Mintich, Mary. Water as a Kinetic Element in Sculpture. (May, 1971) Directed by: Mr. Walter Barker pp. 5

Two all acrylic and one acrylic and chrome plated copper sculptures explore some of the possibilities of water as a visual and physical kinetic force in sculpture.

This thesis was exhibited in Weatherspoon Gallery, University of North Carolina at Greensboro, from May 2 to 9, 1971.

Color slides representing the exhibit are on file at the University of North Carolina Library in Greensboro, North Carolina.

WATER AS A KINETIC ELEMENT IN SCULPTURE

by

Mary Mintich

A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Fine Arts

Greensboro
May, 1971

Approved by

Walter Barker

Thesis Adviser

APPROVAL SHEET

This thesis has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

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May 6, 1971
Date of Examination

Acknowledgements

I would like to express my appreciation to the art department, particularly Mr. Walter Barker, for this period of concept expansion and professional growth. I would also like to thank Barbara Kasler for her interest and encouragement, but I am primarily indebted to my husband, George Mintich, for his unfaltering dedication, enduring patience, encouragement and, especially, his enthusiasm.

CATALOGUE OF EXHIBIT

	Piece	Dimensions
1.	Tidal Sculpture #1 Acrylic sheet 1971	60" x 30" x 18"
2.	Water With Red Plex and Chrome Acrylic sheet with chrome-plated copper 1971	75" x 34" x 13½"
3.	Single Column With Water Acrylic sheet 1971	72" x 8¼" x 19¼"

LIST OF FIGURES

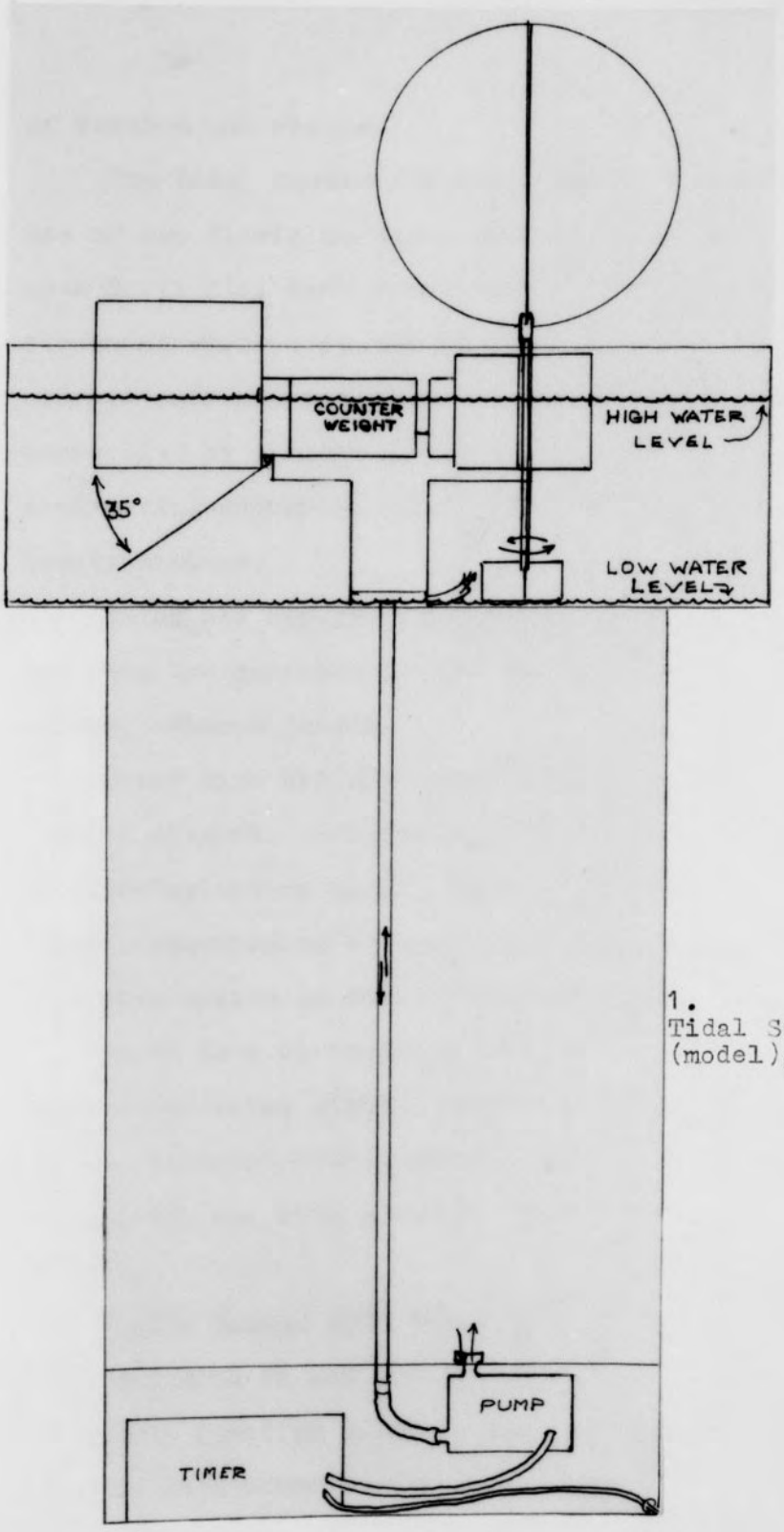
Two all acrylic and one acrylic and chrome plated copper sculptures explore some of the possibilities of water as a visual and physical kinetic force in sculpture.

Tidal Sculpture #1 has been designed to respond in two ways to tidally motivated, eternally activated water and demonstrates a way in which water can be employed as a physical force. Its true environment should be a protected but ocean-fed harbor, inlet or canal. This should dictate its ultimate size, material and engineering specifics.

Since these are unknowns, the model for Tidal Sculpture #1 cannot reflect them but does demonstrate the basic concept of movement by tidal force in two ways:

1. The 12 inch disc responds directly to tidal currents when they engage a submerged wheel connected to this disc by a shaft.

2. A 6½ inch cube with rectangular shank is hinged to the "T" shaped pedestal. Its position is determined by the rise and fall of the water level. Water enters the cube at high tide. As the water receeds, the balance is disturbed, and the form assumes a 35 degree angle. It remains in this position until the captured water drains from the cube and the counterweighted shank restores it to its original position. This movement is perpetual and predictable and brings the sculpture alternating periods



1.
Tidal Sculpture #1 (model), 1971.

of tension and repose.

