A Genetic Basis for the Occurrence of Minus Mutants in Glomerella.

Harry Ernest Wheeler

Louisiana State University and Agricultural & Mechanical College

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A GENETIC BASIS FOR THE OCCURRENCE OF MINUS MUTANTS IN GLOMERELLA

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Botany, Bacteriology, and Plant Pathology

by

Harry Ernest Wheeler
B.S., University of Vermont, 1941
M.S., Louisiana State University, 1947
June, 1949
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Previous studies of a Gnomarella isolated from Ipomoea have indicated that the variants which occur in the ascospore progeny of this fungus arise as the result of single gene mutations. Wild-type cultures of this fungus, called "plus A", produce fertile perithecia in large glomerate masses. Single ascospore transfers from wild-type cultures give rise to large numbers of mutants of a type called "minus" which produce scattered perithecia. Cultures very similar to the wild-type, called "plus B", produce few minus mutants. The present investigation has been chiefly concerned with the determination of the genetic difference between plus A and plus B cultures, since this information seemed vital in relation to the problem of the nature of variation in this fungus. This investigation has also included the identification of certain genes, and preliminary studies of the physiological effects of various genes.

The results of appropriate crosses demonstrated that plus A and plus B cultures of Gnomarella differ genetically by a factor which controls the production of minus type mutants. This factor is inherited as a single gene according to simple Mendelian principles and is independent of the locus at which gene changes from plus to minus occur. The presence of this factor in plus cultures results in the production of a few very large clumps of perithecia, which, on the basis of limited data, contain most of the minus mutants which are produced. It has no phenotypic effect in other types of cultures. The results of attempts
to induce mutations in plus B cultures through the use of extracts from plus A cultures were inconclusive.

Analysis of a mutant gene causing abnormal development of fruiting structures indicated that it was inherited independently of the two previously known loci. In plus type cultures this gene usually completely suppressed the development of perithecia. Ascospores isolated from perithecia formed by mating such abnormal plus cultures with normal conidial B cultures gave rise to both normal and abnormal plus, minus, conidial A, and conidial B cultures. The results of other crosses in which the parents differed by three pairs of factors indicated that no brachymorphic division occurs in this fungus.

Plus and minus cultures, which produce perithecia but few or no conidia on oatmeal or bean agar, produced appreciable numbers of conidia when grown on nutrient agar. Conidial production by plus, minus, and conidial A cultures was reduced by more than 90 per cent when they were grown on nutrient agar stalled by plus cultures. Conidial production in the same cultures was increased on the same medium stalled by conidial cultures, but perithecial formation in plus and minus cultures was retarded. Sterile extracts from plus B cultures which are self-fertile, added to minus A cultures which are normally almost self-sterile, resulted in a considerable increase in the number of fertile perithecia produced by the latter.
INTRODUCTION

Certain characteristics of the genus *Glomerella* seem to make this group of fungi especially well adapted for studies of gene action in relation to mutation and sexuality. Wild-type cultures of *Glomerella* are homothallic, producing self-fertile perithecia in large glomerate masses. Single ascospore transfers from wild-type cultures give rise to large numbers of variants, most of which produce nearly self-sterile, scattered perithecia. Occasionally variants of other types occur. These include homothallic, heterothallic, and sterile types and also exhibit a wide range of variation in cultural characteristics.

Extensive genetical and cytological studies of a *Glomerella* isolated from *Ipomoea* have indicated that the variants which occur in the ascospore progeny of this fungus arise as the result of single gene mutations. Most of these mutants differ from their wild-type parent both culturally and in mating reaction, but one culture has been obtained which differs from the wild-type only in that it produces very few, rather than many, mutants of the scattered perithecial type. The main objective of the present investigation has been the determination of the genetic difference between wild-type cultures which produce large numbers of scattered perithecial mutants and cultures of a similar type which produce few such mutants. This investigation has also included the identification of additional genes and preliminary studies of the physiological effects of certain genes.
HISTORICAL REVIEW

Interest in the genetics of members of the genus *Glomerella* was first aroused by the pioneer studies of Edgerton. In 1908, this investigator reported that in a culture of *Glomerella*, which normally produced fertile perithecia in glomerate masses, there suddenly appeared a dark colony with scattered perithecia which were nearly self-sterile. Edgerton concluded that this variant arose as the result of mutation. Later (1913, 1914), he found similar variants in cultures isolated from several different hosts. When these variants were mated with the normal glomerate cultures from which they arose, a dense ridge of fertile perithecia formed quickly at the line of contact. Single ascii obtained from perithecia formed by these matings were cultured and part of these gave rise to both types, i.e., the normal glomerate type and the variant scattered type. Edgerton considered these results conclusive evidence that cross fertilization occurred when the two types of cultures were mated. Following the terminology of Blakeslee (1904) he called the normal glomerate type "the plus strain" and the variant scattered type "the minus strain". Edgerton's interpretations were questioned by Shear and Wood (1913) and later Dodge (1927) stated that "plus" and "minus" cultures of *Glomerella* should be considered to be homothallic rather than heterothallic.

In 1935, Hütting, who was apparently unaware of Edgerton's work, published the results of his studies of a *Glomerella* isolated from tomato. In addition to types of cultures similar to the plus and minus strains of Edgerton, this German worker obtained several other types which were
sexually and culturally distinct. Hättig isolated single ascospores at random from plus type cultures and found that a large number of these gave rise to minus type cultures. He also made similar isolations from perithecia formed by mating various cultures and obtained recombination of characters which indicated that cross fertilization had occurred. He concluded that plus type cultures were "self-fertile hybrids" from which the other types arose through mutation and hybridization.

Several years elapsed before Hättig's results were brought to the attention of workers in this country. During this interval, Andes (1941), using an apple isolate, analysed individual asci from "light" (plus) and "dark" (minus) cultures grown separately as well as from perithecia at the line formed by mating the two types of cultures. He found that spores from asci produced by cultures of the minus type invariably gave rise to cultures of the same type. Spores from asci produced by plus cultures usually gave rise to four plus and four minus or eight minus cultures, although in a few cases eight plus cultures were obtained. Spores from asci from the line of perithecia formed by mating plus and minus cultures produced essentially the same distribution of types that was obtained from plus cultures grown separately. With one exception, all asci which were analysed from a single peritheciun produced the same distribution of types.

Andes' results raised anew the question of whether cross fertilization occurred when plus and minus cultures were mated. This issue was settled a few years later in a series of papers by Lucas, Chilton, and Edgerton (1944); Edgerton, Chilton, and Lucas (1945); and Chilton, Lucas, and Edgerton (1945). Working chiefly with a Glomerella isolated from Ipomoea, they confirmed Andes' findings and also obtained several variants.
ether than the common minus strain of Edgerton. On the basis of macro-
scopic cultural characters and mating reactions the cultures studied were
of four types which were designated as follows:

Plus - perithecia produced in glomerate masses; lines of perithecia
formed when mated with minus, conidial A, or conidial B cul-
tures.

Minus - perithecia scattered; lines of perithecia formed when mated
with plus or conidial A cultures.

Conidial A - non-perithecial, conidia produced in large masses; lines
of perithecia formed when mated with plus or minus cultures.

Conidial B - non-perithecial, conidia produced in small masses; lines
of perithecia formed when mated with plus cultures.

Wild-type plus cultures which produced many minus type variants were
termed plus A while cultures similar in appearance which did not produce
minus type variants were termed plus B. In the same way, minus cultures
which were nearly self-sterile were designated minus A while self-fertile
minus type cultures were called minus B. When plus A or plus B cultures
were mated with minus A or minus B cultures, a large number of the asci
obtained from perithecia formed at the line of contact contained spores
which gave rise to four cultures of each of the two types which were com-
bined, indicating that cross fertilizations had occurred. Matings of
minus and conidial A cultures yielded still more conclusive evidence of
cross fertilization, since asci obtained from perithecia formed by such
matings commonly contained spores which gave rise to plus, minus, conidial
A, and conidial B types in a 2:2:3:2 ratio.

Analysis of the results of these crosses indicated that genetic
factors at two loci, designated by the symbols "A" and "B", controlled the
expression of cultural characters and mating type reactions in the
cultures studied (Chilton, Lucas, and Edgerton, 1945). In unmated cul-
tures these genes had the following principal effects:

A - controlled the production of perithecia.

a - controlled the production of conidia.

B - determined the arrangement of perithecia or conidia in large
masses.

b - determined the arrangement of scattered perithecia or conidia in
small masses.

