STUDIES CONCERNING THE INHERITANCE OF ASCOSPORE LENGTH IN *NEUROSPORA CRASSA*

I. STUDIES ON LARGE-SPORED STRAINS

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**Summary**

Previous work has shown that the inheritance of ascospore size in certain strains of *Neurospora crassa* is controlled by a polygenic system with a small cytoplasmic component. Various types of selection lines were set up in strains that had been selected for the large-spored character. The results of these selection procedures are discussed. Experimental work which gives a better estimate of the linkage of part of this polygenic system to the albino locus on chromosome I is reported.

I. INTRODUCTION

It has been shown (Pateman 1955, 1959) that the length of ascospores in *Neurospora crassa* is largely controlled by a polygenic system with a small cytoplasmic component. In this work (cf. Pateman 1959), simple selection for increased ascospore length was practised. The mean ascospore length increased from about 18 units in wild-type crosses to a mean ascospore length of about 23 units after eight generations of selection. Further simple selection for increased ascospore length failed to increase the mean ascospore length over a further eight generations. Additional selection experiments with these strains and other studies on the inheritance of ascospore length in *N. crassa* are described in this paper.

II. MATERIALS AND METHODS

The strains used were 232-1, 232-2, 232-3, and 232-4, all of which were albino (*al* 2), large-spored strains obtained from a single ascus at the fifteenth generation of selection for increased ascospore length. The wild-types, St. Lawrence A (St.L.A) and 1196α, were originally derived from the Abbot or Lindegren wild-types or both and are largely isogenic, since 1196α was derived from seven generations of backcrossing to St.L.A (Pateman 1959). The vegetative cultures were maintained on Fries No. 3 (Beadle and Tatum 1945) medium. All crosses were made on agar slopes of a medium favouring sexual reproduction (Westergaard and Mitchell 1947) and normally incubated at 25°C.

The length of the ascospores was measured using a projection microscope and the unit of measurement was approximately 1.73 μ.

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III. Experimental Results

(a) Selection Lines Derived from the Large-spored Genotype

In earlier selection experiments (Pateman 1959) a response to selection was obtained over the first eight generations and although selection for increased ascospore size was practised for a further eight generations no further response was obtained. However, this response to selection was accompanied by a decrease in fertility in the selected lines. Strains from the fifteenth generation of selection for increased ascospore size were taken as a starting point for further selection in order to find out if any genetic variability still existed in them. The following series of selection lines were set up.

(i) The High Line.—The procedure was as follows. Crosses were set up between 232-1 and 232-3, and 232-2 and 232-4. From these crosses phenotypically large ascospores were isolated and the strains thus obtained test-crossed to the large-spored parents. A sample of 50 ascospores was measured from each cross. The strains that gave the highest mean ascospore size in the test crosses were then crossed. Two crosses in which each of the four parents were dissimilar were used for the selection of the next generation. This procedure was used in all succeeding generations.

This form of selection was adopted for the following reasons. First, simple selection had been practised for a prior eight generations without increasing the mean ascospore size. Second, there is more chance of genetic variability showing up in this type of performance test, than in simple selection for ascospore length. Third, the course of selection implies a selection for fertility.

The results of this selection are illustrated in Figure 1. There was an advance of about 2 units over the level of the original selected line. Thus the total response to selection for increased ascospore size is about 9 units of which about 7 units net increase was obtained by Pateman (1959). Crosses between strains in this selection line were very much more fertile than crosses between the strains from which they originated. The regression line through the values of this high line is significantly different ($t_l = 6.44; P<0.001$) from a line of zero slope at the mean value of 14 crosses between the originating strains. This suggests that there was some genetic variability in the original strains even after 15 generations of selection and even though there had been no response for the last eight generations of this selection.

(ii) The Low Line.—In this case a simple selection was practised. In each generation the shortest ascospores were selected and the strains so obtained were intercrossed. From these crosses two fertile crosses in which each of the four parents were dissimilar were selected to carry on the next generation. This procedure was used in all succeeding generations.

