TECHNIQUES AND RELATED GENETIC RESULTS FOR A QUANTITATIVE INHERITANCE STUDY IN *NEUROSPORA CRASSA*

BU-114-M Keith H. Thompson April, 1960

Introduction.

An experiment designed to estimate genetic components of variance of a quantitative character in the haploid organism *Neurospora crassa* is described. The quantitative character used in the study is linear mycelial growth and is experimentally controlled by two unlinked loci.

The mutants adenineless (ad) and methionineless (me) respectively require exogenous sources of adenine, a purine, and methionine, an amino acid, for growth. The normal growth of wild type neurospora is approximated by (ad) and (me) if they are grown upon minimal media to which an organic source of adenine or methionine has been added. In amounts less than optimum, adenine and methionine become limiting factors of growth for the mutants and under specific experimental conditions provide an environment for which genotypic effects and variance components may be defined for a two gene, two allele system.

Two breeding systems, each derived from two haploid parents, are used in the study. Both systems, random mating among full sibs in successive generations and recurrent backcrossing to both parents, are described in detail by Robson (1956a, 1956b, 1958).

Experimental results and discussion.

A purple, adenine requirer, ppl-30a, and a methionine requirer, V-41a, were used as genotypic sources. Both were isolated at Cornell from conidia of 77a, a wild type neurospora, after β-propiolactone treatment. They were selected among several other auxotrophic mutants available on the basis of their consistent performance in preliminary growth experiments at different dilutions of adenine and methionine. Because of their similar origin, they were considered to be reasonably isogenic.

The two standard wild type neurosporas used in the experiment, 77a and 74A, were originally isolated by P. St.Lawrence and were provided to the laboratory by D. Bonner of Yale University.

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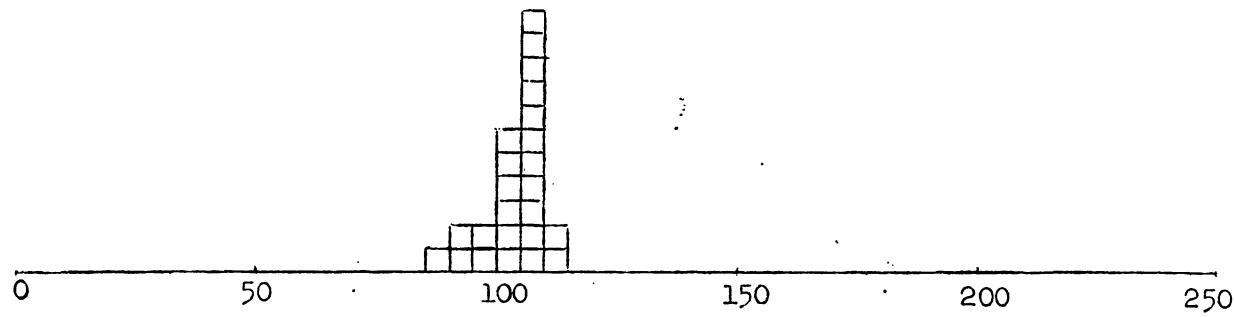
Ppl-30a and V-41a are of identical mating type and hence unsuitable as parents directly. In order to obtain a parent of mating type A, ppl-30a was crossed with 74A and purple, adenine requiring progeny were isolated. One of these, 34A, appeared similar to ppl-30a in growth experiments at different dilutions of adenine and was crossed with V-41a to further determine the suitability of 34A and V-41a as parents for the two breeding systems.

Ninety-six ascospores in approximately equal numbers of each of the four resulting genotypes (++, ad+, +me, adme) were randomly selected from the progeny and along with twenty-four replicates of each of the two parents, 34A (ad+) and V-41a (+me), were grown on specially constructed growth tubes. Minimal media supplemented with 5×10^{-5} gram of dl-methionine and 8.75×10^{-6} gram of adenine sulfate per cc. was used for all four genotypes and the parental controls. The genotypic distributions of the parents and the randomly selected progeny under these conditions are given in Figure 1. The measurements are in millimeters of total linear growth at approximately 25°C during a sixty hour period following a twenty-four hour lag after innoculation.

Figure 1. Genotypic distributions.

41a (+me)



34A (ad+)

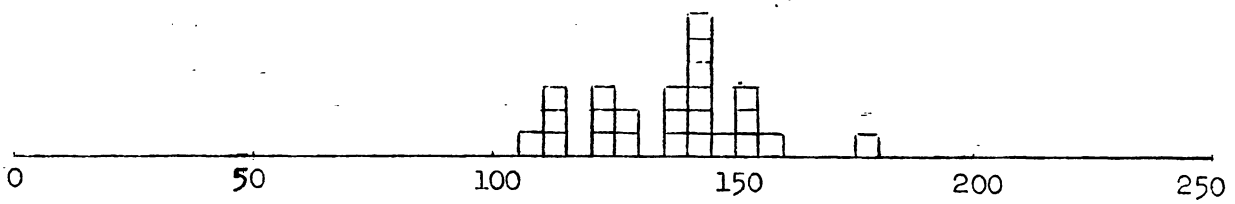
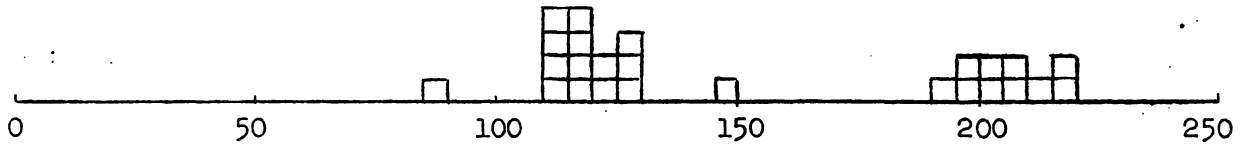
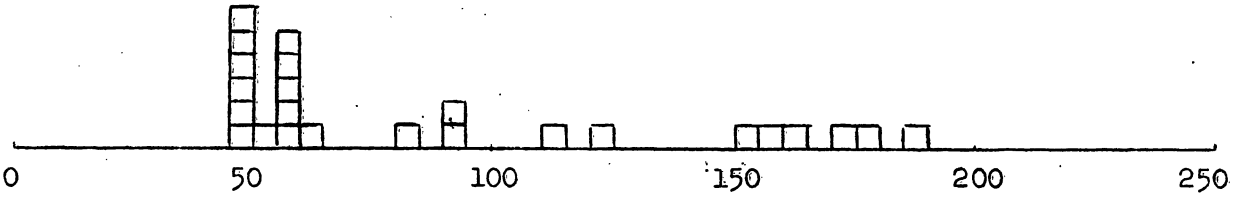