The presence of A and B in some combination was necessary in a mating
for the formation of a definite line of perithecia. A fifth gene, b₁,
either at or closely linked to the B locus, controlled self-fertility of
scattered perithecial (minus type) cultures. Chilton and Wheeler (1949)
confirmed these findings and presented evidence that the two loci were
linked. Wheeler (1947) identified a gene, designated A₁, and located
either at or near the A locus, which had various effects depending upon
which gene was present at the B locus. In the combination A₁ B, this gene
resulted in the formation of small rather than large clumps of perithecia
and such cultures produced lines of perithecia when mated with plus,
conidial A, or conidial B cultures but not with minus cultures, although
cross fertilisations occurred in all of these matings. In the combina-
tion A₁ b or A₁ b₁, this gene caused increased self-sterility and such
cultures formed lines of perithecia with plus, minus, and conidial A cul-
tures as well as with A₁ B cultures. Later Wheeler and Chilton (1945b)
presented evidence that the genes A, A₁, and b comprised a series of mul-
tiple alleles at the A locus and the genes B, b, and b₁ comprised a
similar series at the B locus. Partial analysis of another gene affecting
mating reactions indicated that it was located either at the A locus or on
another chromosome (Wheeler, 1947). Attempted crosses between cultures carrying this gene and plus or conidial A cultures were unsuccessful, although marked ridges of perithecia were formed when these cultures were mated. This cross sterility prevented the complete analysis of this gene. Wheeler (1947) also reported that matings of two non-ascigerous cultures resulted in the formation of perithecia from which spores were obtained which gave rise to both ascigerous and non-ascigerous cultures.

This brief review indicates the complex nature of the problem of sexuality in this fungus. This problem is further complicated by the results obtained from various crosses in which a plus type culture was one of the parents. From all such crosses a large number of the asci contained spores which segregated for two or more cultural types. A few of the remaining asci produced 8 plus cultures but most of them produced 8 cultures of the other parental type, even though this parent was incapable of producing perithecia by itself. It should be noted that only one of these three types of asci was obtained from any single perithegium.

The origin of the variants, especially the large numbers of those of the minus type, which have been found among the ascospore progenies of wild type (plus A) cultures constitutes perhaps the most interesting genetic problem encountered with this fungus. Edgerton (1908), Hättig (1933), and Dodge (1943) suggested that these variants arise as the result of mutations. Chilton and Wheeler (1947) concluded that mutations were responsible and presented the hypothesis that the distribution of types obtained by culturing spores from individual asci was determined by the genetic make-up of the nuclei which entered the ascogenous hyphae, paired, and later fused in the ascus. Lindegren (1948) advanced a similar theory. None of these provided an entirely satisfactory explanation for certain
phases of the problem. Among these were the extremely large numbers of
minus variants obtained from ascospores, the fact that no such variants
were obtained from conidia (Annes, 1941, Lucas 1942), and the apparent
inability of plus B cultures to produce minus variants (Edgerton, et al,
1945). The solution of these problems seemed dependent upon more precise
information concerning the chromosomal complement of this fungus and a
better knowledge of its life history.

In cytological study, Lucas (1946) found that eight ascospores, each
containing a single, haploid nucleus, were delimited in the ascus follow-
ing three nuclear divisions, the first two of which were meiotic and the
third mitotic. He reported four morphologically distinct chromosomes in
the haploid stage in material from plus B and minus B cultures grown
alone and from crosses between plus B and conidial B cultures. Wheeler,
et al. (1949), confirmed these findings and extended them to include plus
A cultures and crosses between plus B and conidial A cultures. They also
reported that the asci arose by the process of crosier formation from
binucleate cells found in young perithecia. In a single perithecium,
only one binucleate cell was observed to give rise to crosiers and asci.
Ernest (1949) reported that in minus B cultures, perithecial initials
arose as lateral branches from adjacent cells of the same hyphae. One of
these initials encircled the other which developed into the "central
coil" described by Wheeler, et al. Later studies (unpublished) have indi-
cated that this "central coil" represents an ascogonial coil.

Chilton and Wheeler (1949) pointed out that the ratios obtained from
various crosses were those expected from crosses between haploid rather
than diploid or heteroploid individuals. Wheeler and Chilton (1949)
reported that plus A cultures produced few minus variants when young
(10 - 15 days) but produced them in increasing numbers as the plus A cultures aged up to 60 days. Plus B cultures produced no minus variants until 30 days old after which a few were produced as the cultures aged. It was also found that conidial A cultures derived from plus A and plus B differed in their ability to produce conidial B variants in the same way that their plus ancestors differed in ability to produce minus variants. Unpublished data indicated that reversion to the original plus type occurred occasionally in minus cultures.

These genetic and cytological studies provided convincing evidence that ascospores from plus A cultures contain a single haploid nucleus and that the minus variants which occur when such ascospores are cultured must, therefore, arise as the result of mutations. The occurrence of reverse mutations, the evidence from the results of crosses that plus and minus cultures differ by a single gene at the B locus, the occurrence of three alleles at this locus, and the fact that no observable differences were found in chromosome numbers or morphology when material from different cultures was studied, all indicate that these mutations involve single gene changes. In a paper now being prepared for publication, Chilton and Wheeler advance the following hypothesis to account for the occurrence of minus variants among the ascospore progenies of plus cultures. Mutant nuclei of the minus genotype (A b) arise in plus cultures (genotype A A) as the result of gene changes from A to b occurring prior to the association of nuclei in pairs in the ascogonial coils of developing perithecia. In the majority of cases A B (plus) nuclei pair with A b (minus) nuclei and asci are produced from which the spores segregate in a 4:4 ratio for the two genotypes. Less frequently two A b (minus) nuclei pair and the asci produced contain 8 spores of the A b (minus) genotype. In a
few cases two A B (plus) nuclei pair and the resulting asci contain 8
spores of the A B (plus) genotype. The frequency of mutation from $B$ to
$b$ in the vegetative stage is approximately 1:1700 in 45 day old cultures
and the large numbers of mutants obtained from ascospores from plus A
cultures are, at least in part, due to differences in the ability of
nuclei of various genotypes to enter into paired associations in the
asconial coils of developing perithecia.

If, as this hypothesis assumes, minus variants arise as the result
of gene mutations, the nature of the genetic difference between plus A
cultures which produce many minus mutants and plus B cultures which pro-
duce few becomes a problem of considerable interest. Wheeler (1947)
studied 163 plus progenies of crosses between plus B and three different
conidial cultures. These differed somewhat in ability to produce minus
mutants but none approached the wild type plus A cultures in this respect.
In view of later studies (Wheeler and Chilton, 1949) which showed that
plus B cultures were capable of producing small numbers of minus mutants,
it seems probable that the differences observed among the plus progenies
studied by Wheeler were no greater than might be expected among plus B
cultures isolated at random.
MATERIALS AND METHODS

In general the methods used were the same as those previously described in detail (Lucas, 1942, Wheeler, 1947). Single ascospores were isolated by means of a Chamber's micromanipulator and cultured on oatmeal agar unless otherwise specified. Care was taken to grow all of the progeny of a single cross on the same batch of medium and all cultures were incubated at 23-35°C. Lucas' (1942) system of classifying cultures by the use of a numeral, followed by a capital letter and a second numeral to designate cross, ascus, and ascospore, was retained. Special techniques are described under the appropriate section in the text.

The cultural types studied, their genotypes, and characteristics have been described in the preceding section. Wild type genes were designated by a capital letter and their mutant alleles by a small letter or a capital letter with a sub-script. Linkage data were calculated by the use of tetrad analyses as described by Mather and Beale (1943).

Plus Progeny Tests by the Dilution Plate Method

Since the determination of the genetic difference between plus A cultures which produce many minus mutants and plus B cultures which produce few such mutants was the main objective of this study, it was necessary to develop and standardize a method for testing the plus progenies of various crosses to determine whether they were capable of producing large numbers of minus mutants. The following modification of the dilution plate method described by Lucas (1942) was used for these
tests. Ten clumps of perithecia were taken from each of the single ascospore isolates of the plus type which were found among the progenies of the various crosses. Each clump was crushed in a drop of sterile water in the bottom of a separate petri dish to liberate the ascospores. Warm agar was then added and the dish agitated to ensure an even distribution of the resulting colonies. These dilution cultures were later examined and each was given a numerical rating from 0 to 3 based on the area of the plate occupied by minus mutants (fig. 1).

![Image of dilution cultures](image)

**Fig. 1.** Illustration of the method used for rating dilution cultures made from plus isolates. Ratings from the left: 0 - no minus colonies; 1 - 1/3 or less of the area of the plate occupied by minus colonies; 2 - 1/3 to 2/3 of the area of the plate occupied by minus colonies; 3 - 2/3 or more of the area of the plate occupied by minus colonies.

