations in asci.

Preliminary evidence bearing on this question was obtained from platings of unisexual and bisexual heterocaryons utilizing unlinked biochemical mutant markers. These results produced no evidence for nuclear fusion followed by chromosomal reassortment such as to give rise to new genotypes, which would be capable of growing on an unsupplemented medium.

Following the previously mentioned report of mitotic reassortment in *Neurospora,* this problem has been investigated further by making the following cross and looking for prototrophs on an adenine supplemented medium. This cross involves three of the seven linkage groups in *Neurospora.* The protoperithecial parent, **hist-2** **pan-2** **ylo** A was crossed utilizing conidia from a heterocaryon **hist-2** a **ad-6** **pan-2** **ylo** a. The **hist-2** and **pan-2** markers are the same mutants in both the protoperithecial and heterocaryon parental strains. The **ad-6** mutant is not linked to these mutants, while **ylo** is linked to **pan-2**. In this manner, fertilization of the protoperithecial parent nuclei by either one of the two parental nuclei in the heterocaryon would constitute a selfing with respect to either the **hist-2** or the **pan-2** locus and no prototrophs would be expected to occur when the cross is plated on an adenine-supplemented medium (Diagram A).

**Diagram A**

<table>
<thead>
<tr>
<th>Protoperithecial parent</th>
<th>X</th>
<th>Heterocaryon parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, <strong>hist-2</strong></td>
<td></td>
<td>a, <strong>hist-2</strong></td>
</tr>
<tr>
<td><strong>ylo</strong>, <strong>pan-2</strong></td>
<td></td>
<td><strong>ylo</strong>+, <strong>pan-2</strong>+</td>
</tr>
<tr>
<td><strong>ad-6</strong>+</td>
<td></td>
<td><strong>ad-6</strong>+</td>
</tr>
</tbody>
</table>

If however, mitotic reassortment has occurred at some point during either the formation or growth of the heterocaryon, or as a result of a triple fusion and reassortment at the time of fertilization, then prototrophs would be recovered in such a plating (Diagram B).