The Design of an Experiment for Studying Quantitative Inheritance in Neurospora

BU-90-M Keith H. Thompson May, 1958

Introduction

Isogenic strains of Neurospora crassa, differing in two unlinked loci, form the experimental basis for a study in quantitative inheritance. Two breeding systems, random mating among full sibs obtained from the cross of two haploid parents and recurrent backcrossing to each of the two parents, are described and are used to empirically test the validity of two genetic models given by Robson (1956a, 1956b).

Such a technique, that of fitting an experiment to a genetic model, particularly the simpler genetic models, may be expected to provide significant information as to the present adequacy of interpretations of quantitative inheritance. The use of different breeding systems will increase the precision and amount of information obtained in such a study.

Materials and Methods

Two biochemical mutants of Neurospora crassa were selected as parents for both breeding systems. An adenine-requiring parent, 19a, and a methionine-requiring parent, 428A, were selected from the progeny of a cross between 34A, adenine-requiring, and 41a, methionine requiring. 34A was a selection from the cross of PPL-30a, an adenineless strain which had proven consistent in previous genetic studies and 74A, a standard wild stock. The methionineless strain, 41a, was also isolated as a mutant and had been tested in previous genetic studies.

It was discovered that the progeny of 34A (ad+A) x 41a (+mea) segregated independently for the adenine locus (ad) and the methionine locus (me), but that ad was linked with mating type. In addition, there was independent segregation for a fourth factor which affected growth by a factor of two at

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the limiting adenine and methionine levels used in the trial. The factor apparently affected all the genotypes equally. The parents, 19a and 428A, were homozygous for this growth factor and were selected partly on this basis.

The quantitative variable under measurement was the length of mycelial advance of a single ascospore down a specially constructed growth tube at a constant temperature. The amount of adenine and methionine added to the minimal media in the growth tube was determined by the performance of the parents 19a and 428A over varying concentrations of adenine sulfate and methionine. Growth of 19a (ad+a), when plotted as mycelial advance per hour against the logarithm of the concentration of the supplement, gave a typical S-shaped growth curve, approaching zero at the lower concentrations and approaching an optimal growth level comparable to that of 74A, a wild strain, at the higher concentrations.

Similarly, 428A (+meA) made no growth unless methionine was added to the media and gave a similar growth curve although even under optimal growth conditions the rate of the wild strain, 74A, was never reached.

Thus, in measuring the growth of progeny in the developing generations, limiting adenine and methionine were added to Fries minimal media in an amount calculated to slightly overlap the tails of the distributions of the four genotypes, (++), (ad+), (+me), and (adme). Environmental variation was, of course, present among progeny of assumed identical genotype.

Special crossing media was necessary to secure all the possible crosses that would be expected randomly, based on the breeding system employed. Ordinary corn meal agar gave good results for most crosses, but if both parents carried the adenineless gene, then corn meal agar supplemented with adenine sulfate was far more successful.

A medium made with minimal Fries, supplemented with sugar, agar, and optimal methionine and adenine sulfate, was found to be generally satisfactory as a germinating medium for the ascospore progeny. Germination of all genotypes was more or less uniform regarding time and was complete in about 72 hours from activation of the ascospores. It was interesting to note that if complete media were used, i.e. minimal media supplemented with yeast and malt extracts, germination took up to fourteen days to complete with (adme) progeny

germinating near the end of the period.

In a few cases, however, severe germination difficulties developed with isolates of certain crosses, notably (admea) x (admeA).

Single ascospore isolates were allowed to grow in individual tubes. This technique then permits the experimenter to use part of the resultant mycelial growth for genotype testing, part for innoculating growth tubes for measurements, part for crossing for the next generation, and yet still maintain the haploid individual without subjecting the parent tube to unusual environmental or selective pressures. In this way the progeny were completely identified for genotype with respect to ad, me, and mating type, on the basis of fairly simple transfer tests.

Random Mating System

Robson (1956a) describes the genetic model based on the restricted random mating among full sibs, which yields estimates of all possible epistatic components of variance.

The breeding system used to verify the unlinked, two-gene special case of this model starts with two parents, each carrying one of the two genes affecting the quantitative variable under consideration. From this cross we get an F_1 population consisting of equal numbers of four genotypes.

P_0	1/2(ad+)		1/2(+me)	
F_1	1/4(++)	1/4(ad+)	1/4(+me)	1/4(adme)

Random mating among the F_1 population produces ten different crosses: (++) x (++), (ad+) x (ad+), (+me) x (+me), and (adme) x (adme) with probability 1/16, and (++) x (ad+), (++) x (+me), (++) x (adme), (ad+) x (+me), (ad+) x (adme), and (+me) x (adme) with probability 1/8.