The rating of each plus isolate was obtained by averaging the ratings of the ten dilution cultures made from that isolate. While this method did not yield exact data concerning the frequency of mutation, it proved to be an adequate means of separating plus A and plus B cultures.
Analysis of a Mutator Gene

Since none of the plus progeny from crosses between plus B and three different conidial cultures approached the original plus A cultures in ability to produce minus mutants (Wheeler, 1947), a new approach to the problem of the genetic difference between plus A and plus B cultures was sought. The original plus B culture (6-15) was isolated from the line of perithecia formed by a cross between minus A and conidial A cultures. Therefore, this cross was repeated, using as parents the minus A culture 59-K-4 and one of the conidial A cultures (36-0-5) which had been crossed with plus B in the studies referred to above. Spores from six asci from this cross (cross 76) were isolated and cultured. The resulting cultures segregated into four cultural types, plus, minus, conidial A, and conidial B, in the manner previously reported (Chilton, et al., 1945) for crosses between minus A (genotype Ab) and conidial A (genotype aB) cultures. Sixteen of the cultures obtained from this cross were of the plus type and these were tested by the dilution plate method when they were 35 days old and rated according to their ability to produce minus mutants by the system previously described (fig. 1). These tests indicated that the plus progeny of this cross had segregated in a clear-cut manner for the ability to produce minus mutants. Six were typical plus A cultures having an average rating of 1.33 while the remaining ten were typical plus B cultures with an average rating of 0.03. The results of the plus progeny tests with cultures from two of the asci from this
cross were typical and are shown in detail (table I) since these cultures were used as foundation stocks for most of the subsequent breeding work.

Table I. Results of plus progeny tests with cultures from ascus A and ascus C from cross 76. The parents of this cross were minus A (69-K-14) and conidial A (36-0-5).

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Cultural type</th>
<th>Rating of each dilution culture</th>
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<tr>
<td>76-A-1</td>
<td>plus</td>
<td>1 0 2 0 1 2 0 0 2 3</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>plus</td>
<td>0 2 0 2 0 1 3 1 3 3</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>con. B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>con. B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>con. A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>con. A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>minus A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>minus A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 76-C-1         | plus          | 0 0 0 0 0 0 0 0 0 0             | 0.0            |
| 2              | plus          | 0 0 0 0 0 0 0 0 0 0             | 0.0            |
| 3              | con. B        |                                 |                |
| 4              | con. B        |                                 |                |
| 5              | con. A        |                                 |                |
| 6              | con. A        |                                 |                |
| 7              | minus A       |                                 |                |
| 8              | minus A       |                                 |                |

The fact that the two plus type isolates from ascus 76-A were typical plus A cultures while those from ascus 76-C were typical plus B cultures (table I) indicated that the conidial B isolates from these two ascid also probably differed in a similar manner. In order to obtain breeding stocks of known pedigrees, several preliminary crosses were made. The plus A culture 76-A-2 was crossed with the conidial B cultures 76-C-3 (cross 82) and 76-C-4 (cross 83). The plus B culture 5-16-1 was crossed with the conidial B cultures 76-A-4 (cross 79) and 76-C-4 (cross 84).

The cultural types obtained from these crosses (table II) were similar to those previously reported for crosses between the genotypes AB (plus) and ab (conidial B) (Chilton and Wheeler, 1949). The plus progenies of
Table II. Segregation ratios obtained from asci from four crosses between plus and conidial B cultures.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Parents</th>
<th>2 plus</th>
<th>2 minus</th>
<th>4 plus</th>
<th>4 minus</th>
<th>8</th>
<th>8</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>5-16-1 x 76-A-4</td>
<td>1</td>
<td>*</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>83</td>
<td>76-A-3 x 76-B-4</td>
<td>0</td>
<td>*</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>84</td>
<td>76-A-2 x 76-C-4</td>
<td>0</td>
<td>*</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>84</td>
<td>5-16-1 x 76-C-4</td>
<td>1</td>
<td>*</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>2</td>
<td>*</td>
<td>12</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>24</td>
</tr>
</tbody>
</table>

Table III. Results obtained from the plus progenies from crosses 79, 82, 83, and 84 when each plus isolate was rated according to its ability to produce minus mutants. Ratings were made by the dilution plate method on two different dates.

<table>
<thead>
<tr>
<th>Cross 79</th>
<th>Isolate Rating number (15) (43)</th>
<th>Cross 82</th>
<th>Isolate Rating number (15) (43)</th>
<th>Cross 83</th>
<th>Isolate Rating number (15) (43)</th>
<th>Cross 84</th>
<th>Isolate Rating number (21) (43)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A-6</strong></td>
<td>0.0 0.0</td>
<td><strong>A-1</strong></td>
<td>0.0 1.1</td>
<td><strong>B-4</strong></td>
<td>0.0 1.2</td>
<td><strong>A-1</strong></td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>8</td>
<td>0.0 0.8</td>
<td>5</td>
<td>0.0 1.4</td>
<td>6</td>
<td>0.0 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D-6</strong></td>
<td>0.0 0.0</td>
<td><strong>D-4</strong></td>
<td>1.1 2.1</td>
<td><strong>C-4</strong></td>
<td>0.0 1.4</td>
<td><strong>D-3</strong></td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>8</td>
<td>0.0 1.4</td>
<td>3</td>
<td>0.4 1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>E-1</strong></td>
<td>0.0 0.0</td>
<td><strong>E-1</strong></td>
<td>0.0 1.4</td>
<td><strong>E-1</strong></td>
<td>0.0 1.4</td>
<td><strong>E-1</strong></td>
<td>0.0 1.4</td>
</tr>
<tr>
<td>2</td>
<td>0.0 0.0</td>
<td>2</td>
<td>0.0 0.6</td>
<td>2</td>
<td>0.0 1.3</td>
<td>2</td>
<td>0.0 0.3</td>
</tr>
<tr>
<td>7</td>
<td>0.0 0.2</td>
<td>3</td>
<td>0.0 1.2</td>
<td>3</td>
<td>0.0 1.9</td>
<td>3</td>
<td>0.0 0.1</td>
</tr>
<tr>
<td><strong>F-1</strong></td>
<td>0.0 0.0</td>
<td><strong>F-1</strong></td>
<td>0.0 1.2</td>
<td><strong>F-1</strong></td>
<td>0.0 1.9</td>
<td><strong>F-1</strong></td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>7</td>
<td>0.3 0.6</td>
<td>6</td>
<td>0.0 1.0</td>
<td>4</td>
<td>Av. 0.00 1.44</td>
<td>5</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td><strong>G-2</strong></td>
<td>0.0 0.0</td>
<td>8</td>
<td>0.0 1.6</td>
<td>6</td>
<td>0.0 0.0</td>
<td>7</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td><strong>Av.</strong></td>
<td>0.03 0.08</td>
<td><strong>Av.</strong></td>
<td>0.03 1.58</td>
<td><strong>Av.</strong></td>
<td>0.03 1.2</td>
<td><strong>Av.</strong></td>
<td>0.03 0.42</td>
</tr>
<tr>
<td>7-1</td>
<td>0.0 1.5</td>
<td>2</td>
<td>0.0 1.2</td>
<td><strong>E-2</strong></td>
<td>0.2 1.7</td>
<td><strong>E-2</strong></td>
<td>0.0 0.8</td>
</tr>
<tr>
<td>3</td>
<td>0.0 1.3</td>
<td>4</td>
<td>0.0 1.4</td>
<td>8</td>
<td>0.0 1.6</td>
<td>Av.</td>
<td>0.03 0.42</td>
</tr>
<tr>
<td>5</td>
<td>0.0 1.6</td>
<td>6</td>
<td>0.0 1.4</td>
<td>7</td>
<td>0.0 1.3</td>
<td><strong>Av.</strong></td>
<td>0.03 0.42</td>
</tr>
<tr>
<td>8</td>
<td>0.0 1.5</td>
<td><strong>G-1</strong></td>
<td>0.0 0.8</td>
<td><strong>Av.</strong></td>
<td>0.05 1.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.0 1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses refer to the age (days) of the isolates when ratings were made.
these four crosses were tested to determine their ability to produce minus mutants by the dilution plate method when they were 15 to 31 days old and again 3 to 6 weeks later (table III). The results of the two series of plus progeny tests differed markedly. With the exception of isolate B-4 from cross 82 and isolate B-4 from cross 84, the plus progenies of these crosses all behaved like plus 3 cultures, producing few or no minus mutants in the first series of tests made when the isolates were relatively young. When the same isolates were tested 3 to 6 weeks later, the plus progeny of cross 79 produced few or no minus mutants, the plus progenies from cross 83 and cross 83 produced many minus mutants, and the plus progeny of cross 84 segregated, isolates B-3, B-4, N-2, and E-8 produced many minus mutants while the other plus isolates from this cross produced few or none. In regard to the ability of plus cultures to produce minus mutants, these results emphasised two points, (1) that the age of the culture was an important factor, and (2) that the ability of plus cultures to produce large numbers of minus mutants was inherited as if controlled by a single genetic factor.