Figure 1 (continued)

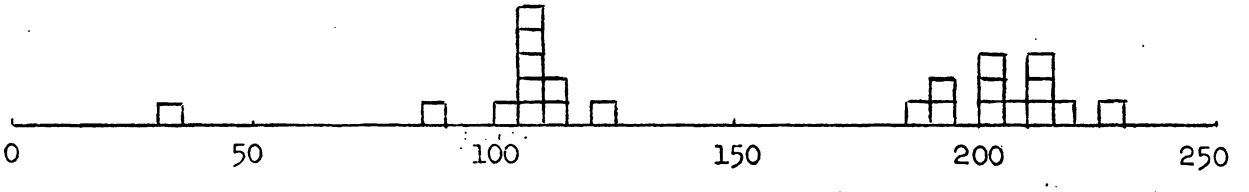
++



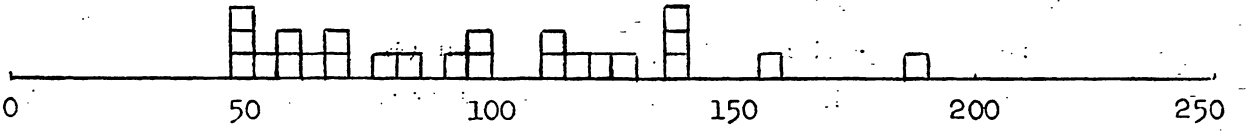
ad+



+me



adme



The parent V-41a performed on the adenine and methionine supplemented media similarly as it had in previous dilution experiments at an identical concentration of methionine alone. This concentration of methionine, 5×10^{-5} gram of dl-methionine per cc. of media, produced maximum growth for V-41a in the earlier experiments at a rate of about half that of normal wild type. Increasing the concentration of methionine beyond this optimum failed to increase the growth rate.

The adenineless parent, 34A, also performed on the adenine and methionine supplemented media as expected on the basis of its previous performance in dilution series on minimal media supplemented with adenine alone. The concentration of adenine used, 8.75×10^{-6} gram of adenine sulfate per cc. of media, was limiting, however, since in preliminary experiments 7.485×10^{-5} gram of adenine sulfate was required to produce growth for 34A at a rate comparable to wild type.

The genotypic distributions of the progeny from the cross V-41a x 34A exhibit great variability when compared to the parents. The (++) progeny appear to be characterized by two different growth rates, one normal and the other approximately half that of normal. Similarly, the (+me) progeny may be classified into two distinct groups based on growth rate, one comparable to the parent V-41a and the other comparable to normal wild type. This dichotomy is not as pronounced in the (ad) progeny, perhaps because the greater variability of the (ad) genotype under the experimental conditions tends to obscure the bimodal distribution characteristic of (++) and (+me). However, the range of growth rates of both (ad+) and (adme) is about twice that of the parent 34A and includes values in the range of, but not exceeding, 34A and values about half as large as the smallest of the 34A controls.

To account for the unexpected variation within genotypes in the progeny, the hypothesis of a growth factor, differing in the two parents and segregating independently of the adenineless, methionineless, and mating type loci, is advanced and appears to be consistent with the data.

Suitable parents were now sought among the (ad+) and (+me) progeny thought to be identical for the unknown growth factor. Two crosses were made: 428A(+me,227) x 19a(ad+,123) and 394a(+me,193) x 499A(ad+,154). The last number in parentheses is the linear growth measurement of the corresponding isolate and is the basis of selection, both (ad+) and both (+me) parents being selected from the faster growing (normal) mode of their respective genotypic distributions.

Four-hundred fifty ascospores from the 394a x 499A cross and 442 ascospores from the 428A x 19a cross were randomly isolated into individual culture tubes on complete media (i.e., minimal media, required by wild type neurospora, to which has been added .75% malt extract and .25% yeast extract). The ascospores were activated shortly after isolation in a water bath for thirty minutes at about 60°C. The two tables (1 and 2) below indicate the germination performance of the eight genotypes over time for the two crosses.

Table 1. Cross 1. 394(+mea) x 499(ad+A)

Days after activation	++A	++a	ad+A	ad+a	+meA	+mea	admeA	admea	Total
1									0
2	8	41							49
3	1	6	7	1	4	13			32
4			16	1			2		19
5			5				2		7
6			4						4
7			2			1	1		4
8			1				1		2
9		1	2	3			2		8
10			3						3
11			3				2		5
12			2				2		4
13			4				1		5
14			3				1		4
15							3		3
16							2		2
17									0
18									0
19			1						1
20									0
Total	9	48	53	5	4	14	19	0	152

Number of ascospores (out of 450) germinating on complete media on a given date after activation.

Table 2. Cross 2. 428(+meA) x 19(ad+a)

	++A	++a	ad+A	ad+a	+meA	+mea	admeA	admea	Total
1									0
2	54	11		1	25	6			97
3				1					1
4				12		1		3	16
5	1		4	12		1		5	23
6				2			1	1	4
7	1		1	4					6
8				3				2	5
9			4	6				5	15
10			1	7			1	1	10
11			1	2				6	9
12				2				1	3
13				1					1
14									0
15				1			1		2
16								1	1
17				1				1	2
18								1	1
19									0
Total	56	11	11	55	25	8	3	27	196

Number of ascospores (out of 442) germinating on complete media on a given date after activation.

