CROSSING PLAN FOR NEUROSPORA EXPERIMENT TO DETERMINE EFFECT OF SELECTION THROUGH RECOMBINATION FREE OF HETEROTIC EFFECTS

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ABSTRACT

A procedure was outlined in BU-103-M for determining the rate of genetic progress attained through selection by recombination of factors free of heterotic effects for an organism such as neurospora. The following is an outline of two experiments designed specifically to develop and to test genetic models related to rate of genetic progress. The first experiment relates to sib matings and the second experiment is concerned with matings using backcrossing techniques. Some preliminary results of selection for fastest growing plus and minus selections of neurospora are included. Because the selections have not been carried through sufficient cycles to fix genotypes, no definite conclusions can be drawn from these data.

CROSSING PLAN FOR NEUROSPORA EXPERIMENT TO DETERMINE EFFECT OF SELECTION THROUGH RECOMBINATION FREE OF HETEROTIC EFFECTS *

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A procedure was outlined in BU-103-M for determining the rate of genetic progress attained through selection by recombination of factors free of heterotic effects for an organism such as neurospora. The following is an outline of two experiments designed specifically to develop and to test genetic models related to rate of genetic progress. The first experiment relates to sib matings and the second experiment is concerned with matings using backcrossing techniques.

Sibbing Series

I. Strains for crossing

Hon 3A and Hon 1a = A
74A and 77a = B
3-723-3A and 3-723-5a = C
Tai I-8A and Tai I-22a = D

II. Crosses

The two strains denoted as A and B and the four families used are A, B, AXB and BXA. More specifically, it involves the following crosses: (1)74A/77a, (2) Hon 3A/77a, (3) 77a/Hon 3A and (4) Hon 1a/Hon 3A. Four additional crosses ((1) 77a/74A, (2) Hon 1a/74A, (3) 74A/Hon 1a, and (4) Hon 3A/Hon 1a) have been included at least until possible cytoplasmic effects, within as well as between strains, can be determined. Reciprocal crosses between strains A and C and B and C have also been made to be handled in the same manner as crosses between A and B. The number of crosses to be used in each of the combinations between strains other than strains A and B will be determined after some information is obtained regarding possible cytoplasmic effects. Additional combinations may be included later, i.e., A and D, B and D, C and D and possibly other cross-compatible strains.

III. Progeny

From a given progeny, proceed as follows:

(i) Obtain a random sample of ten + individuals and a random sample of ten - individuals. (Isolate 40 spores from each cross and select first ten + and first ten - based on mating type tests.)

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(ii) Obtain growth rates for the 20 individuals in (i) using duplicate growth tubes -- 40 growth tubes at each of three temperatures (18° C , 25° C and 35° C) for each cross.

(iii) Select fastest growing + and - individuals based on means of the duplicate tubes and cross these two individuals.

(iv) Grow out 8 tubes (vegetatively) of each of the fastest growing + and - to obtain a better estimate of the mean and an estimate of the environmental variance at each stage and for each progeny.

(v) Repeat (i) on progeny of (iii) and proceed through (i), (ii), (iii) and (iv) until the mean growth rate of a progeny is stabilized.

IV. Do III for each of the progeny from the particular crosses to be used.

V. Repeat III and IV several times starting from the material in II , i.e., the crosses will be made only once. Thus it will involve selecting ten + and ten - individuals again from the original crosses for each of the three temperatures which should give some measure of the efficiency of sampling. The possible number of replications to make will be mainly limited by the length of time in which viable first-generation ascospores can be maintained. Crosses, from which viable first-generation ascospores cannot be maintained long enough for adequate replication, will be re-made. Any differences within strains involved in initial and re-made crosses can be corrected for in the analysis of the data.

VI. Save all stocks of fastest growing individuals from each cycle in order that any phase can be repeated if necessary.

VII. Preliminary results.

The results available at the present time are from crosses between strains A and B. The data from crosses involving these two strains are included in Table 1.