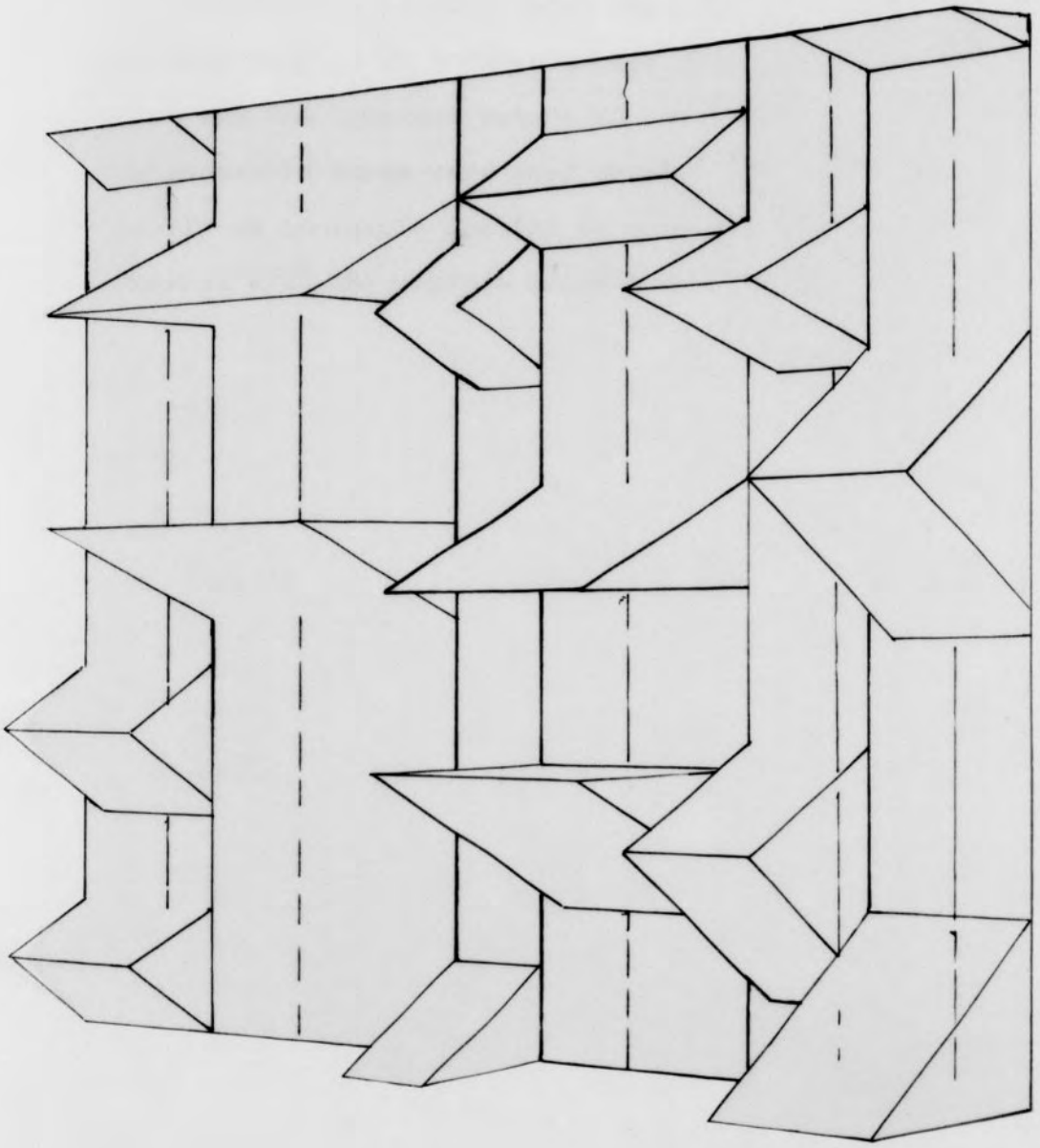
The tidal source for the model is achieved by the use of two Plexiglas tanks with similar volumes. An open horizontal tank functions as the sculpture's environment while a closed vertical one is both water storage unit and pedestal. A submersible water pump is controlled by a timer with a seven and thirteen minute alternating sequence. They fill and empty the tank each twenty minutes.

Color was applied by floating epoxy containing a dye onto the surfaces. This also served to reinforce solvent adhered joints.

Water With Red Plex and Chrome uses water as a visual kinetic element. A contained spray activates the smaller of three cylinders which comprise the finial. Free falling sprays, functioning as synapses, complete the three ovals that give motion to this stationary form.

There is a correlation between this piece and a railroad crossing signal. Both are intentionally kinetic, bright, arrogant with a penchant for control (water in this case), and both could be considered free standing reliefs.

Single Column With Water also employs free falling water but with in and out patterning. This sculpture can either function alone or as a modular unit to be combined with other varied but related columns to form



2. Single Column With Water combined with other varied but related columns.

a relief wall of Plexiglas and falling water. Its size would have few limits.

Transparent acrylic sheet was selected as the major material because it relates to the transparency of the water and the inherent nature of the geometric forms. The geometric forms were used because they seemed to intensify an inorganic quality to make more potent their contrast with the organic quality of water.

MILLER, CARROLL CHRISTIAN. A Modifier of β-Glucosidases in Neurospora crassa. (1971) Directed by: Dr. Bruce M. Eberhart pp. 69

Studies of a new suppressor effect on the gluc-2 gene are reported here. It is believed this effect is due to the su-1 gene. Genetic studies of su-1 indicated initially single gene action at a locus other than gluc-2. The gluc-2 regulatory gene in turn may have a super-repressor effect, since it repressed aryl-β-glucosidase activity to less than one-percent of wild-type levels. The suppressed gluc-2 mutant appeared to be a revertant to wild-type, gluc-2^+.

The phenotypic wild-type mutant strain, 12-2-68FA, was selected by ultraviolet irradiation of conidia from a gluc-2, inos, cot, "A" strain. subsequent screening by a modified "inositol-less death" technique, and recovery of a phenotypic gluc-2^+ colony. The genotype of the mutant strain recovered was gluc-2, su-1, leu, inos, "A". The cot mutation had changed to cot^+. Vegetative reisolation of individual conidia revealed homocaryosis for leu, cot, inos, and gluc-2^+ phenotypes. Heterocaryon tests showed gluc-2 to be dominant to its wild-type allele, gluc-2^+. The wild-type allele, cot^+, was shown to be dominant to cot; su-1 was recessive to su-1^+ in a heterocaryon.

Aryl-β-glucosidase levels regulated by the mutant were initially greater than wild-type as shown by qualitative tests and quantitative enzyme assays. Preliminary electrophoretic studies revealed no different pattern from a normal wild-type strain of N. crassa.

Genetic studies of the mutant gluc-2, su-1 strain, 12-2-68FA, crossed to standard wild-type strains, gluc-2^+, su-1^+, resulted in a 3:1 ratio of

phenotypic wild-types to gluc-2 phenotypes among random progeny of the first generation. In crosses of gluc-2, su-1, to gluc-2, su-1^{+}, (gluc-2 phenotype), a 1:1 ratio of phenotypic gluc-2^{+} to gluc-2 types was observed among random progeny. An attempt was made to prove the genetic nature of su-1 by crossing second generation phenotypic gluc-2^{+} strains selected from a cross of a gluc-2, su-1 x gluc-2^{+}, su-1^{+} to standard wild-type strains and examining progeny for gluc-2 phenotypes. No gluc-2 phenotypes were observed. Phenotypic gluc-2 random progeny were observed, however, when gluc-2^{+} phenotypic progeny from a cross of a second generation gluc-2^{+} phenotypic strain to a gluc-2 phenotypic strain. Among progeny of the third generation, the ratio of gluc-2^{+} to gluc-2 phenotypes varied from 3:1 to 1:1. The presence of the suppressor could be detected for three generations, but could not be detected in fourth generation progeny.

The possible involvement of other mutant markers, leu and cot, was observed. The segregation ratios of these genes indicated a modifier or modifiers of both mutant markers. Because of the extreme lability of su-1 it was impossible to demonstrate the exact mechanisms of suppressor action. Several hypotheses concerning the nature of the suppressor are advanced in this thesis.

The linkage of su-1 to other genes in Linkage Group VI was investigated. The genetic changes in the strain used for ultraviolet irradiation were noted throughout the course of this genetic investigation.