After 12 generations, if there was any response, it was a slight but non-significant increase in mean ascospore size (Fig. 1). However, the decrease in fertility was very rapid and after a few generations it was difficult to obtain enough ascospores to measure and germinate. This decrease in fertility became evident after
two generations and in order to try to restore the fertility, mass culture lines were started at generations 2 and 6. In these lines the fertility improved slightly and the mean ascospore size decreased, but the decrease was not significant.

These results suggested that the amount of genetic variability left in these strains was not sufficient to allow any decrease in ascospore size using this form of selection. Also when selection was relaxed as in the mass culture lines, the strains show a marked stability about the level obtained by Pateman (1959).

(iii) The Variability Line.—In each generation the shortest and longest ascospores were isolated and the strains so obtained were crossed together, so that a strain derived from a short ascospore was always crossed to a strain derived from a long ascospore. Two crosses in which all four parents were dissimilar were used to carry on the next generation. This procedure was used in all succeeding generations.

As can be seen (Fig. 1) a slight but significant increase ($t_6 = 2.6; \ P < 0.05$) in mean ascospore size has occurred in six generations.

The results indicate that there was some genetic variability left in these strains after 15 generations of selection for increased ascospore length. This was sufficient for an increase in mean ascospore length in two of the lines, but not sufficient for a decrease in mean ascospore length. In all selected lines there has been an increase in mean ascospore length. This indicates that, in fact, selection for deviants from the mean ascospore length, i.e. selection for variability of ascospore length, is important in this genetic system.

In the original selection for increased ascospore length, the coefficient of variation had increased from 5 to 17% by the time the line had plateaued (Pateman
1959). In all these selection lines the coefficient of variation remained at or near this value. This indicates that no further instability in ascospore or ascus development over that found by Pateman (1959) has become evident.

In all selection lines, except the high line, the response to selection was accompanied by a decrease in fertility. This was due to three main causes:

1. Fewer perithecia were formed in crosses between selected strains than in crosses between the original strains.
2. Of the ascospores produced in crosses between selected strains about 25% did not mature.
3. Of the mature ascospores produced in crosses between selected strains about 25% did not germinate.

Even in the mass culture lines, where selection for ascospore length was relaxed and selection for fertility practised, the fertility returned to the level of the originating strains only. Thus it seems likely that the genotype has stabilized around the value of the mean ascospore size of the originating strains.

(b) Linkage Experiments

Previous work (Lee and Pateman 1959) indicated that a part of the polygenic system responsible for approximately one-sixth of the total response to selection over 15 generations was apparently linked to the albino (al 2) locus on chromosome I which also contains the mating-type locus (A, a). The initial estimate of the degree of linkage was 9±6 centimorgans (cM). In order to try to locate this genotypic material more accurately, a further series of experiments was undertaken.

The strains 232-1, 232-2, 232-3, and 232-4 were crossed to the appropriate wild type and the crosses incubated at 25°C. The F1 ascospores were normal in size. One hundred ascospores were randomly isolated from each cross. The isolates thus obtained were each backcrossed to the large-spored parent. This gave a total of 73 crosses in which albino F1 strains were backcrossed and 102 crosses in which non-albino F1 strains were backcrossed. A sample of 50 ascospores from each cross was measured. The results are presented in Table 1. The majority of backcrosses involving albino F1 strains had mean ascospore lengths in the range 19·42-22·88 units, while the majority of backcrosses involving non-albino F1 strains had mean ascospore lengths in the range 17·26-18·94 units. The difference, 1·18 units, between the two groups of backcrosses is highly significant (P < 0·001). This indicates that some of the genotypic material controlling ascospore length is linked with the albino locus.