In a study of the influence of age on the production of minus mutants by plus cultures, single ascoospore cultures from the plus A culture 76-A-2, the plus B culture 5-16-1, and a plus A culture recently isolated from soybean tissue were used (Wheeler and Chilton, 1949). Some of the results of this study which have a direct bearing on the present investigation are listed.

(1) Plus A cultures produced few minus mutants when 10 to 25 days old and the numbers produced increased rapidly with age up to 60 days.

(2) Plus B cultures produced no minus mutants until they were 30 days old after which the numbers produced increased gradually with age but never approached those produced by plus A cultures of the same age.
(3) Plus A and plus B cultures could be distinguished most readily if tested when they were 40 to 50 days old. When tested by the dilution plate method at 45 days of age, plus A cultures had an average rating of 1.61 while plus B cultures averaged 0.07.

(4) Ten single ascospore cultures from each of two different ascospore generations from the plus A culture 76-A-3 were studied. All of these behaved as typical plus A cultures, indicating that the ability to produce large numbers of minus mutants was transmitted from generation to generation.

In the course of these studies it was noted that plus A and plus B cultures usually could be distinguished if they were examined closely after they were 40 or more days old. At this age plus A cultures were characterized by a few very large and many small clumps of perithecia while the perithecial clumps produced by plus B cultures were relatively uniform in size (fig. 2).

![Fig. 2. Comparison of plus A and plus B cultures at 45 days of age. Plus A on the left, plus B on the right.]

The results of the preliminary studies summarized in table IV indicated that plus A and plus B cultures differed by a single genetic factor (or possibly a closely linked group of factors) which was inherited according to simple Mendelian principles. Since the presence of this
Table IV. Summary of the results obtained from the plus progenies of crosses 76, 79, 82, 83, and 84.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Parents</th>
<th>Number</th>
<th>Average</th>
<th>Number</th>
<th>Average</th>
<th>Age when tested (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>tested</td>
<td>rating</td>
<td>tested</td>
<td>rating</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>ABM abM</td>
<td>6</td>
<td>1.22</td>
<td>10</td>
<td>0.02</td>
<td>35</td>
</tr>
<tr>
<td>79</td>
<td>ABM abM</td>
<td>...</td>
<td>...</td>
<td>11</td>
<td>0.08</td>
<td>69</td>
</tr>
<tr>
<td>82</td>
<td>ABM abM</td>
<td>22</td>
<td>1.29</td>
<td>...</td>
<td>...</td>
<td>40</td>
</tr>
<tr>
<td>83</td>
<td>ABM abM</td>
<td>7</td>
<td>1.44</td>
<td>...</td>
<td>...</td>
<td>40</td>
</tr>
<tr>
<td>84</td>
<td>ABM abM</td>
<td>4</td>
<td>1.51</td>
<td>12</td>
<td>0.03</td>
<td>43</td>
</tr>
</tbody>
</table>

Key to genotypic symbols

AB - plus cultural type
Ab - minus cultural type
aB - conidial A cultural type
ab - conidial B cultural type

A factor in plus A cultures resulted in the production of large numbers of minus mutants, it was termed the "mutator gene" and was designated by the genotypic symbol "M". The inactive, mutant allele or the absence of this gene was designated by the symbol "m". Thus plus A cultures would have the genotype ABM and plus B cultures the genotype ABm. Since in a previous study (Wheeler, 1947), no plus A cultures were found in the progeny of a cross between the plus B culture 5-16-1 and the conidial A culture 38-0-5, the genotype of the latter may be assumed to be abM.

When this conidial A culture (38-0-5) was crossed with the minus culture 59-K-4 (table IV, cross 76), both plus A and plus B cultures occurred in the progeny, hence the genotype of the minus parent (59-K-4) of this cross was ABM. One of the parents of each of the other crosses listed...
in table IV was either a tested plus A or plus B culture and the genotypes of the conidial B parents of these crosses were readily derived from the results of the plus progeny tests.

Crosses such as those listed in table IV were unsatisfactory for the analysis of the \( H \) gene because only approximately one-fourth of the progenies were plus cultures. The main objective of these preliminary crosses was the development of conidial A cultures of known ancestry which could be crossed with plus A and plus B cultures since half of the progenies of such crosses are plus type cultures. Only plus B cultures occurred in the plus progeny of cross 79 (table IV), indicating that the conidial A progeny of this cross had the genotype \( abm \). Conversely, only plus A cultures occurred in the plus progenies of crosses 82 and 83, indicating that the conidial A cultures from these crosses were genotypically \( abm \). Two conidial A isolates, one from cross 79 (79-D-8) and one from cross 82 (82-A-5), were therefore selected as parents for further crosses.

In addition, a conidial A culture designated 5-16-1-c and a minus A culture designated 5-16-1-m were selected because they arose as mutants from the plus B culture 5-16-1 (genotype \( ABm \)) and would be expected to have the genotypes \( abm \) and \( Abm \) respectively. This made available for breeding purposes the following cultures for which, theoretically, the genotypes were known.

<table>
<thead>
<tr>
<th>Culture number</th>
<th>Cultural type</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>76-A-2</td>
<td>plus A</td>
<td>( ABm )</td>
</tr>
<tr>
<td>5-16-1</td>
<td>plus B</td>
<td>( ABm )</td>
</tr>
<tr>
<td>59-K-4</td>
<td>minus A</td>
<td>( Abm )</td>
</tr>
<tr>
<td>5-16-1-m</td>
<td>minus A</td>
<td>( Abm )</td>
</tr>
<tr>
<td>82-A-5</td>
<td>conidial A</td>
<td>( abm )</td>
</tr>
<tr>
<td>79-D-8</td>
<td>conidial A</td>
<td>( abm )</td>
</tr>
<tr>
<td>5-16-1-c</td>
<td>conidial A</td>
<td>( abm )</td>
</tr>
</tbody>
</table>

These cultures were used as parents for a number of crosses. When the plus progenies of these crosses were 40 to 45 days old they were
Fig. 3. Illustration of the results obtained with the cultures from one ascus from a cross between plus A and conidial A. Above, 4 plus A and 4 conidial A cultures from ascus D, cross 89. Below, each vertical row of plates represents the dilution cultures made from the corresponding plus A culture shown above.
Fig. 4. Illustration of the results obtained with the cultures from one ascus from a second cross between plus A and conidial A. Above, 2 plus A, 2 plus B, and four conidial A cultures from ascus C, cross 90. Below, each vertical row of plates represents the dilution cultures made from the corresponding plus A and plus B cultures shown above.
Fig. 5. Illustration of the results obtained with the cultures from one ascus from a cross between plus 3 and conidial A. Above, 4 plus B and 4 conidial A cultures from ascus A, cross 95. Below, each vertical row of plates represents the dilution cultures made from the corresponding plus B culture shown above.
examined, and, in most cases, they could be separated into plus A and
plus B cultures on the basis of their appearance, especially if all four
plus type cultures from a single ascus were available for comparison
(figs. 3, 4, and 5). The plus progenies were then tested by the dilution
plate method and rated according to the numbers of minus mutants produced.
The results obtained from cultures from three asci (figs. 3, 4, and 5)
illustrate the clear-cut manner in which the plus progenies of these
crosses segregated for the ability to produce minus mutants.

Three series of crosses were made. In the first of these, the plus
B culture 5-16-1 was crossed with the conidial A cultures 79-D-8 (cross
85) and 82-A-5 (cross 87). The latter, 82-A-5, was also crossed with the
plus A culture 76-A-2 (cross 89). The segregations obtained from analy­
ses of asci from these crosses are shown in fig. 6. In addition to those
diagrammed, one ascus from cross 85, one from cross 87, and three from
cross 89 contained spores which all gave rise to cultures of the conidial
A type. These distributions were essentially the same as those previous­
ly reported from crosses between plus and conidial A cultural types
(Chilton, et al, 1945). The results of tests by the dilution plate
method of the plus progenies of these crosses (table V) confirmed pre­
vious indications that plus A and plus B cultures differ by a single
 genetic factor which controls the production of minus type mutants. The
 standard errors of the average (mean) ratings of the plus A and plus B
progenies of these and later crosses were calculated to indicate the
degree of variability within the two groups.