Germination was poor for both crosses (34% for cross 1 and 44% for cross 2) and was particularly selective against methionineless, independent of adenineless. This is evident from the ratio of genotypes ++:ad+:+me+adme which for cross 1 was 57:58:18:19 and for cross 2 was 67:66:33:30. The data from all eight genotypes further indicates a linkage of adenineless with mating type. There are only 12% crossover progeny in cross 1 and 17% for cross 2.

Both crosses reveal a considerable time lag in germination for the adenineless progeny, independent of methionineless. Whereas 83% of the (++) and (+me) genotypes had germinated by the second day following activation and 97% by the third day, both the (ad+) and (adme) genotypes germinated fairly uniformly over a two week period. In an attempt to find a more suitable germination media, one which might permit random survival of genotypes over a shorter period of time, 150 ascospores from the cross 428A x 19a were isolated onto minimal media supplemented with optimum levels of methionine and adenine. The results are tabulated below (Table 3).

Table 3. Cross 1. 428(+meA) x 19(ad+a)

	++	ad+	+me	adme	Total
1					0
2	29	32	18	15	94
3	6	2		2	10
4	1			1	2
5					0
6		1			1
7					0
Total	36	35	18	18	107

Number of ascospores (out of 150) germinating on minimal media supplemented with optimal adenine and methionine on a given date after activation.

The overall germination, 71%, was considerably superior to that observed previously on complete media, 44%, and was 88% complete by the second day after activation and 97% complete by the third day. The ratio of the genotypes ++:ad+ :+me :adme was 36:35:18:18, still showing a marked deficiency of methionineless progeny of about two to one.

This deficiency was striking in view of the excellent germination on complete media of methionineless progeny from a cross of 428A and 77a. Of sixty randomly isolated ascospores 32(++) and 25(+me) germinated, providing no evidence of poor germination of the (+me) genotype.

Sixty ascospores were also isolated at random on complete media from a cross of 19a and 74A. The observed distribution was 26(++) and 24(ad+), about as expected.

To obtain more information about the germination of methionineless, other (+me) x (ad+) crosses were made at random from among the progeny of V-41a x 34A. In fact, the 103:101:51:48 segregation of ++:ad+:+me:adme observed in the two isolations from cross 2 (428A x 19a) and the 57:58:18:19 ratio observed in cross 1 (394a x 499A) prompted the consideration of an hypothesis that a factor, say G, differing in the parents, does not permit germination when associated with (me). That is, if 34A were genotypically constituted (ad+G) and V-41a (+meg), we would expect a 2:2:1:1 ratio of ++:ad+:+me :adme assuming non-

germination of the (+meG) and (admeG) genotypes. Thus, selecting (+me) and (ad+) genotypes for parents among the V-41a x 34A progeny at random should result in two types of crosses in equal proportions : (+meg x ad+G) and (+meg x ad+g). The observed genotypic frequencies of seven such random crosses are tabulated in Table 4, each with a X^2 goodness of fit value to both a 2:2:1:1 and to a 1:1:1:1 ratio. For convenience, the germination results of the parent cross, V-41a x 34A, and the two crosses previously considered are included.

Table 4. Genotypic frequencies of parental and random progeny crosses.

		Genotype					X^2	
	Cross	++	ad+	+me	adme	Total	2:2:1:1	1:1:1:1
	V-41a(+me)x 34A(ad+)	155	159	71	72	457/1050	.92	64.06
	394(+mea) x 499(ad+A)	57	58	18	19	152/450	5.55	40.05
	428(+meA) x 19(ad+a)	103	101	51	48	303/592	.17	36.47
1	193(+meA) x 218(ad+a)	41	30	31	31	133/150	11.92	2.43
2	250(+meA) x 260(ad+a)	31	36	20	22	109/150	1.78	6.27
3	309(+mea) x 314(ad+A)	29	26	33	33	121/150	24.60	1.15
4	325(+meA) x 332(ad+a)	38	32	33	28	131/150	11.31	1.55
5	339(+meA) x 351(ad+a)	27	20	11	13	71/150	1.21	8.94
6	344(+meA) x 360(ad+a)	37	25	15	23	100/134	5.06	9.92
7	380(+meA) x 411(ad+a)	34	33	28	21	116/150	5.43	3.66
	Total	237	202	171	171	781/1034	40.77	15.18

If the seven crosses are classified on the basis of the best goodness of fit to the two hypothetical ratios considered, 1,3,4 and 7 may be classified as 1:1:1:1 and 2,5 and 6 as 2:2:1:1. With the exception of 6 and 7 which are somewhat arbitrary, the crosses give excellent fits to one of the two hypothesized ratios in expected proportions and provide evidence that germination of (me) may depend, at least in part, upon a segregating genetic factor in the population. Certainly, the X^2 values on the totals do not indicate conformity to either a 2:2:1:1 or a 1:1:1:1 ratio whereas the X^2 test for goodness of fit to a 3:3:2:2 ratio on the totals gives a value of 7.29 which is below the tabulated value of $X^2_{3,.05}=7.81$. This is the expected ratio if half of the random crosses segregate according to a 1:1:1:1 ratio and the other half segregate according to a 2:2:1:1 ratio.

Choosing to ignore the preferential survival of (+) to (me), 428(+meA) and 19(ad+a) were selected as genotypic sources, and the performance of each at different dilutions of methionine and adenine respectively was now considered in some detail in order to determine appropriate experimental levels of the two supplements.

Twelve different dilution levels of methionine were used for 428A. The highest concentration, designated 1 in Figure 2, contains 10^{-4} gram of dl-methionine per cc. of minimal media. In these experiments minimal media consists of Fries solution, 1.5% sucrose and 3% Difco-Bacto Agar. Each succeeding level of methionine, except the last which is a zero control containing no methionine supplement, is a dilution by half of the concentration of the preceding level.

The twelve dilution levels of adenine used for 19a were similarly constructed. The highest concentration of adenine, also designated level 1, contains 1.497×10^{-4} gram of adenine sulfate or 10^{-4} gram of adenine per cc. of minimal media.

Four growth tubes of each parent at each dilution level were incubated at 25°C. Two different sections of one incubator were used as replicates, and two tubes of each parent at each dilution level were randomized within a replicate. After a twenty-four hour lag period to overcome as much as possible the initial differences in growth due to unequal size of innoculum, the length of mycelial advance in millimeters was recorded in five successive twelve hour periods. The means for each parent at each dilution level, each based on a total of sixty hours of growth, are presented in Figure 2.