From Table 1, it can be seen that 10 out of 73 albino F1 strains when backcrossed gave a mean ascospore length in the range 17·26-18·94 units and 13 out of 102 non-albino F1 strains when backcrossed gave a mean ascospore length in the range 19·42-22·88 units. It is reasonable to regard these exceptions as due to crossing-over between the albino locus and the site of the genotypic material controlling this part of the response to selection for increased ascospore size. If this is so, then this genotypic material is located about 13±5 cM from the albino locus, calculated on the basis of 23 recombinants in a total of 175 backcrosses.
The ascospore length in the majority of backcrosses fell into one or other of two distinct classes. There were, however, two albino F1 strains where the mean ascospore length was intermediate between the two classes. These two intermediate cases are probably due to the segregation of the genotypic material controlling the rest of the response to selection for increased ascospore size. These intermediate cases have not been included in the estimation of the degree of linkage of the genotypic material to the albino locus. Attempts to locate this genotypic material using other biochemical markers have not yet been successful. So the following method of establishing the exact location is the best available at the moment.

| Table 1 |
|-----------------|-----------------|
| **backcrosses of al and al+F1 strains to large-spored parents** |
| 1 unit = 1.73μ. For difference between means \( t_{175} = 7.5; P < 0.001 \) |
|                              | al F1 | al+F1 |
| No. of backcrosses with mean in the range 17.26-18.94 units | 10    | 89    |
| No. of backcrosses with mean in the range 19.42-22.88 units | 61    | 13    |
| No. of intermediate backcrosses | 2     | 0     |
| Total number of backcrosses    | 73    | 102   |
| Mean ascospore length (units)  | 19.68 | 18.50 |

Considering the cross 232-1×1196a, the genotypic material linked to the albino locus was called L and in backcrosses to the large-spored parent gave a mean ascospore size in the range 19.42-22.88 units. If L is assumed to be proximal then the cross can be represented as \( A-L-al \times a-L+al^+ \). The region between A and L was called region I, while the region between L and al was called region II. Then, in this cross when only strains of mating-type a were backcrossed to the large-spored parent, the following genotypes resulted and represent the following crossover types: \( a-L^+al^+ \), parental, \( a-L-al^+ \) crossover in regions I and II, \( a-L^+al \) crossover in region II, and \( a-L-al \) crossover in region I. Applying this argument to each of the other sets of backcrosses, when L is assumed to be proximal to al, the genotypes and crossover classes are as presented as in Table 2.

Again, if L is assumed to be distal to al, then the cross 232-1×1196a may be represented as \( A-al-L \times a-al^+-L^+ \). The region between A and al was called region III and the region between L and al was called region II. Then, when strains of mating-type a only were backcrossed to the large-spored parent, the following genotypes resulted and represent the following crossover types: \( a-al^+L^+ \), parental, \( a-al^+-L \) crossover in region II, \( a-al-L^+ \) crossover in regions II and III, and \( a-al-L \).
crossover in region III. Applying this argument to each of the other sets of backcrosses, if \( L \) is assumed to be distal to the \( al \) locus, the genotypes and crossover classes may be tabulated as in Table 3.

### Table 2
**Genotypes and Crossover Classes when \( L \) is assumed to be proximal to \( al \)**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Region where Crossover Occurred</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A\cdot L^+\cdot al^+ ) ( a\cdot L^+\cdot al^+ )</td>
<td>None</td>
<td>89</td>
</tr>
<tr>
<td>( A\cdot L\cdot al^+ ) ( a\cdot L\cdot al^+ )</td>
<td>I and II</td>
<td>13</td>
</tr>
<tr>
<td>( A\cdot L^+\cdot al ) ( a\cdot L^+\cdot al )</td>
<td>II</td>
<td>10</td>
</tr>
<tr>
<td>( A\cdot L\cdot al ) ( a\cdot L\cdot al )</td>
<td>I</td>
<td>61</td>
</tr>
</tbody>
</table>

In the genotypes represented in Tables 2 and 3, all strains of mating-type \( a \) were backcrossed to either 232-1 or 232-2 and all strains of mating type \( A \) to either 232-2 or 232-4.