The segregation obtained from analyses of asci from the second
series of crosses are shown in fig. 7 and a summary of the results of
tests of the plus progenies of these crosses is presented in table VI.
Six asci from cross 95 from which all of the spores produced minus A
Fig. 6. Segregations obtained from analyses of ascii from three crosses between plus and conidial A type cultures. Cross 85, plus B (ABm) x conidial A (aBM); cross 87, plus B (ABm) x conidial A (aBM); cross 89, plus A (ABm) x conidial A (aBM).

Table V. Summary of the results obtained from the plus progenies from three crosses between plus and conidial A cultures.

| Cross | Parental genotype | Plus A (ABm) | | Plus A (ABm) | | Plus B (ABm) | | Age when tested (days) |
|-------|------------------|--------------|----------------|--------------|----------------|----------------|-------------------|
|       |                  | Parental number | Tested | Average | rating | Tested | Average | rating | Tested |                |
| 85   | ABm x aBM       | 25            | 0.04±0.016 |       |       | 39     |       |       |       |                |
| 87   | ABm x aBM       | 22            | 0.07±0.032 |       |       | 40     |       |       |       |                |
| 89   | ABm x aBM       | 37            | 0.75±0.041 |       |       | 45     |       |       |       |                |
cultures and four ascii from cross 90 from which the spores produced either all plus or all conidial A cultures were not included in the diagram. The results of these crosses showed that only plus B cultures were obtained when the minus A culture 5-16-1-a and the conidial A culture 5-16-1-c were back-crossed (crosses 95 and 93) to the plus B culture 5-16-1 from which they arose. When the same conidial A culture, 5-16-1-c, was crossed with the plus A culture 76-A-2 (cross 90), both plus A and plus B cultures were obtained. From this last cross, one ascus was analysed from which three plus A cultures and one plus B culture were obtained. While this distribution could be attributed to an error in isolation or possibly considered as evidence of a brachymeiotic division, it seems more likely that a mutation from M to m or from m to M, occurring after the second nuclear division in the ascus, was responsible. The results of these crosses furnished further evidence of a single gene difference between plus A and plus B cultures. In addition, the results of cross 95 demonstrated that when a minus A culture, which arose as a mutant from a plus B culture, was backcrossed to its plus B parent, the ratios of cultural types obtained were similar to those obtained from crosses between other plus and minus cultures (Edgerton, et al, 1945).

In the third series of crosses (fig. 8, table VII), the plus B culture 5-16-1 was crossed first with the minus A culture 59-K-4 (cross 88). The occurrence of both plus A and plus B cultures in the progeny of this cross demonstrated that the minus A parent carried the M gene since otherwise only plus B cultures would have occurred in the progeny as was the case with cross 95 (fig. 7). One ascus from cross 86, the last one diagrammed, gave rise to four minus A and four plus A cultures. Since the four plus A cultures carried the M gene from the minus A parent, it was
Fig. 7. Segregations obtained from analysis of ascii from crosses between plus type cultures and minus A and conidial A cultures which arose as mutants from a plus B culture. Cross 90, plus A (ABM) x conidial A (aBm); cross 93, plus B (ABm) x conidial A (aBm); cross 95, plus B (ABm) x minus A (Abm).

Table VI. Summary of the results obtained from the plus progenies of crosses between plus cultures and minus A and conidial A cultures which arose as mutants from a plus B culture.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Parental genotype</th>
<th>Plus A (ABm)</th>
<th>Plus B (ABm)</th>
<th>Age when tested (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parental genotype</td>
<td>Number tested</td>
<td>Average rating</td>
<td>Number tested</td>
</tr>
<tr>
<td>90</td>
<td>ABM x aBm</td>
<td>18</td>
<td>1.49±0.095</td>
<td>28</td>
</tr>
<tr>
<td>93</td>
<td>ABm x aBm</td>
<td>..</td>
<td>.............</td>
<td>38</td>
</tr>
<tr>
<td>95</td>
<td>ABm x Abm</td>
<td>..</td>
<td>.............</td>
<td>50</td>
</tr>
</tbody>
</table>
Fig. 8. Segregations obtained from analyses of ascii from four crosses between plus and minus type cultures. Cross 88, plus B (ABm) x minus A (AbM); cross 91, plus A segregant backcrossed to its minus A parent; cross 92, minus A segregant backcrossed to its plus B parent; cross 94, intra-ascus cross.

Table VII. Summary of the results obtained from the plus progenies of four crosses between plus and minus cultures.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Parental genotype</th>
<th>Plus A (ABm)</th>
<th>Plus B (ABm)</th>
<th>Age when tested (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number tested</td>
<td>Average rating</td>
<td>Number tested</td>
</tr>
<tr>
<td>88</td>
<td>ABm x Abm</td>
<td>27</td>
<td>1.33±0.169</td>
<td>16</td>
</tr>
<tr>
<td>91</td>
<td>ABm x ABm</td>
<td>43</td>
<td>1.41±0.140</td>
<td></td>
</tr>
<tr>
<td>92</td>
<td>ABm x Abm</td>
<td></td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>94</td>
<td>ABm x Abm</td>
<td>32</td>
<td>1.45±0.041</td>
<td>27</td>
</tr>
</tbody>
</table>
The plane 34 culture was developed from the parent of cross 34 when the plane 34 culture was produced from the parent of cross 34. Both planes and singles type cultures were obtained from one source, the 34 type cultures. The singles type cultures were obtained from the two planes 34 and singles type cultures from the two planes 34. singles type cultures were tested and all of these 34 cultures were tested and all of these 34 cultures were tested.

These 34 cultures from each of these 34 cultures were tested and all of these three cultures were tested and all of these three cultures were tested.

When the 34 cultures were tested and all of these 34 cultures were tested, the 34 cultures were tested and all of these 34 cultures were tested.

The singles type cultures were obtained from the parents of cross 34. When the singles type cultures were obtained from the parents of cross 34, the singles type cultures were obtained from the parents of cross 34.

The singles type cultures were obtained from the parents of cross 34, and only the singles type cultures were obtained from the parents of cross 34. The singles type cultures were obtained from the parents of cross 34, and only the singles type cultures were obtained from the parents of cross 34.
conidial A parent. Crosses between plus A and the same conidial A cultures either yield only plus A cultures (fig. 6, cross 89) or both plus A and plus B cultures (fig. 7, cross 90). If minus A rather than conidial A cultures are used as one of the parents, the same segregations for plus A and plus B cultures occur in the progenies (fig. 8). A total of 486 plus cultures from 18 different crosses were tested and rated by the dilution plate method. With the exception of the cultures from one ascus from cross 90 (fig. 7), the plus progenies of these crosses segregated for the production of minus mutants according to simple Mendelian principles. These results demonstrate clearly that plus A and plus B cultures differ genetically by a factor which in some manner controls the production of minus mutants. This factor is inherited as a single gene and segregates in the progenies of appropriate crosses as readily as any other character.

A summary of the results of crosses between cultures differing by two pairs of factors is presented in table VIII.

The parents of cross 67 and cross 90 differed by the genes Aa and Ma. A total of 22 asci were analyzed from these two crosses. These represented 176 ascospores of which 84 were parental types and 92 were recombination types. Twenty-eight asci were analyzed from crosses 88 and 94 where the parents differed by the genes Bb and Ma. These represented 224 ascospores of which half were parental types and half were recombination types. These rather limited results indicated that M was inherited independently of either A or B. Since A and B are widely separated loci on the same chromosome, (Chilton and Wheeler, 1949), it is probable that the M gene is on a separate chromosome.
Table VIII. Summary of the segregation ratios obtained from crosses involving the M gene in which the parents differed by two pairs of factors.

<table>
<thead>
<tr>
<th>Parental Genotypes</th>
<th>Number of asc in each segregation group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross genotypes</td>
<td>4:4 parental</td>
</tr>
<tr>
<td>87 Abm x AbM</td>
<td>2</td>
</tr>
<tr>
<td>90 AbM x Abm</td>
<td>1</td>
</tr>
<tr>
<td>Between Ab and Wm</td>
<td>3</td>
</tr>
<tr>
<td>88 Abm x AbM</td>
<td>1</td>
</tr>
<tr>
<td>94 AbM x Abm</td>
<td>4</td>
</tr>
</tbody>
</table>

Key to genotypic symbols

AB - plus cultural type
Ab - minus cultural type
ab - conidial A cultural type

M - active allele of mutator gene
m - inactive allele of mutator gene

The Relation of the Size of Perithecial Clumps to the Occurrence of Minus Mutants in Plus A Cultures

In the course of tests by the dilution plate method of plus A cultures it was noted that more minus mutants were obtained when large rather than small clumps of perithecia were used. To obtain more information on this point, a single spore transfer from the plus A culture 76-A-2 was grown in a petri dish for a period of 40 days. At this time a few very large clumps of perithecia had formed but most of the clumps were small. The large clumps were broken up into fragments about the size of the small clumps and these were used to make seven dilution cultures from each large clump. At the same time dilution cultures were made by the usual method from small clumps. The average rating obtained from 10 large clumps was 1.60 while 50 small clumps averaged 0.13. These limited results indicated that most of the minus mutants which are obtained from plus A cultures occur in the large clumps of perithecia which
Preliminary Studies of the Mode of Action of the Mutator Gene

The fact that the numbers of minus mutants obtained from plus A cultures increased markedly as the cultures aged (Wheeler and Chilton, 1949) suggested the possibility that the mutator gene produced its effect through the elaboration of some substance which became mutagenic when present in sufficient concentration in the substratum. If this were the case, the presence in a substratum of metabolic or staling products of cultures carrying the M gene would, theoretically, induce mutations in cultures grown on such a substratum. A number of preliminary experiments designed to test the validity of this hypothesis were conducted.