A MODIFIER OF β-GLUCOSIDASES IN *NEUROSPORA CRASSA*

by

Carroll Christian Miller

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the Faculty of the Graduate School at
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INTRODUCTION

Cellulose metabolism has been studied in Neurospora crassa from the standpoint of the role of genetic systems and their regulation of the metabolic pathway (Eberhart et al., 1961). The enzymes that attack cellulose include a swelling enzyme that destroys the crystalline aggregation of the long chains. These chains are then broken down by an internal cellulase to polyglucosides of eight to ten units in length. These products are then broken down to glucose by cellobiase (Reese, 1963).

Mahadevan and Eberhart reported in 1962 a dominant regulatory gene for aryl-β-glucosidase in N. crassa. This regulatory gene, gluc-1, was dominant to its wild-type allele, gluc-1^{+}. The situation suggested a repression of the wild-type by the mutant. The gluc-1 gene was concerned with regulation of aryl-β-glucosidase synthesis rather than with its structure (Mahadevan and Eberhart, 1962).

Another dominant regulatory gene for aryl-β-glucosidase has been isolated by ultraviolet irradiation of a gluc-1 strain by a method suggested by Myers in 1963. This mutant gene was called gluc-2 (Eberhart and Miller, unpublished). It was allelic with gluc-1 and repressed aryl-β-glucosidase activity to less than one-percent of wild-type levels. Both gluc-1 and gluc-2 seemed to have a super-repressor effect. Cellobiase activity and cellulase activity were not affected by the gluc-1 mutation. (Eberhart, Cross, and Chase, 1964).

Another mutation was induced, cell-1, which caused simultaneous constitutive production of both cellulase and cellobiase activity in N. crassa (Eberhart and Myers, 1966). This recessive gene was probably regulatory in function.

The purpose of this genetic study was to determine the nature of a modifier of gluc-2, a mutant gene regulating aryl-β-glucosidase synthesis in N. crassa by appropriate crosses, biochemical tests, heterocaryon studies, and vegetative reisolation of conidia.

MATERIAL AND METHODS

Chemicals

All vitamins used for growth media and for test media were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. The amino acids, leucine and tryptophan, used in nutritional test media were supplied by Eastman Chemical Company, Rochester, New York, and Nutritional Biochemicals Corporation, respectively.

The sugars, glucose and fructose, were supplied by Matheson, Coleman, and Bell, East Rutherford, New Jersey. Sorbose and fructose were obtained from Nutritional Biochemicals Corporation, while cellobiose was from Calbiochemical Company, Los Angeles, California.

Media

Stock cultures of N. crassa were maintained on a glycerol complete medium (GCP) (Eberhart, Cross, and Chase, 1964). For GSCP, glycerol sucrose complete medium, sucrose (1%) was added to GCP.

Vogel's minimal medium was prepared according to the procedure used by H.J. Vogel, 1956.

The vitamin stock solution used in complete media was prepared according to the procedure of Eberhart, Cross, and Chase, 1964.

Synthetic sorbose medium (Lester and Gross, 1958) was prepared in 800 ml quantities, and after autoclaving and cooling 12-15 ml were poured into individual petri plates.

For quantitative and qualitative testing of auxotrophs, the following minimal medium was used: 20 ml of Vogel's 50X and 780 ml of

distilled water. Sugar solutions were made up separately by making 200 ml of a 5% sugar concentration, 10 g of sugar (glucose, sucrose, etc.), and 200 ml of distilled water. The two solutions were autoclaved separately and while still hot, the two were mixed together and dispensed aseptically.

For qualitative testing of N. crassa strains for their aryl-β-glucosidase activity, glycerol complete medium (GCP) containing esculin monohydrate and ferric ammonium citrate was used. The concentrations of esculin and ferric ion were 20 mg and 100 mg per 100 ml GCP, respectively. Since esculin is decomposed rather quickly by heat, it is added to hot GCP media, dispensed while hot and autoclaved only a few minutes in 10 x 75 mm test tubes plugged with cotton.

Linear growth rates of putative mutant strains were measured on a purified Noble agar minimal media prepared as follows: 15 g Noble agar; 20 ml Vogel's 50X; 10 g sugar (1%); and 980 distilled water; plus any additional growth requirement such as an amino acid or vitamin, in the appropriate quantity. This media was autoclaved, cooled somewhat, and dispensed to growth tubes (Ryan et al., 1943).

Corn meal agar minimal media was used to cross strains of N. crassa. The media was prepared by adding 1.7 g corn meal agar, 1 ml vitamin stock solution to 100 ml distilled water, autoclaving, and dispensing while warm to sterile 17 x 150 mm tubes. Steep slants were made.

For forced heterocaryon studies, a Noble agar minimal medium in petri dishes was used. The media was prepared according to the method described for linear growth rates, but without any added vitamins or other growth factors. About 12-15 ml per plate were used. One-percent glucose was the carbon source.

Maintenance and Growth of Cultures

All strains of N. crassa used in this genetic study were maintained on a glycerol complete medium (GCP). They were transferred routinely every few days to maintain a fresh supply of conidia. To avoid possible contamination of the cultures by repeated vegetative transfer, a freshly conidiated culture of each strain was kept moisture-free at 4 C. For indefinite storage silica gel cultures were stored moisture-free at 4C (Perkins, 1962).

As conidia were needed for experiments, a glycerol complete agar slant was inoculated with the desired strain and placed in a 25 C incubator for optimal growth of five to seven days.

For colonial growth, that is, restricted mycelial growth of the fungus, two methods of producing colonies were employed. The first method made use of the colonial temperature sensitive (cot) mutation. The second method utilized sorbose as a means of producing colonial growth (de Serres and Kølmark, 1958).

By diluting filtered conidia to a concentration of 10^3 per ml sterile distilled water, pipetting a 0.1 ml sample of the conidial suspension onto the surface of a GCP agar petri plate, spreading this suspension evenly over the surface, and placing the petri plate in a 33 C incubator for twenty-four to forty-eight hours, individual colonies could be easily recovered and isolated in GCP slants for wild-type growth.

The sorbose media was inoculated by spreading a 0.1 ml sample of a 10^3 per ml concentration of conidia on the agar surface, and placing the plates in a 25 C incubator for forty-eight hours. Individual colonies were isolated into GCP slants for wild-type growth.

Selection of Strains

Standard St. Lawrence wild-type strains of N. crassa, STA4, 74-OR8-1a, and 74-OR23-1A were used in this genetic study to insure heterocaryon compatibility of the mutant strains (Figure 1). One Emerson wild-type strain was used, 692a. Wild-type strains having the prefix CM are random ascospore isolates of a cross made between 74-OR8-1a and 74-OR23-1A. They are CM-343(10)a, CM-343(16)A, CM-343(13)A, and CM-343(27)A.

The maternal irradiation strain, CM-329A(20)A was isolated as a random ascospore isolate of a cross between CM-75(9-5)a and 89601A. Genotypes of these strains are listed in Table 1. The genetic background of the irradiation strain is shown in Figure 2.

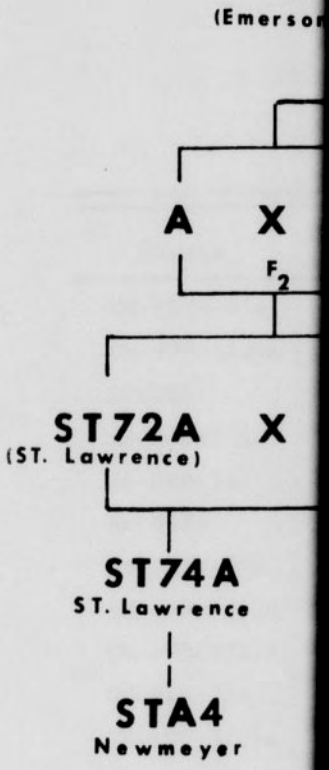
Mutant strains designated gluc-2 contain less than 1% of wild-type aryl-β-glucosidase activity as determined by qualitative and quantitative tests. Those strains designated gluc-2, su-1 have been shown to have greater than wild-type enzyme activity as determined by qualitative and quantitative tests. They also have produced gluc-2 progeny when crossed to wild-type strains.