In addition to the graph of the means, analyses of variance for the two parents based on the experimental design employed are presented in Table 5.

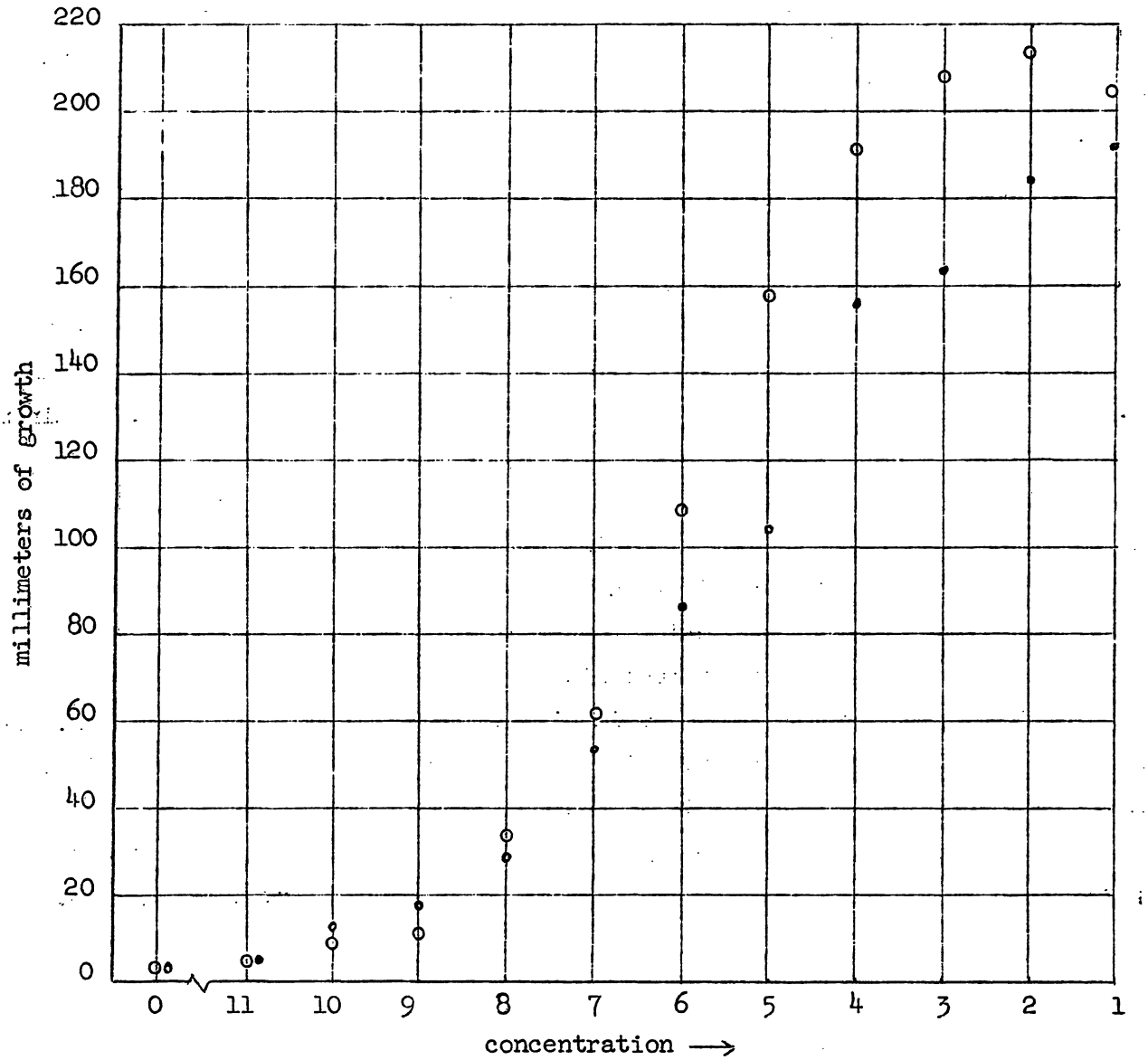


Figure 2. Average linear growth in millimeters for a sixty hour period at successive dilutions of adenine for 19(ad+a)(•) and methionine for 428(+meA)(°).

Table 5. Analyses of variance on the data for the two parents, 428(+meA) and 19(ad+a).

428(+meA)

Source	df	SS	MS	F
Replicates=R	1	2.40	2.40	< 1.00
Dilution levels=D	11	70,796.90	6,436.08	202:20 **
DxR	11	350.10	31.83	
Periods=P	4	816.52		
Linear=P_L	1	785.41	785.41	280.50 **
Residual	3	31.11	11.13	3.98
PxR	4	11.18	2.80	
DxP	44	945.68		
DxP_L	11	842.34	76.58	45.05 **
Residual	33	103.34	3.13	1.84 *
DxPxR	44	74.82	1.70	
Error	120	454.00	3.78	
Total	239	73,451.60		

19(ad+a)

Replicates=R	1	26.00	26.00	1.24
Dilution levels=D	11	48,029.05	4,366.28	208.81 **
DxR	11	230.05	20.91	
Periods=P	4	880.73		
Linear=P_L	1	869.41	869.41	269.17 **
Residual	3	11.32	3.77	1.17
PxR	4	12.93	3.23	
DxP	44	1,187.27		
DxP_L	11	1052.14	95.65	39.36 **
Residual	33	135.13	4.09	1.68
DxPxR	44	106.77	2.43	
Error	120	516.50	4.30	
Total	239	50,989.30		

The pattern of variation over the different sources is consistent for the two parents. Those sources which are important for 428A are also important and of approximately the same magnitude for 19a, and those sources which are negligible for one are found to be negligible for the other.