Although these data are too fragmentary to draw any conclusions at this time, it is quite obvious that in the crosses in which two or three cycles of selection have been completed, there has been an increase in growth rate. The mean growth rate for the 20 individuals at each temperature in all first cycles of selection have not been included due to the great amount of variation in growth rate in the first cycles. After more cycles have been completed, meaningful comparisons can be made between the growth rates of the fastest growing individuals of each mating type from successive cycles.

The differences, if any, between reciprocal crosses within and between strains are difficult to assess at this time with the limited amount of data which has been obtained.

Table 1. Rate of growth (mm 1 hr.) at three temperatures (18°C , 25°C , and 35°C) of 20 individual isolates from crosses between two strains of Neurospora. Selected individuals for subsequent cycles are based on crosses between fastest individuals of opposite mating type. Means of each cycle are based on 40 individuals in each cycle at each temperature, i.e., 20 individuals in duplicate tubes.

Cross	Cycle of Selection	Growth rate (mm 1 hr.)					
		18° C		25° C		35° C	
		Mean	Fastest +x-	Mean	Fastest +x-	Mean	Fastest +x-
74A/77a	1	--	2.36 x 2.42	--	3.88 x 3.96	---	4.96 x 4.96
77a/74A	1	--	2.27 x 2.27	--	3.96 x 3.94	--	4.50 x 4.68
	2	2.30	2.40 x 2.40	4.00	4.06 x 4.09	5.23	5.28 x 5.37
Hon 1a/Hon 3A	1	--	2.49 x 2.46	--	4.43 x 4.50	--	5.33 x 5.50
	2	2.45	2.54 x 2.52	4.27	4.46 x 4.46	5.06	5.22 x 5.47
	3	2.53	2.59 x 2.60	4.44	4.61 x 4.55	5.50	5.74 x 5.63
Hon 3A/Hon 1a	1	--	2.44 x 2.53	--	4.15 x 4.31	--	5.35 x 5.09
Hon 3A/77a	1	--	2.44 x 2.30	--	4.52 x 4.36	--	5.20 x 5.50
	2	2.65	2.82 x 2.87	4.40	4.52 x 4.62	5.34	5.44 x 5.79
77a/Hon 3A	1	--	2.38 x 2.51	--	4.11 x 4.43	--	4.30 x 5.47
	2	2.57	2.80 x 2.73	4.23	4.27 x 4.42	5.05	5.37 x 5.35
Hon 1a/74A	1	--	2.38 x 2.34	--	4.06 x 4.01	--	4.72 x 4.64

Backcrossing series

The following individual progenies will be available from the first sibbing cycle:

+A x -B → fastest + (AB+) and fastest-- (AB-)

+B x -A → fastest + (BA+) and fastest - (BA-)

In the sibbing series, the cytoplasms are taken into account. If it is desired to do so in this series, each of these 4 progenies must be backcrossed using the appropriate strain as the protoperithecial parent. "Side by side" crosses will obscure some cytoplasmic differences.

Following crosses:

				Final Genotype	
				Nuclear	Cytoplasmic
(1)	AB+	x B-		B	A
(2)	AB-	x A+	side by side	A	A
(3)	BA+	x B-	side by side	B	B
(4)	BA-	x A+		A	B

Because the + parent of AB was A, backcrosses to A need only be side by side. Because the + parent of BA was B, backcrosses to B need only be side by side also. There will be 4 progenies, at each of 3 temperatures, or 12 in all.

Do III (i) and III (ii) as for the sibbing series, then select fastest + from backcross series to B- and fastest - from backcross series to A+, and again backcross these to their recurrent parent. To select fastest individual obtain a random sample of 10 individuals, test these in duplicate growth tubes. A total of (10 x 2) x 12 = 240 tubes will be needed for each cycle; each of the selected strains should be retested with 8 replications, with 4 x 3 strains, total of 8 x (4 x 3) = 96 tubes.

Repeat these cycles until growth rate stabilizes, as in the sibbing series. Because only one mating type need be tested in growth tubes, the b. c. series will involve less work per cycle than the sibbing series.

As stated under V for the sibbing series, select spores at random from the initial crosses and repeat the cycles again until growth rate stabilizes. This should be done several times.