At first, attempts were made to grow plus A and plus B cultures on opposite sides of a membrane which would allow the passage of metabolic products but prevent the mycelia of the two cultures from intermingling. These were not successful because no such membrane could be found. In later studies, cultures were grown on media which had been previously staled by the growth products of other cultures. Media staled for periods ranging from 10 to 40 days by cultures carrying the M gene and then sterilised by heat had no detectable effect upon plus A and plus B cultures other than a retardation of the rate of growth. This led to the use of bacteriological filters made of fritted glass for the sterilisation of staled media. This method of sterilisation required the use of liquid media, several types of which were tried. These included green bean infusion, oat flake infusion, potato broth, and nutrient broth. None of these were entirely satisfactory, since the fungus did not fruit well on any of them after they were passed through a fritted glass filter, especially if they also contained staling products. This could
be remedied to some extent by mixing the staled filtrates with an equal quantity of fresh media. No results were obtained which furnished any conclusive evidence that mutations could be induced by the use of staled media. The results of two tests, however, were somewhat promising. In these, plus A and plus B cultures were grown for a period of thirty days on oat flake infusion medium in 600 ml. flasks. The staled medium was then clarified by several passages through filter paper and sterilized by vacuum filtration through a fritted glass filter. The sterilized filtrates were added to an equal quantity of an oatmeal medium containing three per cent agar. Single spore plus A and plus B cultures were grown on this mixture in petri dishes. These were tested by the dilution plate method and rated according to the number of minus mutants produced after 25 and 35 days (table IX).

Table IX. Comparison of the occurrence of minus mutants in plus A and plus B cultures grown on media staled by the same cultures.

<table>
<thead>
<tr>
<th>Culture Tested</th>
<th>Staling Agent</th>
<th>Test No.</th>
<th>Dilution culture ratings After 25 days</th>
<th>Avg.</th>
<th>After 35 days</th>
<th>Avg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plus A</td>
<td>plus A</td>
<td>1</td>
<td>20200300211</td>
<td>1.1</td>
<td>220121311</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3001123202</td>
<td>1.4</td>
<td>3203311210</td>
<td>1.6</td>
</tr>
<tr>
<td>Plus A</td>
<td>plus B</td>
<td>1</td>
<td>3300131011</td>
<td>1.3</td>
<td>3201120231</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2011301120</td>
<td>1.1</td>
<td>303120320</td>
<td>1.4</td>
</tr>
<tr>
<td>Plus B</td>
<td>plus A</td>
<td>1</td>
<td>0000000000</td>
<td>0.3</td>
<td>0000000000</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0000000000</td>
<td>0.1</td>
<td>0000000000</td>
<td>0.2</td>
</tr>
<tr>
<td>Plus B</td>
<td>plus B</td>
<td>1</td>
<td>0010000200</td>
<td>0.3</td>
<td>0000000000</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0000000000</td>
<td>0.0</td>
<td>0000000000</td>
<td>0.0</td>
</tr>
</tbody>
</table>

These results, particularly those obtained after 35 days, showed that plus cultures grown on media staled by plus A cultures produced a few more minus mutants than did the same cultures grown on media staled
by plus B cultures. These differences were considered to be too small for significance in view of the very limited data available.

Analysis of a Gene Causing Abnormal Development of Fruiting Structures.

In a previous study (Wheeler, 1947), the occurrence of a non-ascigerous, nonconidial mutant, which arose as a sector in a plus B culture, was reported. When this mutant was mated with a conidial B culture of the genotype ab_1, perithecia formed at the line of contact, but few of these contained viable ascospores. Single ascospores isolated at random from these perithecia gave rise to cultures of eight different phenotypes (fig. 9). Two of these were like the parents of the cross, while three of the others were easily identified as plus B, minus B, and conidial A. Of the remaining three types, one resembled minus B except that the perithecia produced were much smaller and apparently infertile. The other two resembled conidial A and conidial B, respectively, but did not produce as great an abundance of conidia. In addition, these three types were characterised by dark streaks which appeared as the cultures aged.

These results indicated that ascigerous cultures occurred in the progeny of a cross between two non-ascigerous cultures and the occurrence of eight different phenotypes in the progeny indicated that the parents differed by three factors. Since the genotype of the conidial B parent of this cross was ab_1, the occurrence of plus B cultures in the progeny indicated that the other parent carried the genes AB. The mutant form of the third gene involved in this cross resulted in abnormal development of fruiting structures. This gene was designated by the genotypic symbol n and its wild-type or normal allele by the symbol N. On this basis, the genotypes of the cultures obtained from this cross were those listed under fig. 9.
Fig. 9. Phenotypic effects of a gene causing abnormal development of fruiting structures. The left member of each pair of cultures is normal, the one on the right carries the gene for abnormal development. Genotypes from the left: ABN (plus) and ABn, AbH (minus B) and AbH, aBN (conidial A) and aBN, abH (conidial B) and abH.

Table X. Number of cultures of each of eight genotypes obtained from ascospores isolated at random from crosses between two non-ascigerous cultures, conidial B and a mutant from plus B.

<table>
<thead>
<tr>
<th>Parents</th>
<th>Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABn x abH</td>
<td>ABN</td>
</tr>
<tr>
<td>Cross 1</td>
<td>3</td>
</tr>
<tr>
<td>Cross 2</td>
<td>5</td>
</tr>
<tr>
<td>Totals</td>
<td>8</td>
</tr>
</tbody>
</table>

Analysis of asci from perithecia formed by the cross ABn (abnormal plus) x abH (normal conidial B) was not possible because very few of these perithecia matured ascospores. A summary of the results of random ascospore isolations from this cross is presented in table X. The cultures obtained segregated into the eight genotypes expected, but a large
excess of cultures like the conidial B parent (ab₁N) occurred. The significance of this distribution will be discussed in a later paragraph.

Table XI. Segregation ratios obtained from ascii from two crosses involving a gene causing abnormal development of fruiting structures.

<table>
<thead>
<tr>
<th>Parents</th>
<th>ab₁N</th>
<th>ab₁n</th>
<th>ABN</th>
<th>ABn</th>
<th>AB₁N</th>
<th>AB₁n</th>
<th>AB₁¹</th>
<th>AB₁n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABN x ab₁n</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>aBN x AB₁n</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Key to genotypic symbols

AB - plus  
ab₁ - conidial A  
N - normal  
Ab₁ - conidial B  
n - abnormal

Table XII. Segregation ratios obtained from ascii from the cross ABN (normal plus) x aBN (abnormal conidial A).

<table>
<thead>
<tr>
<th>Parents</th>
<th>4 ABN</th>
<th>2 aBN</th>
<th>4 ABn</th>
<th>8 ABN</th>
<th>Total</th>
<th>ascii</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABN x aBN</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>19</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

The segregation ratios obtained from ascii from the cross ABN (normal plus) x ab₁n (abnormal conidial B) and the cross aBN (normal conidial A) x Ab₁n (abnormal minus B) are shown in table XI. The ratios obtained were those expected from crosses between cultures differing by three factors except for the large number of ascii from the first cross which
yielded only ABN (normal plus) cultures. Spores from a large number of
the ascii from a cross between a normal plus culture (ABN) and an abnormal
conidial A culture (aBn) also gave rise to only normal plus cultures
(table XII). These results were of interest since from previous crosses
between normal plus and normal conidial A or conidial B cultures, asci
were frequently found which gave rise to only conidial cultures but ascii
which gave rise to only plus cultures seldom occurred. Also, the majority
of the ascospores isolated from crosses between abnormal plus and normal
conidial B (table X) or conidial A (Wheeler, 1947) cultures gave rise to
cultures like the normal conidial parent. In regard to these distribu­
tions, it should be noted that dense ridges of fertile perithecia formed
quickly when normal plus cultures were mated with either normal or abnor­
mal conidial B cultures. Most of the perithecia formed by mating abnor­
mal plus and normal conidial B cultures were infertile while in matings
between two abnormal cultures no perithecia were formed (fig. 10).