For both experiments the average mean square for replicates ($\frac{2.40+26.00}{2} = 14.20$) is only about one-half of the average replicates x dilution mean square ($\frac{31.81+20.91}{2} = 26.36$), indicating that the incubator provided a reasonably uniform environment for the measurement of growth. This result was substantiated in other experiments where levels of the incubator were used as replicates. This was considered important, since if the temperature were not uniform over different areas of the incubator, large differences in growth could be expected from this source.

As expected, differences among the twelve dilution levels accounted for most (approximately 95%) of the total variation. These differences resulted from varying the amount of the adenine and methionine supplements from a concentration permitting optimum growth to a concentration which permitted scarcely any growth at all.

An important source of variation for both parents is the linear regression comparison for periods. This comparison is found to account for nearly all of the variation among the five, twelve hour periods; i.e., the deviations from linear regression are approximately equal to error. Hence, there is an increasing growth rate with time as the mycelium advances down the tube, and this increase appears to be constant.

A study of the interaction of the linear effect of periods with dilution levels (DxP_L) reveals that this increase in growth rate is not homogeneous over the different dilution levels. In particular, while there is a fairly constant growth rate for the lower dilutions over periods, the greater the concentration of the supplement, the greater the constant increase in growth rate appears to be. This result (DxP_L), similar in both parents, accounts for most of the interaction between dilution levels and periods (DxP).

The segregating progeny from the cross 428(+meA) and 19(ad+a) were now measured for linear growth on minimal media supplemented with 4.6875×10^{-6} gram of methionine and 1.4034×10^{-5} gram of adenine sulfate per cc. of media. These levels represent 5.5 and 4.5 respectively on the dilution axis of Figure 2 and determine approximately that part of the curve for which the rate of change in growth with respect to supplement is greatest. In the dilution experiments, 428(+meA) was grown on differing levels of methionine, independent of adenine, and 19(ad+a) was grown on differing levels of adenine, independent

of methionine. One of the purposes of testing the progeny of the 428A x 19a cross was to observe the performance of (ad) and (me) on media supplemented with a specific level of both adenine and methionine.

Growth was measured in specially constructed growth tubes approximately one cm. in diameter, open and bent upwards on the ends. When filled with ten cc. of media, they present a surface about one cm. wide and thirty cm. long. Conidia from each individual isolate were transferred to one end of a growth tube, and after a 24 to 48 hour lag period, until the mycelium was established and advancing down the tube, growth was measured for a sixty hour period at 25°C. Measurements were made at twelve hour intervals during this period. The varying lag period was allowed because differences in genotype and unequal size of the initial innoculum greatly affect the first few centimeters of growth. In general the (++) progeny establish a characteristic growth rate more quickly than (ad+) and (+me), and the (adme) genotype was typically the slowest.

Twenty-four replicates of each parent, 428A and 19a, were included at random with the progeny in a completely randomized design. The frequency distributions of the parents and progeny are presented (Figure 3) to show the variation within genotypes at the supplemental levels of adenine and methionine used.

Figure 3. Frequency distributions of growth (mm) for parents and progeny.

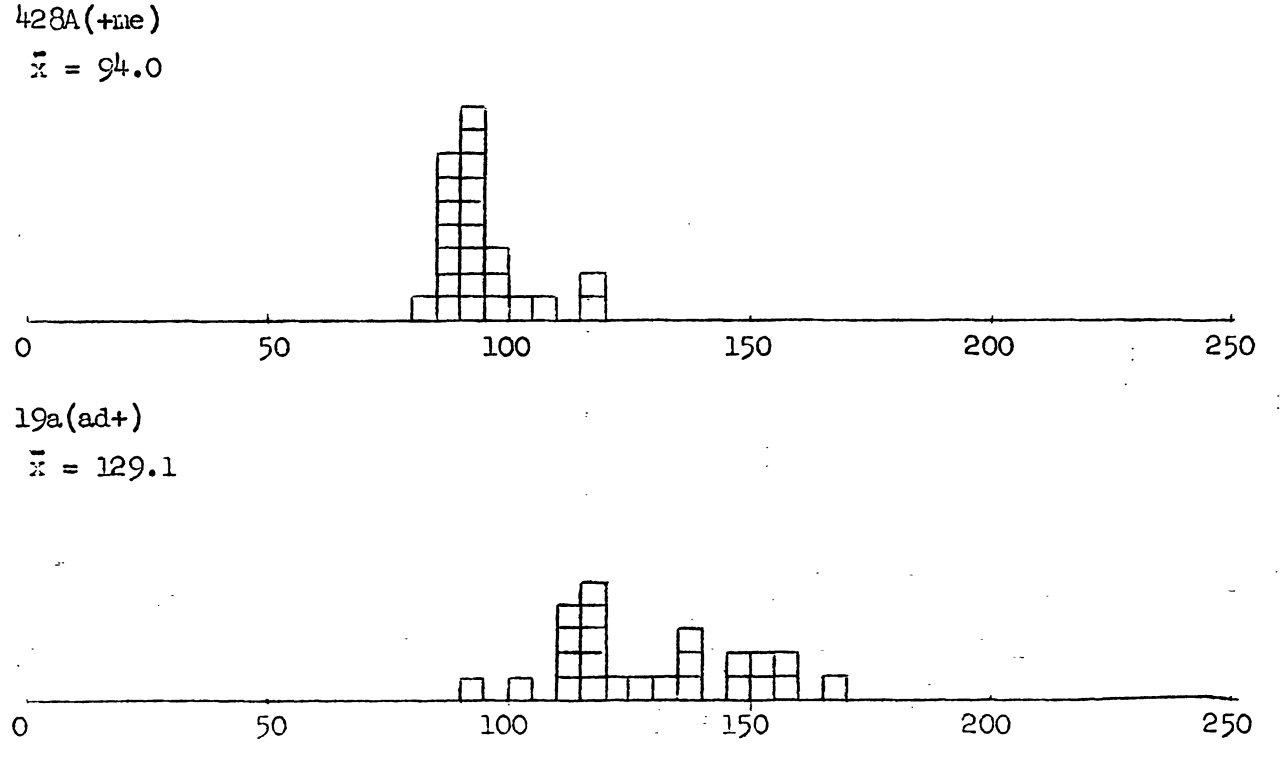
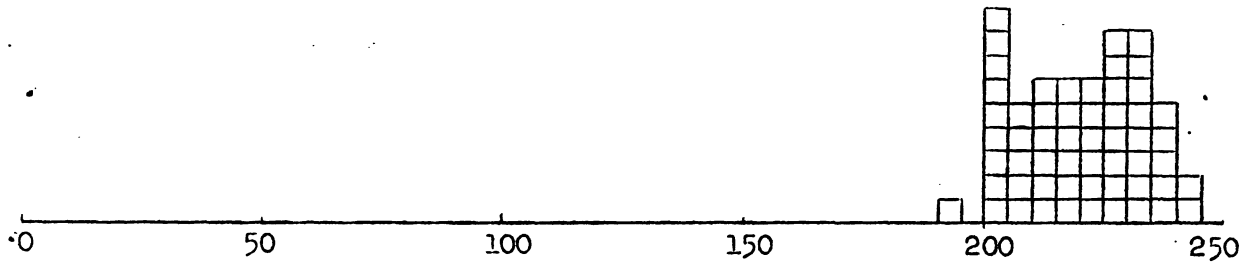


