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

II. SELECTION EXPERIMENTS WITH WILD-TYPE STRAINS

By B. T. O. Lee*

[*Manuscript received July* 17, 1961]

Summary

Various types of selection with respect to ascospore length were practised with wild-type strains of *Neurospora crassa*. The effect of all types of selection over 11 generations was essentially the same. A significant increase in mean ascospore length, a tendency for a decrease in the coefficient of variation, and a decrease in fertility were observed in each case. These results are discussed and a hypothesis to explain them is put forward.

I. Introduction

Earlier work on the inheritance of ascospore length in *Neurospora crassa* (Lee and Pateman 1959; Pateman 1959) was carried out with strains which initially showed an abnormally high percentage of large ascospores. It seemed possible that at least a part of the response to selection for increased length which was obtained by Pateman (1959) was due to an increase in variability, that is a lack of canalization of ascospore development. The coefficient of variation of ascospore length increased from the original 5% to 17% by the time the line had plateaued after eight generations of selection.

It was considered that the appropriate selection experiments with wild-type strains might provide information on the following points:

(1) Do wild-type strains possess genetic variability, with respect to ascospore size, which can be exploited by selection?

(2) Does the response to selection involve canalization?

(3) Is the genetic variability in wild-type strains, if any, similar to that in the large-spored strains?

II. Methods

The strains used have complicated pedigrees, but all were probably derived originally from the Abbot or Lindegren wild-types or both of these. The vegetative cultures were maintained on agar slopes of 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 incubated at 25°C. The length of the ascospores was measured on a projection microscope and the unit of measurement was approximately $1 \cdot 73\ \mu$.

* Botany School, University of Melbourne; present address: Botany Department, Queen's University, Belfast.

III. Selection and Mating Procedure

From a particular cross between two wild-type strains, a sample of about 2000 ascospores were taken, and 5 of the longest and 5 of the shortest ascospores were selected. Using the strains obtained from those ascospores, the following selection lines were set up:

(1) A high line, in which phenotypically long ascospores were selected and the resultant strains crossed to give the next generation.

(2) A low line, in which phenotypically short ascospores were selected and the resultant strains crossed to give the next generation.

(3) A so-called variability line, in which strains derived from phenotypically long ascospores were crossed with strains from phenotypically short ascospores to give the next generation.

In addition, crosses between the original wild-type strains were used at each generation of selection. The mean lengths of samples of 50 ascospores from each of these crosses were used as controls.

The following mating procedure was used with all three lines throughout the experiment. From two crosses in which all four parents were dissimilar, five ascospores of the appropriate phenotype were selected from a sample of about 2000. The cultures so obtained were crossed and two crosses in which all the four parents were dissimilar were used to obtain the next generation of ascospores:

Crosses	1st Generation	Crosses	2nd Generation
1×2	5 and 6	5×7	9 and 10
3×4	7 and 8	6×8	11 and 12

In order to ascertain if the results obtained were merely a reflection of the mating procedure, a second control line was established utilizing a random selection of ascospores at each generation. From each of the two original crosses five random ascospores were selected. These were crossed and intercrossed randomly and two crosses in which each of the four parents were dissimilar were selected randomly to give the next generation of ascospores.

The response to selection was followed by measuring samples of 50 ascospores from each of the two crosses which were used to obtain the next generation.

IV. Experimental Results

The results of selection over 11 generations are presented in Table 1. The mean ascospore length is plotted against the number of generations (Fig. 1). From an analysis of the regression lines (Table 2) it can be seen that each of them is significantly different from a line of zero slope, but not significantly different from one another. The control lines are almost identical with lines of zero slope so that in effect each of the selected lines is different from the control lines. There was a significant increase in mean ascospore length from about 18·7 to about 19·6 units in all three selection lines. There is a tendency for the coefficient of variation to decrease in all three selection lines, but the decrease is not significant.

The decrease in the coefficient of variation would have been significant except for generations 2 and 9. Since the coefficient of variability of all the lines suddenly

TABLE 1

MEAN ASCOSPORE LENGTH AND COEFFICIENT OF VARIATION IN SELECTED AND UNSELECTED LINES

Generation	High Line		Low Line		Variability Line		Control Line 1 (asexual)		Control Line 2 (random selection)	
	Mean	Coeff. of Variation	Mean	Coeff. of Variation	Mean	Coeff. of Variation	Mean	Coeff. of Variation	Mean	Coeff. of Variation
0	18·71	7·9	18·71	7·9	18·71	7·9	18·34	7·9	18·71	7·9
1	18·63	6·7	19·54	8·3	19·45	7·2	18·68	8·5	18·20	6·7
2	19·19	5·7	18·35	6·8	19·12	9·7	18·10	7·3	18·31	8·5
3	19·47	8·2	19·56	6·7	19·57	5·8	18·66	6·5	18·26	7·3
4	19·75	6·8	19·13	5·9	18·83	6·2	18·72	6·7	18·13	8·6
5	18·97	7·5	18·77	7·5	19·14	6·9	18·68	9·4	18·06	7·1
6	19·77	6·7	18·15	7·6	20·14	6·6	18·24	6·5	18·62	6·6
7	19·73	6·8	19·73	6·5	19·67	6·4	18·40	8·0	18·51	8·1
8	19·42	4·8	19·85	8·1	19·73	5·6	18·38	7·1	18·37	6·9
9	20·45	9·1	19·83	8·1	19·96	9·2	18·24	7·5	18·53	7·2
10	20·22	6·7	19·76	6·3	19·81	5·4	18·40	8·0	18·41	8·0
11	19·78	5·5	19·73	5·9			18·56	8·1	18·21	8·1

increased in these two generations this may have been due to a common environmental factor. The most interesting fact that emerged from these results was that the three different forms of selection all had the same result.