Fig. 10. Normal and abnormal plus and conidial B cultures mated in all
possible combinations. Top, normal plus; bottom, abnormal plus;
left, normal conidial B; right, abnormal conidial B.
A summation reveals the following facts in regard to crosses between normal and abnormal plus and conidial B cultures:

1. **normal plus x normal conidial B** — many fertile perithecia produced; all of the progeny normal, spores from most of the ascii segregate for two or more genotypes, some produce eight conidial B cultures, and a few produce eight plus cultures.

2. **normal plus x abnormal conidial B** — many fertile perithecia produced; spores from the majority of the ascii produce eight normal plus cultures, the remainder segregate for the expected normal and abnormal types.

3. **abnormal plus x normal conidial B** — few fertile perithecia produced; most of the ascospores produce normal conidial B cultures, the remainder segregate for the expected normal and abnormal types.

4. **abnormal plus x abnormal conidial B** — no perithecia produced.

The results of the crosses involving three pairs of heterozygous factors (table XI) furnished conclusive evidence that no brachymiotic division occurs in this fungus, since in no case were more than four types of cultures obtained from a single ascus. It should be noted that a few ascospores were found in three of the abnormal plus (AB+) cultures and in five of the abnormal minus B (AB-A) cultures from this series of crosses; however, these occurred only after the cultures were more than sixty days old. Some of these spores were isolated, but since none of them germinated, their origin remained unknown. Apparent reversions to normal in the form of sectors have been noted when mass transfers have been made from old abnormal cultures. Spores from ten ascii from the line of perithecia formed by mating a normal minus A culture with an abnormal plus culture produced only normal minus A cultures.

As noted previously, dark streaks appear in cultures which carry the mutant gene causing abnormal development of fruiting structures. In old cultures these become very pronounced and take the typical pie-shaped sector form (fig. 11). A number of mass and single conidium transfers
were made from the light and dark areas of an abnormal conidial A culture. All of these gave rise to cultures which again produced dark sectors like the parent culture.

Fig. 11. Dark sectors characteristic of cultures carrying a gene which causes abnormal development of fruiting structures. On the left, abnormal conidial A; on the right, abnormal conidial B.

Table XIII. Summary of linkage data between the loci A and B, A and N, and B and N.

<table>
<thead>
<tr>
<th>Parental genotypes</th>
<th>4:4 parental</th>
<th>2:3:2:3 recombination</th>
<th>Total asci</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB N x ab_1n</td>
<td>2</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Ab_1n x AB_1n</td>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td><strong>Between A and B</strong> total</td>
<td><strong>5</strong></td>
<td><strong>21</strong></td>
<td><strong>30</strong></td>
</tr>
<tr>
<td>AB N x AB N</td>
<td>1</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>AB N x ab_1n</td>
<td>5</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Ab_1n x AB N</td>
<td>1</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td><strong>Between A and N</strong> total</td>
<td><strong>7</strong></td>
<td><strong>27</strong></td>
<td><strong>40</strong></td>
</tr>
<tr>
<td>AB N x ab_1n</td>
<td>3</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Ab_1n x AB N</td>
<td>1</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td><strong>Between B and N</strong> total</td>
<td><strong>4</strong></td>
<td><strong>22</strong></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>

Key to genotypic symbols

AB - plus
Ab\_1 - minus
aB - conidial A
ab\_1 - conidial B
N - normal
n - abnormal
A summary of the results of crosses involving the A, B, and N loci (tables XI and XII) is presented in table XIII. These data show no evidence of linkage between the A and N loci or the B and N loci. Since A and B are loosely linked (Chilton and Wheeler, 1949), these data indicate that N is on a separate chromosome.

Studies of the Physiological Effects of Certain Genes

Chilton, et al. (1945), pointed out that ascigerous cultures of *Glomarella* isolated from nature may or may not produce conidia. Those from *Ipomoea* which were used in the present investigation produce few or no conidia when grown on oatmeal or bean agar. However, mutations from A to a in these wild-type ascigerous (plus) cultures from *Ipomoea* give rise to non-perithecial cultures called conidial A which produce an abundance of conidia on these media. Since mutations usually involve the loss of a character or function rather than the acquisition of a new character, it was difficult to account for the inability of plus type cultures to produce conidia. During the course of the present investigations, plus cultures were grown on nutrient agar and on this medium they produced appreciable numbers of conidia. It was also noted that in matings between plus and conidial cultures, no conidia were produced on the conidial cultures for a short distance back of the line of perithecia formed by the mating. These observations seemed to indicate that plus cultures carried the genetic factors necessary for the production of conidia, but that the elaboration of some substance prevented their development in plus cultures. The results of a series of experiments supported this hypothesis.

Ascigerous, non-conidial plus and non-ascigerous conidial A cultures were grown for 10 days on nutrient broth in 500 ml. flasks, each of which
contained 60 ml. of the medium. The media staled by the two cultures was sterilized by passage through a fritted glass filter and each of the two lots was then mixed with enough 5 per cent water agar to make a medium containing 1.5 per cent agar. Plus, minus, and conidial A cultures were then grown in triplicate on each lot of staled medium as well as on fresh nutrient agar. This experiment was repeated three times and the results were in general agreement (fig. 12). In order to obtain quantitative data, uniform samples were taken after 10 days from the cultures from one of these trials and these were used to make water suspensions of conidia. The number of conidia in each of ten small drops from each of these suspensions was determined and the results were then averaged (table XIV).

Table XIV. Effect of staling products from plus and conidial A cultures on the production of conidia by plus, minus, and conidial A cultures.

<table>
<thead>
<tr>
<th>Type of culture</th>
<th>Av. colony diameter</th>
<th>Staling agent</th>
<th>Av. No. of conidia per drop</th>
<th>Per cent of check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Test 1</td>
<td>Test 2</td>
</tr>
<tr>
<td>Plus</td>
<td>60.3 mm</td>
<td>check</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Plus</td>
<td>45.3 mm</td>
<td>con. A</td>
<td>13.3</td>
<td>12.2</td>
</tr>
<tr>
<td>Plus</td>
<td>45.0 mm</td>
<td>plus</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Minas</td>
<td>61.7 mm</td>
<td>check</td>
<td>25.9</td>
<td>26.5</td>
</tr>
<tr>
<td>Minas</td>
<td>45.7 mm</td>
<td>con. A</td>
<td>47.7</td>
<td>45.3</td>
</tr>
<tr>
<td>Minas</td>
<td>45.2 mm</td>
<td>plus</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Conidial A</td>
<td>64.3 mm</td>
<td>check</td>
<td>139.6</td>
<td>106.3</td>
</tr>
<tr>
<td>Conidial A</td>
<td>48.7 mm</td>
<td>con. A</td>
<td>123.5</td>
<td>139.8</td>
</tr>
<tr>
<td>Conidial A</td>
<td>46.7 mm</td>
<td>plus</td>
<td>7.6</td>
<td>5.7</td>
</tr>
</tbody>
</table>

These results demonstrated that media staled by plus cultures reduced the production of conidia in plus, minus, and conidial A cultures by more than 50 per cent as compared to nutrient agar which served as a check. Media staled by conidial A cultures increased the production of conidia, especially in plus and minus cultures. Plus and minus cultures produced no perithecia on media staled by conidial A cultures until they
Fig. 12. Effect of staling products from plus and conidial A cultures upon the development of perithecia and conidia. On the left, minus cultures; on the right, conidial A cultures.
were more than ten days old (fig. 12). Staled media sterilised by autoclaving for 40 minutes at 15 pounds pressure had no detectable effect upon plus, minus, or conidial A cultures other than a retardation of the rate of growth, which also occurred on staled media sterilised by filtration (table XIV).

These results indicated that the wild-type gene A, which controls the production of perithecia, also controls the synthesis of some substance which inhibits the development of conidia. The mutant allele of this gene permits the production of conidia, but apparently produces some substance which retards the development of perithecia.