### Table 3
**Genotype and Crossover Classes when \( L \) is assumed to be distal to \( al \)**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Region where Crossover Occurred</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A\cdot al^+\cdot L^+ ) ( a\cdot al^+\cdot L^+ )</td>
<td>None</td>
<td>89</td>
</tr>
<tr>
<td>( A\cdot al^+\cdot L ) ( a\cdot al^+\cdot L )</td>
<td>II</td>
<td>13</td>
</tr>
<tr>
<td>( A\cdot al\cdot L^+ ) ( a\cdot al\cdot L^+ )</td>
<td>II and III</td>
<td>10</td>
</tr>
<tr>
<td>( A\cdot al\cdot L ) ( a\cdot al\cdot L )</td>
<td>III</td>
<td>61</td>
</tr>
</tbody>
</table>

When \( L \) is assumed proximal to the albino locus, the map distances are \( A \) to \( L \) 42 cM and \( L \) to \( al \) 13 cM. Now the map distance between \( A \) and \( al \) is known to
be 38 cM (Barratt et al. 1954), so assuming this value, the expected number of double crossovers between \( al \) and \( A \) would be \( 3.2\% \). In fact that actual value is \( 7.5\% \). Again, the distance between \( A \) and \( al \) calculated on the assumption that \( L \) is proximal to \( al \) is 55 cM, which does not correspond with the known value of 38 cM. This cannot be due to any chromosomal abnormalities since the same data when \( L \) is assumed to be distal to \( al \) gives a very reasonable value of 41 cM.

If, on the other hand, \( L \) is assumed to be distal to the albino locus, the map distances are \( A \) to \( al \) 41 cM, and \( al \) to \( L \) 13 cM. So assuming the distance between \( al \) and \( A \) is 38 cM, the expected number of double crossovers would be \( 5.0\% \) and in fact the value obtained from the data is \( 5.8\% \). Also, the calculated distance between \( al \) and \( A \), 41 cM, corresponds quite well with the known value of 38 cM. So the assumption that \( L \) is distal to the albino locus is much more likely than the assumption that \( L \) is proximal.

IV. Discussion

In the selection lines derived from the large-spored genotype, only the high line and variability line showed any significant response over the level of ascospore size obtained by Pateman (1959). Although environmental variation has a large effect on these large-spored strains, there is still some genetic variability left, but not enough to respond to simple selection, either for large or small ascospores.

In the low and variability lines developmental instability occurred as a result of selection. This was reflected in a decrease in the number of ascospores produced, an increase in the number of aborted ascospores and asci, and a decrease in the number of mature perithecia produced. This decrease in fertility is similar to that obtained by selecting for differences in bristle number in *Drosophila melanogaster* (Mather and Wigan 1942; Harrisson and Mather 1950) and can probably be regarded as a correlated response. The mass culture lines set up to try to improve the fertility of the low line gave an increase in fertility, yet no significant drop in mean ascospore length. This indicates that the selection for ascospore size practised by Pateman (1959) has been effective in increasing the mean ascospore length for these strains to about 23 units and stabilizing the genotype at this level. This is further substantiated by the fact that no further changes in the coefficient of variation over that found by Pateman (1959) occurred in these selection lines even though further developmental instability resulted.

The establishment of a linkage relation between a part of this polygenic system and the albino \((al\, 2)\) locus on chromosome I has been previously reported (Lee and Pateman 1959). This part of the polygenic system is responsible for about one-sixth of the total response to selection for large ascospore size over 15 generations. This cannot be due to pleiotropic effects of the albino locus because recombination between the polygenic material and the albino locus has been demonstrated.

Apparent linkage between polygenes and major genes has been shown in *Phaseolus vulgaris* (Sax 1923) between seed size, a polygenic character, and the colour gene \( P \). This may have been due, as Haskell (1959) suggests, to pleiotropic effects of this colour gene. However, the linkage shown in *Pisum* (Rasmusson 1935) between polygenes controlling flowering time and a major gene governing pi-
tation is free from this objection since recombination between the polygenes and the major gene locus has been shown. Again in *D. melanogaster* many cases are known of the location of areas of polygenic activity. Mostly these cases are concerned with the location of polygenes controlling bristle number (Mather 1941, 1942, 1944; Wigan 1949). So far, additional work with other markers has failed to locate this genotypic material with greater accuracy. This is probably because of interaction between the marker alleles used and the polygenic material. If this material can be located and bounded by marker alleles which do not interfere with its phenotypic expression, then it may be possible to make a direct study of recombination within a group of polygenes which exhibit linkage in a crude analysis.

V. Acknowledgments

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VI. References


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