Figure 3 (continued)

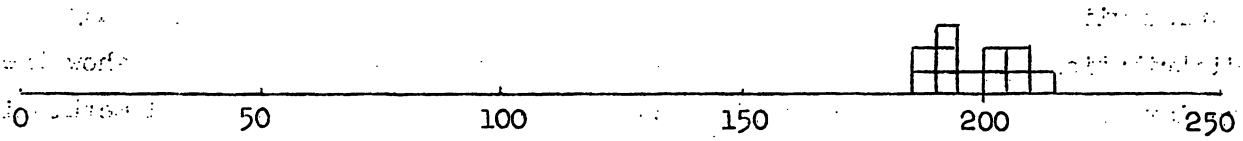
++A

$\bar{x}$ = 219.4



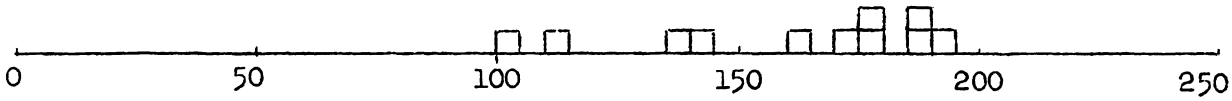
++a

$\bar{x}$ = 198.4



ad+A

$\bar{x}$ = 153.4



ad+a

$\bar{x}$ = 120.5

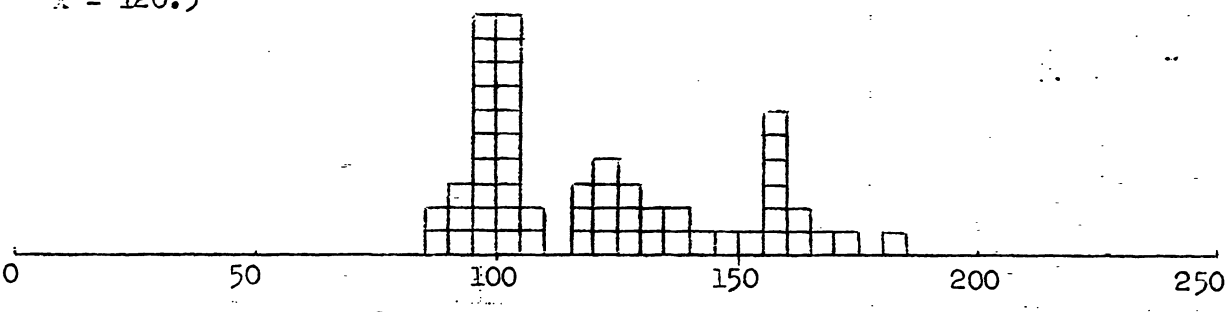
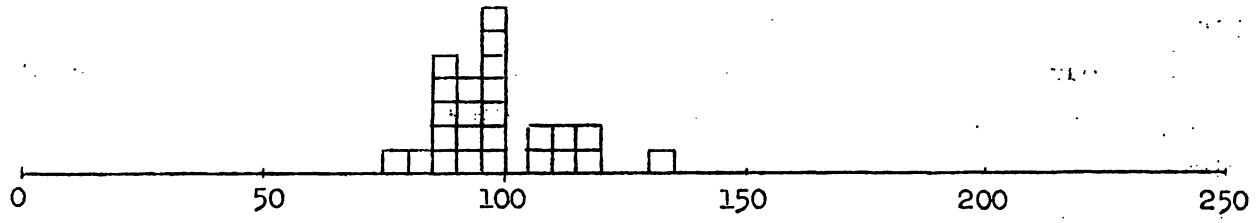


Figure 3 (continued)

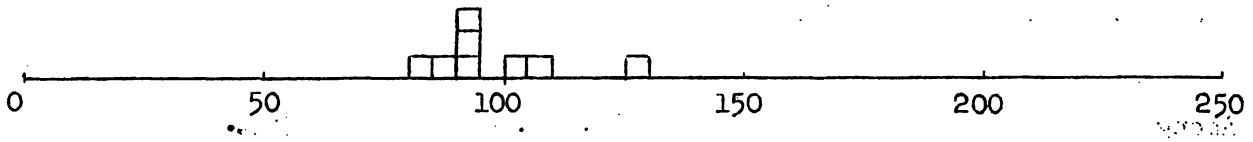
+meA

$\bar{x}$ = 97.2



+mea

$\bar{x}$ = 98.9

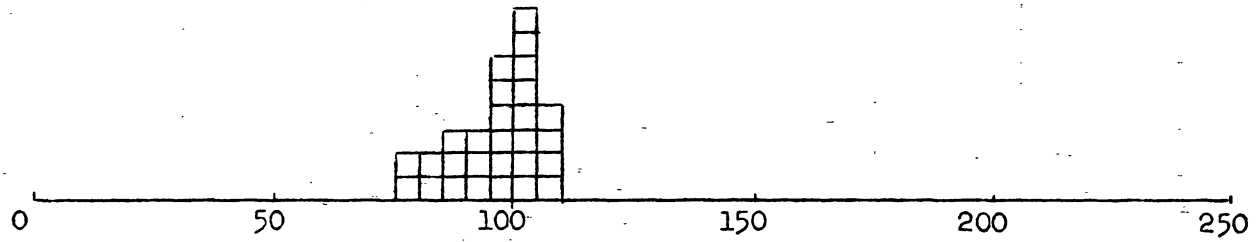


admeA

$\bar{x}$ = 93.3

admea

$\bar{x}$ = 96.4



Differences among the genotypic means, ignoring mating type, were expected since the supplemental levels of adenine and methionine were chosen to differentiate between (ad) and (me). Growth of (adme) appeared to be controlled by the more severe of the two limiting supplements, methionine. This effect was independent of the allele at the adenine locus since both (+me) and (adme) performed similarly to the methionineless parent 428A.