Another problem of considerable interest concerns the nature of the self-sterility exhibited by minus A cultures grown alone. When such cultures are mated with plus cultures approximately 30 per cent of the perithecia formed at the line of contact contain only ascii from which all of the spores produce typical self-sterile minus A cultures. This seemed to indicate that minus A cultures were capable of producing fertile perithecia if grown in close association with plus cultures. A number of experiments were conducted which demonstrated that extracts from plus cultures increased the production of fertile perithecia in minus A cultures. Best results were obtained by adding a few drops of a sterile extract from a plus culture grown on an oat flake infusion medium to a minus A culture grown on oatmeal agar. Drops were added at two day intervals until the minus A cultures were 10 days old. Examination of the minus A cultures at 15 days of age showed a considerable increase in the number of fertile perithecia as compared to either untreated checks or checks treated with extracts from minus A cultures. When the treated minus A cultures which showed increased self-fertility were transferred they immediately reverted to the original, nearly self-sterile type.
DISCUSSION

The results of the present investigation have shown that plus A and
plus B cultures differ genetically by a factor which in some manner
controls the production of minus mutants. This factor is inherited as
a single gene and has been termed the "mutator" or "M" gene. Previous
studies have indicated that mutations from plus to minus involve gene
changes at a locus designated "M". Since plus A cultures, which produce
large numbers of minus mutants, carry the active form of the M gene, while
plus B cultures, which produce few minus mutants, either lack this gene
or carry its inactive allele, it might be assumed that the active form
of the M gene produces its effect by increasing the rate of mutation at
the B locus. There is some evidence which indicates that this is only
partly correct. The marked stimulation of perithecial production which
occurs when plus and minus cultures are mated, and the distribution of
types which have been obtained from crosses between these two types of
cultures, indicate that mutant minus nuclei tend to enter into the forma-
tion of perithecia and asci more readily than do non-mutant plus nuclei.
It is therefore apparent that a decrease in the number of minus mutants
obtained from ascospores from plus cultures might result from a gene
change which increased the ability of plus nuclei to enter into the
formation of asci with each other. There is some evidence that the
absence of the active form of the M gene results in such a change in plus
B cultures, since observations have indicated that plus B cultures are
somewhat more highly self-fertile than plus A cultures. The fact that
very large clumps of perithecia, in which most of the minus mutants in
plus A cultures occur, are not formed in plus B cultures also suggests
that plus B nuclei tend to enter into the formation of asci with each
other more readily than do plus A nuclei. However, the action of the M
gene cannot be explained solely on this basis, since similar distribu-
tions of types were obtained from asci produced by crossing plus A (ABM)
and plus B (ABm) cultures with minus cultures of the genotypes AbM and
Abm. Furthermore, the results obtained from conidial A cultures (Wheeler
and Chilton, 1949) indicate that in the vegetative stage, mutations at
the B locus occur about 13 times more frequently in cultures which carry
the active allele of the M gene than in cultures lacking this gene or
carrying its inactive allele.

The evidence available at present indicates that the three factors
listed below are together responsible for the large numbers of minus
mutants which have been obtained from the ascospore progenies of plus A
cultures.

(1) The active form of the M gene induces mutations in plus A cultures
at a locus "B" which result in the production of mutant nuclei of the
minus genotype.

(2) These mutant nuclei tend to enter into the formation of asci, either
with each other or with plus nuclei, more readily than do non-mutant plus
nuclei. The presence of the active form of the M gene apparently increases
this tendency.

(3) This results in a rapid build-up of perithecia in a culture wherever
mutant nuclei occur and, together with a natural tendency on the part of
the investigator to select these large clumps of perithecia for sampling,
results in the recovery of very large numbers of minus mutants from ascospores.
The demonstration that plus A and plus B cultures differ by a factor which is inherited as a single gene raises two questions, first, how did the first plus B culture originate and second, how does this gene produce its effect. Although no satisfactory answer can be given for either of these, a brief discussion of certain facts which seem to have a bearing on these problems may be of some value.

The original plus B culture, 5-16, was obtained from the line of perithecia formed by mating a minus and a conidial A culture both of which arose as mutants from a plus A culture. Since in other organisms double mutations are extremely rare (Beadle, 1945), it seems probable that both of the parents of this cross carried the active form of the M gene. It would therefore appear that the original plus B culture arose either as the result of a gene change or through the loss of one or more genes. In this regard it should be noted that no genes have been identified which are linked with the M locus and the symbol M as used in this study may represent several closely linked genes or possibly a portion of a chromosome. The fact that no reversions to the original plus A form have been observed in plus B cultures suggests that plus B originated as the result of a loss of a gene or part of a chromosome. The fact that plus B cultures have never arisen directly from plus A cultures but only from crosses might be interpreted as evidence that plus B cultures result from the loss of a portion of a chromosome. If this were the case, a cyto- genetic study should yield valuable information.

If, as the available evidence indicates, the active form of the M gene increases the frequency of mutation at the B locus, a knowledge of the mode of action of this gene should provide basic information concerning the nature of mutation as well as an insight into the nature of the
gene itself. If Beadle's (1945) interpretations of the results obtained with Neurospora are correct, it would seem that the M gene produces some substance which is antagogenic at the B locus. Preliminary experiments have failed to demonstrate the existence of such a substance, but the results obtained concerning the action of other genes suggest that further studies of this type might be extremely fruitful. The results obtained by Lucas, et al (1944) and Chilton, et al (1945) with ascospores, and those by Wheeler and Chilton (1949) with conidia, indicate that the M gene has no effect upon mutations other than those which occur at the B locus. Whatever its mode of action, the M gene is apparently specific in its effect.

Although a general discussion of sexuality in this fungus is beyond the scope of this study, one or two points in regard to this problem seem worthy of mention. Many definitions of sex, most of which are highly superficial, occur in the literature. The concept that sex in fungi is comprised of the total of those events and activities which lead up to and result in the fusion and reduction of two nuclei and that the ability of two nuclei to fuse and reduce is the essence of the sexual process seems as satisfactory as any.

In Glomerella, eight mutant genes at four different loci on at least two different chromosomes, have been identified. All of these genes affect the sexual process in some respect, but none has been demonstrated which affects the ability of nuclei to fuse and reduce. More specifically, in unsated cultures, three genes which control the production of perithecia and three which control the fertility of perithecia are known. Seven different genes are known which affect mating reactions and one gene, the M gene, apparently induces sex mutations. It seems evident that
this fungus offers excellent material for studies concerning the nature of sex in fungi.
SUMMARY

The results of appropriate crosses demonstrated that plus A and plus B cultures of a *Gloeospora* from *Ipomoea* differ genetically by a factor which controls the production of minus type mutants. This factor is inherited as a single gene according to simple Mendelian principles and is independent of the locus at which gene changes from plus to minus occur. The presence of this factor in plus cultures results in the production of a few very large clumps of perithecia, which, on the basis of limited data, contain most of the minus mutants which are produced. It has no phenotypic effect in other types of cultures. The results of attempts to induce mutations in plus B cultures through the use of extracts from plus A cultures were inconclusive.

Analysis of a mutant gene causing abnormal development of fruiting structures indicated that it was inherited independently of the two previously known loci. In plus cultures this gene usually completely suppressed the development of perithecia. Asciopores isolated from perithecia formed by mating such abnormal plus cultures with normal conidial B cultures gave rise to both normal and abnormal plus, minus, conidial A, and conidial B cultures. The results of other crosses in which the parents differed by three pairs of factors indicated that no brachy- meiotic division occurs in this fungus.

Plus and minus cultures, which produce perithecia but few or no conidia on oatmeal or bean agar, produced appreciable numbers of conidia when grown on nutrient agar. Conidial production by plus, minus, and
Conidial A cultures were reduced by more than 90 per cent when the fungi were grown on nutrient agar staled by plus cultures. Conidial production in the same cultures was increased on the same medium staled by conidial cultures, but perithecial formation in plus and minus cultures was retarded. Sterile extracts from plus cultures which are self-fertile, added to minus A cultures which are normally almost self-sterile, resulted in a considerable increase in the number of fertile perithecia produced by the latter.
LITERATURE CITED


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- - - - - - , and - - - - - - - 1948b. Multiple alleles and factor interaction in Glomerella. (Abstract) Phytopath. 36: 28.

- - - - - - , and - - - - - - - - 1949. Production of mutants in Glomerella as influenced by age of culture. (Abstract) Phytopath. 39: 25.

Doctor of Philosophy in June, 1949.

He is a candidate for the degree of

Master of Science in August, 1947. He is a student at the University of

Another with the U. S. Army Air Forces. Upon release from active

institution from January 1946 until April 1, 1946, he served as a

be released a bachelor of science degree in agriculture from

and graduated the University of Vermont in September, 1937. In June, 1941

Vermont. He was graduated from Chathamton High School in June, 1929.

Henry Ernest Wheeler was born January 26, 1919 at West Chathamton,
PUBLICATIONS


EXAMINATION AND THESIS REPORT

Candidate:  Harry Ernest Wheeler

Major Field:  Botany

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Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

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[Signatures]