The mean ascospore length in the control lines has remained steady at or below its original value of about 18·7 units. The coefficient of variation also has maintained its original value at or about 7·9% and has shown no significant deviations from this value.

In all three selected lines, as selection progressed, the fertility of the strains decreased. The main cause of this decrease was a reduction in the number of perithecia produced.

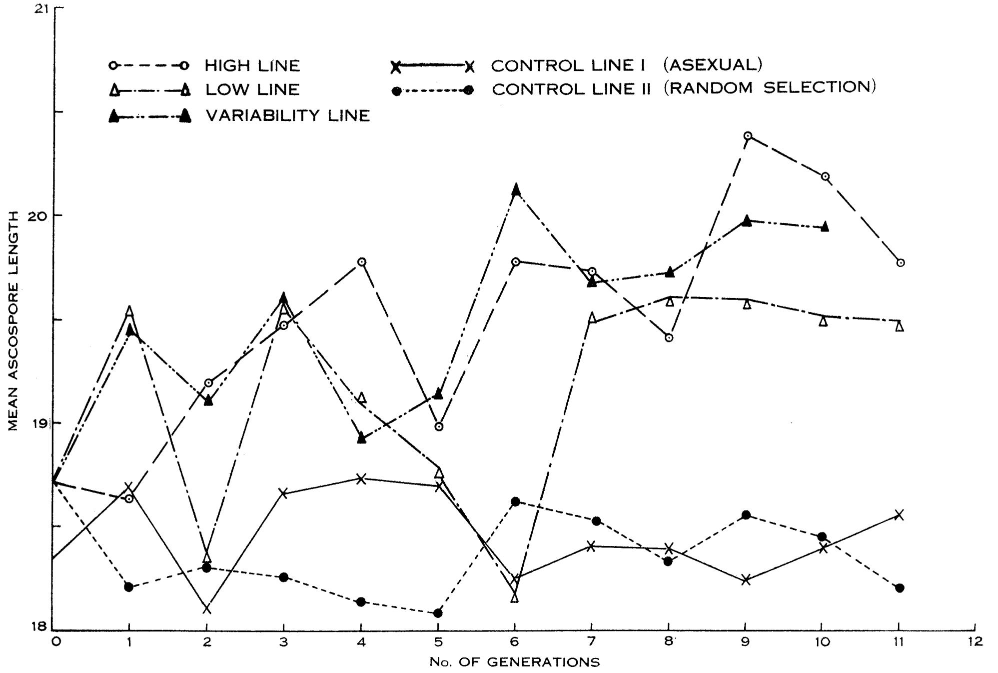

Fig. 1.—Effect of selection on wild-type strains of *Neurospora crassa*.

V. Discussion

The following features emerge from a consideration of the experimental results:

(1) All three types of selection resulted in the same significant response.

(2) The only common feature of the three modes of selection is that all were for extreme deviants from the mean.

(3) The simplest hypothesis is that deviants, whether large or small, represent a breakdown in the canalization system affecting ascospore length. The lack of canalization in the deviants was at least to some extent genetically determined, since there was a significant response in all three selection lines.

TABLE 2

ANALYSIS OF LINEAR REGRESSION OF ASCOSPORE LENGTH ON NUMBER OF GENERATIONS

	High Line			Low Line			Variability Line		
	S.S.	n	M.S.	S.S.	n	M.S.	S.S.	n	M.S.
Regression	297	1	297	222	1	222	220	1	220
Residual	3990	1898	2·102	3876	1898	2·053	4391	1798	2·442
Total	4287	1899		4098	1899		4611	1799	
t_{1898}*	11·88			10·39			9·49		
P	<0·001			<0·001			<0·001		

	Regression Coefficients				Intercepts			
	Difference	n	t	P	Difference	n	t	P
High v. low	0·01497	3796	1·15	>0·2	0·158	3796	1·51	>0·1
High v. variability	0·00543	3696	0·38	>0·7	0·034	3696	0·30	>0·7
Low v. variability	0·00954	3696	0·67	>0·5	0·124	3696	1·12	>0·2

* No. of degrees of freedom for the variability line is 1798.

Thus it seems that to date selection has produced changes in the genetic system which determines the canalization of ascospore length in wild-type strains around a mean of 18·7 units. It might be expected that such changes, produced as a result of selection for deviants, would result in more variation, that is less canalization around the same mean. In fact, it was the mean that changed in the selection lines, while if anything the variance decreased. However, in the absence of any knowledge of the physiological mechanism of canalization of ascospore length, it must be considered a possibility that initial changes in the genotype determining canalization could result in a change in the mean without appreciably affecting the variance. It may be that so far selection has only produced genetic changes to which the system can adjust by a change in the mean. Continued selection may eventually produce genotypic changes which result in a breakdown of the canalization system. This kind of situation is known (Waddington 1953, 1956). In this connection, it may be of significance that in the last few generations of selection abnormally large ascospores have begun to appear, while they are extremely rare in the control crosses. These may be the result of extremely rare recombinational events. It is possible that after further selection they may give rise to ascospores phenotypically and genotypically similar to those reported by Pateman (1959).

VI. Acknowledgments

I wish to thank Dr. J. A. Pateman, and Professor J. S. Turner for help and advice during this work; also the University of Melbourne for the Caroline Kay and Frederick Charles Williams Scholarships.

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