Crosses and Ascospore Isolation

Crosses between strains of N. crassa of opposite mating type were made on corn meal agar (CMA) slants in 16 X 150 mm test tubes for random or linear ascospore isolation. A small inoculum of the proterithecial, or maternal parent, was inoculated on the corn meal agar slant, and after a period of seven to eight days of protoperithecial formation at 25 C, conidia of the opposite mating type were "rubbed" over the entire surface of the slant by using a platinum wire moistened with sterile distilled water. Subsequent perithecial formation indicated that a cross had occurred.

Figure 1. GENETICS OF WILD-TYPE STRAINS

The origin of the Oak Ridge and St. Lawrence wild-type strains is shown in this diagram from the Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire. STA4, 74-OR8-1a, and 74-OR23-1A are heterocaryon compatible.



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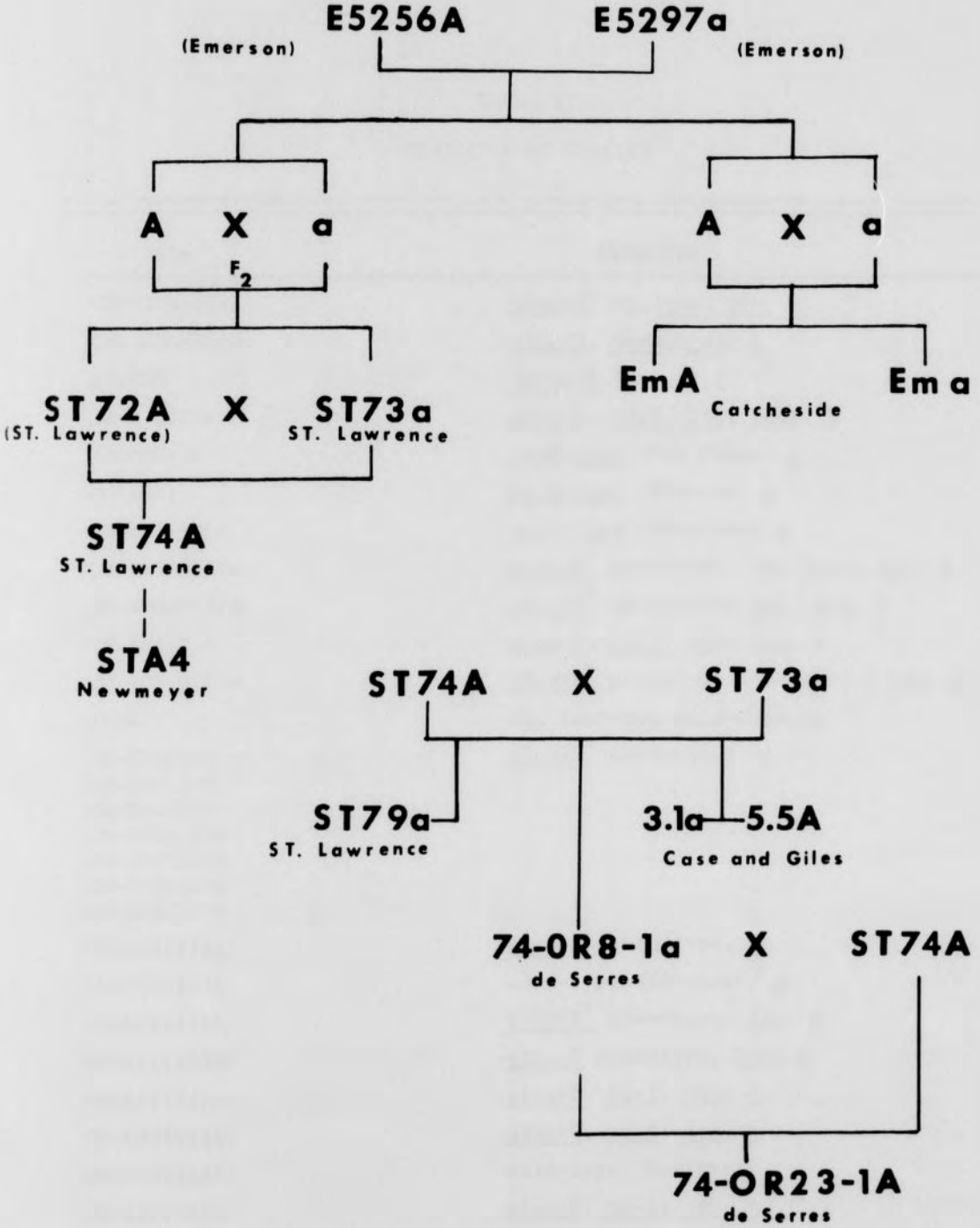


Department of Biological Sciences, Dartmouth College, Hanover, N.H.

TABLE 1

GENOTYPES OF STRAINS

Strain	Genotype
CM-75(9-6)a	gluc-2, nt, cot, ylo, a
CM-329A(20)A	gluc-2, inos, cot, A
89601A	inos, A
12-2-68F A	gluc-2, su-1, leu, inos, A
74-OR8-1a	wild-type (Oak Ridge) a
Em 692a	wild-type (Emerson) a
CM-343(10)a	wild-type (Eberhart) a
CM-400-9(7)A	gluc-2$^+$ phenotype, nt, inos, cot, A
CM-400-9(5)A	gluc-2$^+$ phenotype, cot, ylo, A
CM-356(2)A	gluc-2, su-1, cot, vlo, A
CM-400-9(1)a	gluc-2$^+$ phenotype (intermed.) ylo, a
STA4	St. Lawrence wild-type, A
CM-350(1)A CM-350(2)A CM-350(3)A CM-350(13)A CM-350(16)A CM-350(18)A CM-350(19)A	gluc-2$^+$ phenotypes, A
CM-422(11)a	gluc-2$^+$ phenotype, a
CM-343(13)A	wild-type (Eberhart) A
CM-422(12)A	gluc-2$^+$ phenotype, leu, A
CM-422(13)A	gluc-2 phenotype, leu, A
CM-422(21)A	gluc-2, su-1, leu, A
CM-422(28)a	gluc-2, su-1, leu, a
CM-343(16)A	wild-type (Eberhart)
CM-422(36)a	gluc-2, su-1, leu, a
CM-343(27)A	wild-type (Eberhart), A
CM-422(16)a	gluc-2 phenotype, a

TABLE 1

GENOTYPES OF STRAINS (CONTINUED)

Strain	Genotype
CM-422(26)A	*gluc-2* phenotype, *A*
CM-422(27)A	*gluc-2* phenotype, *A*
CM-350(18)A	*gluc-2*, *inos*, *cot*, *A*
CM-108(64)A	*gluc-2*, *tryp-2*, *A*
CM-356(40)A	*gluc-2*, *nt*, *cot*, *A*
451A	*ad-8*, *A*
CM-438(33)A	*gluc-2*$^{+}$ phenotype, *leu*, *A*
CM-440(21)a	*gluc-2*$^{+}$ phenotype, *leu*, *a*
74-OR23-1A	wild-type (Oak Ridge), *A*