In the experiment, individuals were selected at random from the F_1 population and crossed until the theoretical frequencies were exactly obtained. The ten different crosses of this type produce nine distinct F_2 "families", since the crosses (++) x (adme) and (ad+) x (+me) both generate the F_1 distribution.

F_2 $1/16(++)$ $1/8\{1/2(++)+1/2(ad+)\}$ $1/8\{1/2(++)+1/2(+me)\}$

$1/8\{F_1\}$ $1/16(ad+)$ $1/8\{F_1\}$

$1/8\{1/2(ad+)+1/2(adme)\}$ $1/16(+me)$

$1/8\{1/2(+me)+1/2(adme)\}$ $1/16(adme)$

It is to be noted that the genotypic frequencies are equal in the F_2 to that of the F_1 generation. Indeed, they will remain equal in all succeeding generations. Among the F_2 full sibs, random mating will again produce only nine genetically distinct F_3 families, and here, as before, the progeny of each cross are mated among themselves at random to produce the F_3 generation. In the experiment, random selection of individuals for crossing was made according to the theoretical expectation.

F_3 $1/16(++)$ $1/16(ad+)$ $1/16(+me)$ $1/16(adme)$

$1/8\{1/4(++)+1/2[1/2(++)+1/2(ad+)]+1/4(ad+)\}$

$1/8\{1/4(++)+1/2[1/2(++)+1/2(+me)]+1/4(+me)\}$

$1/8\{1/4(ad+)+1/2[1/2(ad+)+1/2(adme)]+1/4(adme)\}$

$1/8\{1/4(+me)+1/2[1/2(+me)+1/2(adme)]+1/4(adme)\}$

$1/4\{F_2\}$

Twelve individuals were tested from each specific cross, although where different genotypes were expected, the twelve crosses were made up to theoretical expectation regarding genotypic proportions which included mating type. At least two of each of the nine genetically distinct families were tested in each generation in order to estimate all of the possible genetic components of variance.

In this way the breeding system may be carried to the F_k generation although the number of progeny to be isolated, tested, and crossed soon reaches unwieldy proportions.

Backcross System

Using the same two parents, an F_1 population is obtained of equal proportions of the genotypes (++), (ad+), (+me), and (adme) as before, by crossing the adenineless and methionineless strains. If now, crossing occurs at random between individuals of the F_1 population and the two parents, we will have eight different back-crosses, four with each parent, occurring with equal probability. These are $(++)_{F_1}$ x (ad+), $(ad+)_{F_1}$ x (ad+), $(+me)_{F_1}$ x (ad+), $(adme)_{F_1}$ x (ad+), $(++)_{F_1}$ x (+me), $(ad+)_{F_1}$ x (+me), $(+me)_{F_1}$ x (+me), and $(adme)_{F_1}$ x (+me). Genetically there are only seven distinct B_1 families since $(+me)_{F_1}$ x (ad+) and $(ad+)_{F_1}$ x (+me) give the same genotypic distribution.

Twelve individuals were again saved from each cross at random in accordance to theoretical expectation, and no fewer than two of each genetically distinct cross was made to insure adequate estimation of genetic variability.

To obtain B_2 and later backcross generations, individuals were randomly selected from B_1 x P_0 crosses and backcrossed again to P_0 in the appropriate frequencies. The number of individuals carried in each generation by such a breeding system also becomes very large after the first few generations. Another property that both the random mating system and the backcross system impose upon the experimental material is that the frequency of the genotypes remains unaltered from the F_1 through the subsequent generations.

It will be noted that both breeding procedures make use of the genotypic information available from the progeny in selecting individuals to be tested and in insuring that expected random frequencies are adhered to. It was hoped to compare an actual random experiment with a theoretically restricted one, but the nature of the experimental material made this impractical.

The fact that adenine is linked with mating type (approximately 16% crossing over occurs between these two loci) automatically restricts random crossing, since all genotypes do not occur with equal frequency in the F_1 and

hence later generations will show an altered genotypic structure. In addition, differential germination of the genotypes may have the same effect, and this phenomenon is noted to a severe degree in certain (admea) x (admeA) crosses in later generations.

Although the two genes involved, adenineless and methioneless, were selected to test as simple a model as possible, in the course of the experiment a great many modifying effects were observed. One already mentioned was the detection and elimination of a single gene factor which affected growth by a factor of two.

What appeared to be a single gene factor affecting the differential germination of methionine mutants of unlike mating type in certain crosses but not in others, was also discovered and would, of course, upset genotypic frequencies if purely random mating were allowed.

Although data is, at present, only available up to the F_3 and B_3 generations, it now appears that mating type A versus a may have an independent effect on growth as defined for the purpose of these experiments. The effect of mating type cannot be eliminated from the experiment, and may have to be incorporated into it.

References

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