Although an effect due to mating type cannot be detected in the (+me) or (adme) progenies, (++A) has a higher mean growth than (++a), and (ad+A) has a higher mean growth than (ad+a). An analysis of variance was performed to test this interaction; that is, to see if the four genotypes ++,ad+,+me,adme retain the same order and magnitude of performance for A as for a. Since disproportionate subclass numbers were obtained due to linkage of adenineless with mating type and due also to the preferential survival of normal to methionineless, the method of fitting constants was used to obtain an unbiased estimate and test of the interaction.

Table 6. Analysis of variance on gentoypes and mating types.

Source	df	SS	MS	F
Among genotypes	7	520,678.38	74,382.62	
++,ad+,+me,adme	3			
A,a	1			
Interaction	3	7,198.50	2,399.50	7.19 **
Within genotypes	188	62,730.33	333.67	
++A	55	8,667.12	157.58	
++a	10	812.55	81.26	
+meA	24	3,670.56	152.94	
+mea	7	1,248.87	178.41	
ad+A	10	9,552.55	955.25	
ad+a	54	36,803.71	681.55	
admeA	2	.67	.33	
admea	26	1,974.30	75.93	

Thus, there does appear to be an effect associated with mating type A and the normal allele of methionineless, independent of the adenine locus, which is not manifested with methionineless. This effect is different from that of the growth factor noted previously in the progeny of the cross 41(+mea) x 34(ad+A) which was independent of mating type. In fact, 428(+meA) and 19(ad+a) were selected from the progeny of that cross, hoping they would both carry the "normal" allele for the factor which differed in 41a and 34A. An examination of the frequency distributions for both crosses shows that this selection was successful.

Table 7 shows the difference in the change of growth rate over time for 428A when grown on minimal media supplemented with both methionine and adenine, as compared to minimal media supplemented with methionine alone.

Table 7. Mean linear growth of 428(+meA) in mm. for successive twelve hour intervals at differing concentrations of methionine and adenine.

Reps.	adenine sulfate gm./cc.	dl-methionine gm./cc.	Interval 1	2	3	4	5	6	7
4	0	1.0000×10^{-4} (1)*	34.00	37.75	41.25	45.50	46.50		
4	0	6.2500×10^{-6} (5)	28.25	30.00	33.00	34.00	31.50		
24	1.4034×10^{-5} (4.5)	4.6875×10^{-6} (5.5)	26.25	22.12	19.29	19.46	18.42	18.08	17.71
4	0	3.1250×10^{-6} (6)	20.00	21.50	21.25	22.75	22.00		

Thus, on a media containing methionine alone, 428A has a constant or increasing growth rate. On the media containing both adenine sulfate (1.4034×10^{-5} gm./cc.) and methionine (4.6875×10^{-6} gm./cc.), 428A has a constantly decreasing growth rate.

For 19a, Table 8 indicates that an increasing growth rate is typical for this parent, whether grown on minimal media supplemented with both adenine and methionine or grown on minimal media supplemented with adenine alone.

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* The number in parentheses corresponds to the concentration level designated in Figure 2.

Table 8. Mean linear growth of 19(ad+a) in mm. for successive twelve hour intervals at differing concentrations of methionine and adenine.

Reps.	adenine sulfate gm./cc.	dl-methionine gm./cc.	Interval 1	2	3	4	5	6	7
4	1.4970×10^{-4} (1)	0	32.00	36.00	40.25	41.75	42.75		
4	1.8712×10^{-5} (4)	0	23.50	26.25	31.00	36.25	39.75		
24	1.4034×10^{-5} (4.5)	4.6875×10^{-6} (5.5)	17.78	20.91	23.75	24.79	24.54	27.08	28.35
4	9.3562×10^{-6} (5)	0	19.50	22.00	19.00	22.00	21.75		

For purposes of taking the actual measurements on the segregating progeny in the different generations, it is desirable to maintain as constant an environment as possible within each generation as well as from one generation to another. A single incubator maintained at 25°C. was used for making all of the measurements, but the size of all generations beyond the F_1 made it necessary to make several runs over a period of days and even weeks to measure all of the segregating progeny.

It was shown (Table 5) that there is little variation among different levels or sections within an incubator for a sixty hour period. Since it appeared possible to maintain the temperature very uniformly at 25°C. over long periods of time, it was felt that there would be little variation from run to run, even though they might be weeks or months apart. An opportunity to test this assumption was afforded by the parental controls which were randomly included with the segregating progeny in each generation. A table of means and an analysis of variance for each parent are presented below to show the variation among and within different runs over a period of approximately three weeks.

Table 9. Growth in mm. for a sixty hour period on minimal media supplemented with 1.4034×10^{-5} gm./cc. of adenine sulfate and 4.6875×10^{-5} gm./cc. of dl-methionine.

Means		18 June	26 June	3 July	4 July	4 July	5 July
428A	no.	9	9	4	3	4	8
	$\bar{x}$	157.7	170.2	101.7	128.0	126.0	124.1
19a	no.	10	9	3	4	4	7
	$\bar{x}$	179.5	196.8	164.7	187.7	178.5	174.9

Table 9. (continued)

Analysis of variance for 428A.

Source	df	MS	F
Among dates	5	4,003.94	19.13 **
Within dates	31	209.26	

Analysis of variance for 19a.