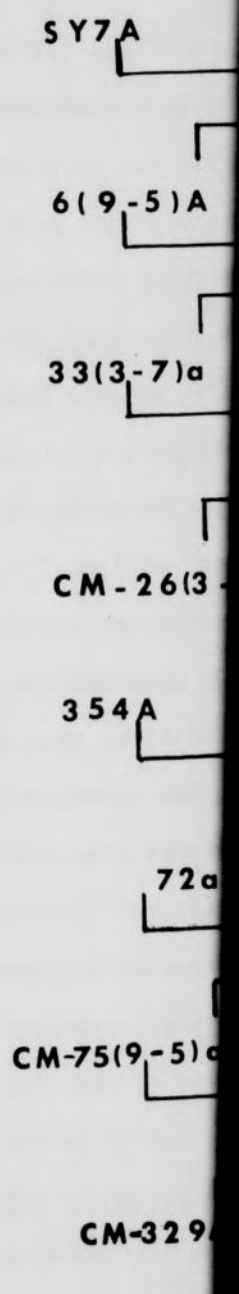
As the ascospores ripened and began to shoot spontaneously from the perithecia, linear dissections of individual asci were made by using techniques suggested by Emerson (1955). The individually isolated linear ascospores were allowed to ripen at 25 C for at least a week before heat-shock in an effort to increase germination. Heat-shock was accomplished by placing racks of small GCP tubes containing the individual ascospores in a constant 60 C temperature waterbath for twenty minutes. After heat-shock, the tubes containing the ascospores were placed in a 25 C incubator to germinate and conidiate.

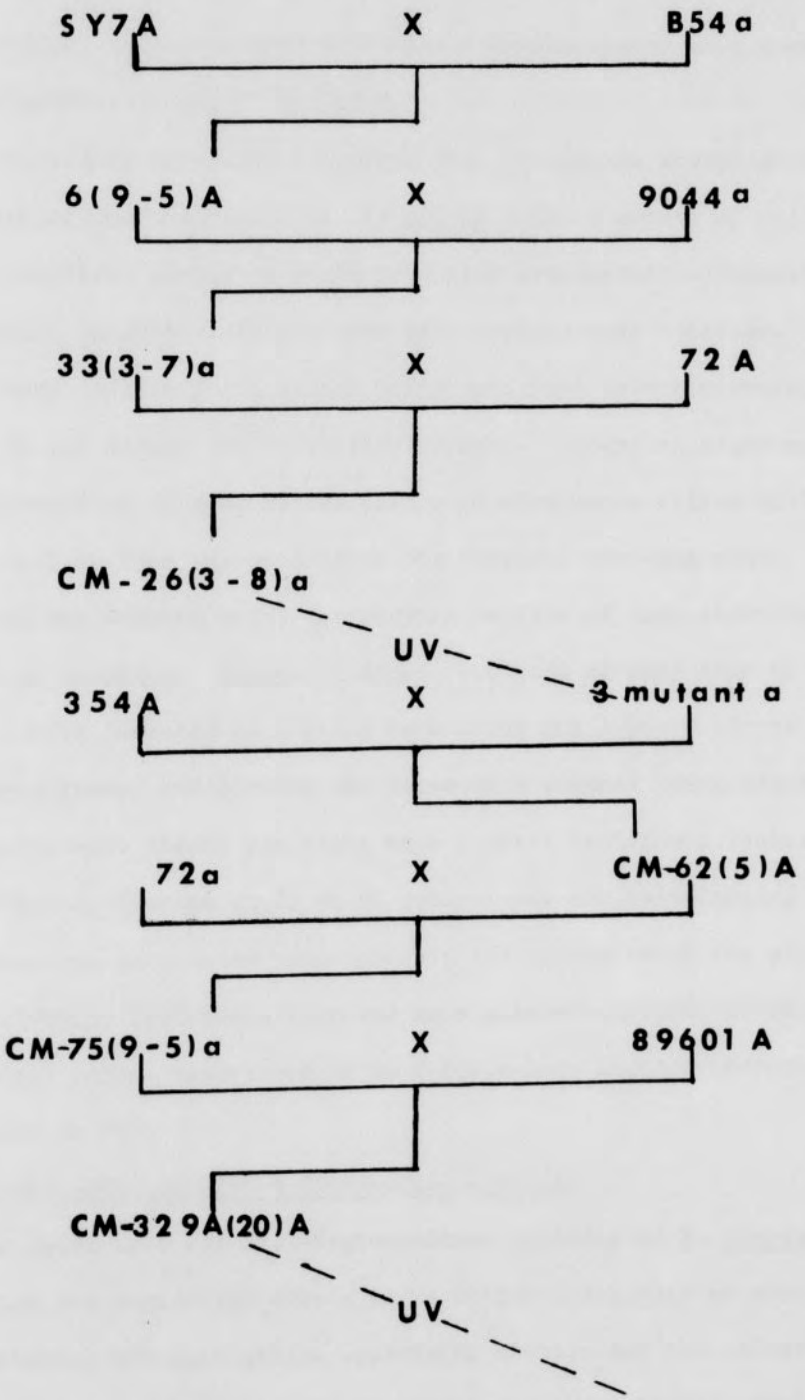
Random ascospores were collected approximately three weeks after perithicial formation to insure maximum germination. By adding a drop of sterile distilled water by pipette to the inside of the crossing tube in order to suspend a large number of ascospores, they could easily be

Figure 2. ORIGIN OF CM-329A(20)A, THE IRRADIATION STRAIN

Genotypes of strains shown in the diagram

SY7A	wild-type, A
B54A	Original gluc-1, a
6(9-5)A	gluc-1, A
9044a	cot, inos, nt, ylo, a
33(3-7)a	gluc-1, nt, cot, ylo, a
72A	pyr-1, A
CM-26(3-8)a	gluc-1, cot, a
354A	wild-type, A
No.3 mutant, a	Original gluc-2, cot, a
CM-62(5)A	gluc-2, A
CM-75(9-5)a	gluc-2, nt, cot, ylo, a
89601A	inos, A
CM-329A(20)A	gluc-2, inos, cot, A




SY7A
X
B54a
6(9-5)A
X
9044a
33(3-7)a
X
72A
CM-26(3-8)a
UV
3 mutant a
354A
X
72a
X
CM-62(5)A
CM-75(9-5)a
X
89601A
CM-329A(20)A
UV

removed and placed on a 6% agar block treated with a 10% Clorox solution for individual isolation into GCP tubes. Random spores were usually heat-shocked immediately after isolation.

In an effort to obtain tetratype asci during the investigation into the nature of the suppressor of the gluc-2 gene, a method of collecting asci as unordered groups of eight projected ascospores as suggested by D.D. Perkins (unpublished) was used with certain modifications. The crosses were made in petri plates which were kept inverted throughout incubation and during the collection of asci. Groups of eight ascospores were collected on 6% agar blocks placed on microscope slides built up to within 1 - 2 mm from the ostiole on the inverted crossing plate. Each agar block was exposed once, for varying periods of time depending upon the rate of shooting. Groups of eight that were clearly seen to be from one ascus were isolated as a group by cutting out a piece of the agar bearing the group, and placing the piece in a covered petri plate. Several asci were stored per plate at 4 C until individual isolation could be done. Storage at 25 C, in groups, was not satisfactory because some ascospores germinated spontaneously and contaminated the plates. The individually isolated ascospores were allowed to ripen at 25 C for several days before heat-shock at 60 C for twenty minutes and subsequent germination at 25 C.

Qualitative Tests for Aryl-β-Glucosidase Activity

As a quick test for aryl-β-glucosidase activity of N. crassa, a small inoculum of the conidiated strain to be tested was placed on the surface of an unslanted GCP agar medium containing esculin and the ferric ion (Fe^{+++}). The enzyme, if present in normal wild-type levels, would split

the esculin, and the resulting esculetin in combination with the ferric ion would form a black complex readily visible in a few hours. This very sensitive test was employed to detect mutant strains, that is, strains showing an abnormally low level of enzyme activity, such as gluc-2, or abnormally high levels of enzyme activity, such as gluc-2, su-1 strains. Because of the simplicity of the test, large numbers of progeny, or large numbers of putative mutants following ultraviolet irradiation of conidia could be tested quickly for their enzyme activity. The "esculin and iron" test was usually scored eighteen to twenty-four hours after inoculation. Wild-type, or gluc-2^+ strains gave a somewhat slower test than the so-called su-1 strains. The gluc-2 strains did not begin to split the esculin for at least forty-eight hours after inoculation.

As an additional check of enzyme activity, the degree of fluorescence that the inoculated "esculin and iron" test media showed several hours after inoculation was observed by viewing the tubes under a long-wave ultraviolet lamp (Ultra-Violet Products, Inc., San Gabriel, California). The esculin fluoresced in the blue range under ultraviolet light until the enzyme attacked the β-glucosidic linkages of the esculin, causing the fluorescence to be "quenched". The degree of "quenching" was directly proportional to the enzyme activity of the strain being tested.

Heterocaryon Tests

In order to establish the dominant or recessive nature of the gluc-2 and gluc-2^+ genes, heterocaryons were forced between strains of the same mating type, having different nutritional requirements. The method used was to inoculate each strain separately on a Noble agar minimal medium in a petri dish containing esculin and iron, and also to inoculate both

strains together on a third plate and observe the phenotypic response of the resulting heterocaryon on "esculin and iron", as opposed to the individual gluc-2 and gluc-2$^+$ phenotypes in the control plates.

In order to establish the dominant or recessive nature of su-1, essentially the same method was used as described above. Both gluc-2$^+$, su-1$^+$ and gluc-2, su-1$^+$ genotypes were forced to form heterocaryons with gluc-2, su-1 types, and the phenotypic response to "esculin and iron" media was observed and compared to the individual controls.

Vegetative reisolation of conidia was used as a method of detecting heterocaryosis in N. crassa. Five to seven day old cultures of N. crassa were used to obtain conidia for reisolation. These conidia were suspended in sterile distilled water, filtered through glass wool, diluted, counted by means of a Hausser Hi-Lite Counting Chamber, and plated on sorbose medium so that approximately 100 individual colonies would result per plate. The plates were put in a 25 C incubator, and after 48 hours individual colonies were isolated to GCP slants and allowed to conidiate. After conidiation of individual reisolates, they were tested for homocaryosis or heterocaryosis of a particular mutant gene.

Qualitative and Quantitative Nutritional Tests

For the qualitative testing of strains of N. crassa for possible nutritional requirements, a Vogel's minimal medium containing a 1% sugar concentration was used. The majority of the mutant strains used in this genetic study were auxotrophs and had to be tested for their individual nutritional response. Nutritional tests were made by placing a small inoculum of each isolate in 2.5 ml Vogel's minimal medium in 13 X 100 mm culture tubes. The inoculated media was observed about twenty-four hours later, and again in forty-eight hours for growth.

This simple test was used in several ways to test individual strains. Before they could be crossed, they were tested for their particular nutritional requirements by inoculating minimal media with and without the nutrient required and scoring the amount of growth.

After the ultra-violet irradiation of conidia from CM-329A(20)A, gluc-2, inos, cot, putative mutants that would not grow on cellobiose as a carbon source were selected. All possible mutants were tested for growth simultaneously on a 1% glucose and a 1% cellobiose minimal medium.

After it had been established that there were no significant growth differences by qualitative methods, linear growth rates of the putative mutant strains were measured by scoring the rate of growth of the mold in a growth tube (Ryan et al., 1943) at certain time intervals. The media used was a purified agar (Noble) minimal containing the required nutrient needed by the mold. The putative mutant strains were tested against standard wild-type strains on five different carbon sources, namely, one percent concentrations of glucose, sucrose, fructose, glycerol, and cellobiose.

Another quantitative nutritional test used to detect any nutritional differences in the mutant strains was a method whereby the mold was grown in a still liquid culture medium for forty-eight hours, and the resulting mycelial weights were compared to those of a standard wild-type and to those of the irradiation strain. Each strain was grown on four carbon sources, 1% glucose, fructose, sucrose, and cellobiose. The strains to be tested were allowed to conidiate for five to seven days on GCP media. At the end of this time the conidia were suspended in sterile distilled water and filtered through sterile glass wool. Fairly uniform conidial

concentrations were made of each strain, and one drop of the conidial suspension was added to 20 ml of medium in a 125 ml flask plugged with cotton.

Auxanographic plate testing was used to determine the amino acid requirement of the gluc-2, su-1 strain isolated from the ultra-violet irradiation of gluc-2, su-1^{+} conidia from CM-329A(20)A. A 1.5% Vogel's minimal medium containing one-percent glucose and fifty micrograms per ml of inositol was prepared, autoclaved, and allowed to cool to 40 C. Meanwhile, conidial suspensions of the strains to be tested were made. The conidial suspensions were added to the warm agar media (de Serres, Kølmark and Brockman, 1962) and each poured into five petri dishes. The agar was allowed to cool and harden. Rather concentrated solutions of each of the twenty amino acids were prepared. A small circle of sterile filter paper was soaked in each amino acid solution and placed on top of the agar surface. Four circles were used per plate. The plates were stored at 25 C for twenty hours. At the end of this time they were observed, and the amino acid causing wild-type growth was noted.

The "Inositol-less Death" Technique

The suppressor of gluc-2 was isolated by a technique known as "inositol-less death" (Lester and Gross, 1958). Certain changes were made in this method in an effort to uncover mutants in the regulation or structure of the cellobiase enzyme or aryl-β-glucosidase enzyme. Other changes were made in order to reduce the number of amino acid and vitamin requiring mutants usually selected by this method. The colonial temperature sensitive (cot) mutant marker was used in the recovery of putative mutants rather than the sorbose plating method used by Lester and Gross.

The strain, CM-329A(20)A, whose genome included gluc-2, cot, inos, and "A" mating type was selected to be the irradiation strain.

A fresh culture of the irradiation strain grown for five days on a GCP slant in a 17 X 150 mm culture tube was used to obtain conidia for ultra-violet irradiation. To ensure maximum growth, the GCP media was supplemented with 32 µg/ml inositol. The conidia were harvested by adding several ml sterile distilled water to the slants agitating on a Vortex mixer, and filtering the resulting suspension through glass wool into a sterile conical screw-cap centrifuge tube and spun in a Sorvall table-top centrifuge (Serial No. 4804329) at 2500 RPM for five to ten minutes. The supernate was decamted. The conidia were resuspended again using the Vortex. The conidia were washed twice by decanting the supernate, resuspending in distilled water, and recentrifuging. It has been shown that exogenous aryl-β-glucosidase is removed from the conidial surface by washing (Eberhart, 1961).

The conidia were counted by using a Hausser Hi-Lite counting chamber, and the concentration was adjusted to 40 X 10^7 per ml. The conidia were kept agitated as much as possible during preparation for irradiation to discourage heterocaryon formation. The conidia were acid treated with 0.1 N HCl by adding 0.5 ml of 0.2 N HCl to 0.5 ml of the conidial suspension and shaking rather vigorously for four minutes to remove still further any traces of exogenous enzyme. At the end of this period the acid was neutralized by adding 19 ml of a 0.1 M phosphate buffer, pH 6.0. This neutralized suspension containing approximately 10^7 per ml conidia was ready for ultra-violet irradiation.