Source	df	MS	F
Among dates	5	692.31	3.60 *
Within dates	31	191.91	

Thus, the variation from date to date did appear to be significant when compared to the variation among controls within a date. Two contributing factors suggest themselves; differences in media from date to date and differences in temperature over time.

The media was prepared separately for each run, and although considerable effort was spent in perfecting the technique of identical preparation, particularly the specified amount of adenine sulfate and methionine, it is possible that this source of error resulted in greater growth differences than expected. It is also possible that the temperature inside the incubator was subject to greater fluctuation than the periodic checks revealed, or that greater differences in growth may result from smaller changes in temperature than previously thought. In any event, elimination of these differences to provide as constant an environment as possible over time would be very desirable.

In summary, two different breeding systems were initiated by the cross of the two haploid parents 428(+meA) and 19(ad+a). In one system, random mating among full sibs, family structures are built up in succeeding generations by mating progeny at random only within the individual crosses from the preceding generation. In the other system, recurrent backcrossing to both parents, individuals at random within each backcross from the preceding generation are backcrossed to both original parents in each generation.

Techniques.

Difco-bacto corn meal agar (17 gm./liter H_2O) was used as a crossing media and generally gave excellent results for crosses involving most of the different genotypes. Crosses were made by streaking conidia of the two parents involved side by side on an agar slant. If, however, both parents carried the gene for adenineless, corn meal agar supplemented with adenine sulfate (.5 gm./liter H_2O) and a base (35 cc. 1M NaOH/liter H_2O) proved much more successful. In fact, this was the only media upon which crosses of the double mutant (admeA x admea) were observed to produce viable ascospores. The addition of the base with the adenine sulfate gave a resulting p_H of approximately 6.8 to the crossing media. This was necessary since the supplementation of adenine sulfate alone lowered the p_H to a level which did not permit satisfactory crossing.

Ascospores from each cross were isolated individually into small culture tubes with the aid of a dissecting microscope. In most crosses, after a lapse of time, ascospores lie in profusion on the side of the test tube, blown there presumably at random from many perithecia. If ascospores were not found on the glass, ripe perithecia were selected at random and opened.

In practice, the actual selection of individual ascospores is based to some extent upon spore size, shape and color. Plump, dark ascospores are generally considered to be more viable than shrivelled, immature green ones, the assumption being that these characteristics are independent of genotype. This assumption is, in all probability, not completely true. To help eliminate the possibility that preferential selection is made for some genotypes because one shape or color is easier to isolate or considered more viable than another, exhaustive individual isolation is performed on large numbers of ascospores which are selected as a unit as much at random as possible.

The isolating media used for all ascospores is composed of minimal Fries solution supplemented with 1.5% sucrose, 3.0% agar, 7×10^{-5} gram of adenine sulfate per cc. and 5×10^{-5} gram of dl-methionine per cc. of media. This media proved superior to complete media, both in the length of time required to complete germination and in the percentage of overall germination (Tables 1,2,3).

As soon as possible after isolation, the ascospores were activated in a hot water bath at approximately 60°C. for thirty minutes. Germination was virtually complete by the fourth or fifth day after activation.

One advantage of an asexual organism like N. crassa is that it is possible to take several isolates from each individual to use for separate tests or making crosses and still maintain the individual without subjecting it to unusual environmental or selective pressure. If it becomes necessary to transfer the individual to another culture tube as the agar dries out and the nutrients are depleted, this also is readily accomplished.

Each individual in each generation for which a growth measurement was made was identified and labelled for mating type and genotype. To test for mating type, conidia of each individual were applied to protoperithecia of 74A, a wild type, growing on corn meal agar slants in small culture tubes. These tubes were prepared by innoculating each one with a spore suspension of 74A from a 1 cc. sterile pipette. Protoperithecia suitable for testing were obtained in eight to ten days after innoculation when incubated at 25°C. If fertile perthecia developed after four days upon exposure to the conidia of unknown mating type, the individual was classified as mating type (a); if not, then mating type (A). This test proved quite definitive and easy to apply.

Classification as to gentoype (++,ad+,+me,adme) was facilitated in the respect that progeny which carry the gene for adenineless (ad) are purple in color and can be easily distinguished from progeny carrying the normal allele for adenineless (+) which are orange.

To further classify an individual as to methionineless (me) versus its normal allele (+), conidiaspores from orange progeny were applied to approximately one cc. of liquid minimal Fries solution supplemented with 1.5% sucrose in a small culture tube. Methionineless progeny (+me) are unable to grow on this media while individuals carrying the normal allele for methionineless (++) grow quite rapidly, permitting classification after two or three days of incubation at 25°C. In a similar way conidiaspores from purple progeny were applied to liquid minimal Fries solution supplemented with 1.5% sucrose and 7×10^{-5} gm./cc. of adenine sulfate. Methionineless individuals (adme) are unable to grow and can be readily distinguished from the (ad+) individuals.

Linear growth was measured in the specially constructed growth tubes discussed previously which when filled with ten cc. of minimal Fries solution, 1.5% sucrose, 3.0% agar, 4.6375×10^{-6} gm./cc. of dl-methionine and 1.4034×10^{-5} gm./cc. of adenine sulfate, permit growth of the different genotypes at different rates (Figure 3).

Discussion.

It is evident from the various tests involving the two parents used in the experiment and their progeny that there exist some important factors which will affect the simple two gene, two allele model proposed. The artificial experimental control of growth by limiting the supply of exogenous nutrients necessary for growth, appears quite reasonable if the experimental conditions can be duplicated over the period of time necessary to measure all of the generations. There are some indications (Table 9) that this aspect of technique can be improved.

The preferential survival of individuals carrying the normal allele of methioneless to methionineless makes it necessary to supplement deficiencies of the (+me) and (adme) genotypes by selection. Such deficiencies would occur if progeny were selected completely at random without regard for expected segregation ratios.

The effect of mating type, or a factor associated with it (Table 6), complicates the two gene model proposed to the extent of the effect of this factor upon growth. However, it appears that this effect is small and perhaps negligible.

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