A 1 ml sample of the neutralized conidial suspension was taken and diluted serially with sterile distilled water to a concentration of

10^3 per ml. By plating 0.1 ml of this dilution on a GSCP (glycerol sucrose complete media) and placing the petri dish at 32 C for forty-eight hours, the percentage rate of survival after irradiation could be calculated easily by comparing the number of viable colonies recovered from a similar dilution of irradiated conidia plated. About 25% survival was considered optimal by Lester and Gross.

The remaining 19 ml of the conidial suspension was poured into the bottom half of a sterile petri dish and exposed to ultraviolet light (GE germicidal lamp - 3 w) for sixty seconds. The suspension was swirled constantly during this time at a distance of about ten centimeters from the light source. Immediately following irradiation a 1.0 ml sample was taken, serially diluted to 10^3 per ml, and a 0.1 ml sample was plated on GSCP media. The petri dish was placed in a 32 C incubator.

The remaining 17 ml portion was poured into a flask containing 153 ml Vogel's minimal medium supplemented with 1% sucrose, 25 µg/ml inositol, and 50 µg/ml chloramphenicol. The flask was placed in a 25 C waterbath and shaken for six hours to allow germination of wild-types. At the end of this time the suspension was filtered through glass wool and washed three times by alternate centrifugation and resuspension in sterile distilled water using plastic screw-cap centrifuge tubes in a Sorvall table-top centrifuge at 4500 RPM for about twenty minutes.

One milliliter allquot portions were spread over the surface of a highly purified (Noble) agar minimal medium containing 1% cellobiose. Ten plates were placed in a 32 C incubator for different periods of starvation. At the end of forty-eight hours two plates were supplemented with 1% glucose and 100 µg inositol and replaced in the 32 C incubator.

The same procedure was repeated on four plates at the end of seventy-two hours of starvation, and on four plates at the end of ninety-six hours of starvation. As the mutant colonies grew colonially they were dissected from the plate and individually isolated into GSCP tubes.

No amino acids or vitamins were added to the recovery plates. This was done in order to reduce sharply the number of amino acid and vitamin requiring mutants usually selected by this "inositol-less death" technique of mutant selection.

As mutants unable to synthesize cellobiose were being sought, each mutant colony recovered was tested qualitatively on 1% cellobiose minimal and 1% glucose minimal simultaneously. Each mutant selected for its slow growth on cellobiose was tested for its "esculin and iron" reaction also.

RESULTS

The Isolation of a Suppressor of the gluc-2 Regulatory Gene

In an attempt to induce mutations in the genes regulating cellobiase enzyme activity, two mutant strains were found that appeared to be revertants to the wild-type in their ability to attack β-glucosidic linkages. Conidia from the gluc-2, inos, cot strain, CM-329A(20)A were irradiated by ultra-violet light, and mutants were screened by using a modified "inositol-less death" technique. Two hundred colonies were recovered (Materials and Methods, p. 17).

Each colony was transferred to a GSCP slant and allowed to conidiate at 25 C. All were tested for their growth on 1% cellobiose and 1% glucose simultaneously. This was accomplished by placing a very small inoculum directly into 2.5 ml liquid medium. No significant differences were noted in seven of the nine colonies tested. Two of the nine failed to grow on either carbon source, indicating an amino acid or vitamin requirement. Preliminary testing by adding vitamin stock solution to inositol supplemented medium revealed that the nutritional requirement was not a vitamin. Auxanographic plate testing of the mutant strains indicated that an amino acid, leucine, was required for growth (Material and Methods, p. 17). A heterocaryon test was made by simultaneously inoculating the two leucine requiring mutants on Vogel's minimal Noble agar containing one-percent glucose. There was no growth, indicating that they were probably allelic or functionally identical.

More exhaustive nutritional tests were made by growing one of the leucine requiring mutants, 12-2-68FA, an Oak Ridge wild-type, 74-OR23-1A,

and the irradiation strain, CM-329A(20)A, in 20 ml Vogel's minimal liquid containing four different carbon sources, namely, one-percent concentrations of glucose, cellobiose, fructose, and sucrose, supplemented with 50 mg/l inositol, and 0.5 mg/ml leucine. Each strain was tested by filtering conidial suspensions of each and adding one drop of the suspension to each of four 125 ml flasks containing the supplemented minimal media. These flasks were allowed to stand for forty-eight hours without shaking. At the end of this time the mycelia was harvested, washed, and dried. No significant differences in weight were noted between the three strains (Table 2).

The nine strains selected were also tested for the "Es-Fe" reaction (Materials and Methods, p. 13). Three of the nine putative mutants were phenotypically gluc-2^+ or wild-type in their β-glucosidase activity. One of the three, 12-2-68FA, having the additional leucine requirement was selected for genetic studies. Its exact genome was uncertain, but it had additional markers of leu, inos, and cot^+. By way of comparison, the strain whose conidia were irradiated, CM-329A(20)A had a genome that included gluc-2, inos, and cot. To explain the cot^+ phenotype of 12-2-68FA vegetative reisolates of conidia of the maternal irradiation strain were tested for their cot character. It was found that a small percentage of conidial reisolates were cot^+, indicating heterocaryosis of the strain that was irradiated, CM-329A(20)A (Table 3). Conidial reisolates of the mutant strain, 12-2-68FA, were all cot^+.

The heterocaryotic nature of the irradiation strain, CM-329A(20)A offers one explanation of the recovery of a leu mutant from the screening and recovery of mutant colonies by a modified "inositol-less death" method.

TABLE 2

QUANTITATIVE NUTRITIONAL TESTS

Strain	Genotype	Dry mycelia wt. in mgs			
		1% glucose	1% fructose	1% sucrose	1% cellobiose
12-2-68FA	gluc-2, su-1, leu, inos, A	44	28	33	28
CM-329A(20)A	gluc-2, inos, cot, A	40	28	33	31
74-OR8-1a	wild-type Oak Ridge	45	26	31	32

Each strain was inoculated into 20 ml Vogel's minimal medium in a 125 ml flask and allowed to grow in still cultures for forty-eight hours. The resulting mycelia were filtered, washed, dried at 60 C, and weighed.

I was trying to avoid selection of amino acid or vitamin requiring mutants that are selected in large numbers by the Lester and Gross technique, so the recovery plates were not supplemented with amino acids or additional vitamins. If the leu mutant colony were cot^+ some growth at 33 C might be expected so that it could be selected from the recovery plate as a colony. It should be mentioned that Lester and Gross report a large number of leu mutants by their "inositol-less death" method.

Since the mutant strain, 12-2-68FA, appeared to have even greater than wild-type levels of β-glucosidase activity, further quantitative experiments were made. Induction experiments (Table 4) verified initial observations that aryl-β-glucosidase activity was greater than wild-type (Beck, unpublished). Preliminary electrophoretic studies revealed no new or different electrophoretic pattern from the normal wild-type strains of N. crassa (Madden, unpublished).

TABLE 3

SUMMARY OF VEGETATIVE REISOLATES OF CONIDIA

Strain	Genotype	No. colonies isolated from sorbose at 25 C			"Es-Fe" test at 25 C of individual reisolates			Cot test at 33 C of individual reisolates			Nut. tests of individual reisolates		
	Tests:	1	2	3	1	2	3	1	2	3	1	2	3
CM-329A(20)A	gluc-2, inos, cot, A	26	58	62	26^{-}	58^{-}	62^{+}	26^{-}	54^{-a} 4^{+}				62^{-b}
1st. gen.													
12-2-68FA	gluc-2, su-1 leu, inos, A	48	60		48^{+}	59^{+} 1^{-}		47^{+} 1^{-}	60^{+}				
2nd. gen.													
CM-356(2)A	gluc-2, su-1 cot, ylo, A	14	60		14^{+}	60^{+}			60^{-}			60^{+}	
Unordered TT ascus.													
CM-400-9(1)a	gluc-2^{+} phenotype, ylo, cot^{+}	47			47^{-+}								
CM-400-9(3)a	gluc-2, cot^{+}	44			44^{-}								
CM-400-9(5)A	gluc-2^{+} phenotype, cot^{+}	41			41^{-+c}								
CM-400-9(7)A	gluc-2^{+} phenotype, cot	49			47^{+} 1^{-+} 1^{--}			49^{-}					

TABLE 3

SUMMARY OF VEGETATIVE REISOLATES OF CONIDIA (CONTINUED)

Strain	Genotype	No. colonies isolated from sorbose at 25 C			"Es-Fe" test at 25 C of individual reisolates			Cot test at 30 C of individual reisolates			Nut. tests of individual reisolates		
Tests:		1	2	3	1	2	3	1	2	3	1	2	3
Linear ascus													
CM-370(1-1)	gluc-2^{+} phenotypes	48			48^{+}								
CM-370(1-3)		57			57^{+-}								
CM-370(1-5)		44			44^{+-}								
CM-370(1-8)		30			30^{+}								
3rd. gen.													
CM-422(21)A	gluc-2, su-1 leu, A	40			40^{+}			40^{+}					
CM-422(36)a	gluc-2, su-1 leu, a	35			35^{+}			35^{+}					
CM-422(28)a	gluc-2, su-1 leu, a	41			41^{+}			41^{+}					
CM-343(10)a	wild-type (Eberhart)	58			58^{+}			58^{+}			58^{+}		

The method is described in Material and Methods.

[a] The minus sign indicates colonial growth. The plus sign indicates wild-type growth.

[b] The minus sign indicates no growth on minimal medium. The plus sign indicates growth on minimal medium.

[c] The minus-plus or plus-minus sign means an intermediate reaction.

TABLE 4

β-GLUCOSIDASE AND CELLOBIASE ACTIVITY OF SUPPRESSED *GLUC-2* STRAINS

Strain No.	Genotype	Es-Fe test at 25 C	Freezing and PEA Intact cells X	Freezing and PEA Intact cells Y	Cell free ext. X	Cell free ext. Y
			O.D. at 410 if O.D. at 600=1.0 X 1000		O.D. / 10 min. X 1000	
1st. gen.						
12-2-68FA	*gluc-2*, *su-1*, *leu*, *inos*, *A*	+ +	575	800	45	50
2nd. gen.						
CM-356(2)A	*gluc-2*, *su-1*, *cot*, *ylo*, *A*	+ +				
Control						
74-OR23-1A	*wild-type* Oak Ridge, *A*	+	175	450	18	12
			non-induced, non HCl treated O.D./min. 10^8 cells X 1000 all Y activity			
3rd. gen.						
CM-422(21)A	*gluc-2*, *su-1*, *leu*, *A*	+ +				250.0
CM-422(28)a	*gluc-2*, *su-1*, *leu*, *a*	+ +				225.0
CM-422(36)a	*gluc-2*, *su-1*, *leu*, *a*	+ +				190.0
Control						
74-OR23-1A	*wild-type* Oak Ridge	+				51.0
			Units/10^8 cells/min. filtered, frozen, washed, conidia all Y activity			
4th. gen.						
CM-438(33)A*	*gluc-2*$^+$ phenotype, *leu*, *A*	+ +				27.3
CM-440(21)a	*gluc-2*$^+$ phenotype, *leu*, *a*	+ +				23.0
Control						
74-OR23-1A	*wild-type* Oak Ridge	+				9.7

TABLE 4

β-GLUCOSIDASE AND CELLOBIASE ACTIVITY OF SUPPRESSED GLUC-2 STRAINS (CONTINUED)

* See Table 3

* These strains do not produce gluc-2's when crossed to a wild-type. For enzyme assays see Reta Beck (unpublished): 1-27-69; 2-19-69; 10-9-69; and 10-27-69; Dept. of Biology, UNC-G, Greensboro, N.C.

Genetic studies were begun in an effort to determine the nature of the mutation or reversion of the gluc-2 regulatory gene to a gluc-2^+ phenotype. Vegetative reisolation of individual conidia of 12-2-68FA did not give strong evidence for heterocaryosis of gluc-2 and gluc-2^+. The dominance of gluc-2 over its wild-type allele was established, and it will be discussed later in this thesis.

Genetics of the Suppressor Mutation

The mutant strain, 12-2-68FA, was crossed to a standard wild-type strain, 74-OR8-1a, and was also crossed to a gluc-2 strain, CM-75(9-6)a. These crosses were CM-350 and CM-356 as listed in Table 5. The genotypes of the parental strains are found in Table 1. The genetic background of the wild-type and the gluc-2 strains is shown in Figures 1 and 2.

The results of crosses, CM-350 and CM-356 are found in Table 5. The ratio of gluc-2^+ to gluc-2 phenotypes among random progeny from CM-350 was approximately 3:1, indicating the action of a suppressor gene. This suppressor of gluc-2 was called su-1. The suppressed gluc-2 strains were tentatively given the genotype, gluc-2, su-1.

The 3:1 ratio of gluc-2^+ to gluc-2 phenotypes among random progeny could have been due also to heterocaryosis of the mutant strain, 12-2-68FA,

TABLE 5

LIST OF CROSSES

Cross No.	Parents	No. picked	% germination	No. tested	Segregation of mutant markers gluc-2	gluc-2+	leu	leu+	cot	cot+
1st. gen.										
CM-350	12-2-68FA X 74-OR8-1a	50	80%	40	13	27	18	22	18	22
CM-356	CM 75(9-6)a X 12-2-68FA	100	70%	70	37	33			17	16
CM-365	Em 692a X 12-2-68FA	one linear ascus			4	4				
CM-370	CM-343(10)a X 12-2-68FA	two linear asci			0	16			0	16
CM-397	CM-343(10)a X 12-2-68FA	5 unordered asci				all+				
CM-400	CM-75(9-6)a X 12-2-68FA	9 unordered asci			7 gluc-2 asci one 1:1 ascus one TT ascus					
CM-417	CM-343(10)a X 12-2-68FA	100	57%	57	0	57			1	56
2nd. gen.										
CM-420	CM-343(10)a X CM-400-9(7)A	100	73%	73	1	72			35	38
CM-421	CM-343(10)a X CM-400-9(5)A	100	25%	25	0	25			7	18
CM-422	CM-343(10)a X CM-356(2)A	100	36%	36	10	26	8	28	12	24
CM-431	CM-400-9(1)a X STA4	100	62%	62	1	58			13	44
CM-432	CM-356(2) X 74-OR8-1a	100	60%	60	0	60	0	60	27	33
CM-453	CM-350(1)A X 74-OR8-1a	50	72%	36	0	36				
CM-454	CM-350(2)A X 74-OR8-1a	50	56%	28	0	28				
CM-455	CM-350(3)A X 74-OR8-1a	50	62%	31	0	31				
CM-459	CM-350(13)A X 74-OR8-1a	50	92%	46	0	46				
CM-460	CM-350(16)A X 74-OR8-1a	50	62%	31	0	31				
CM-461	CM-350(18)A X 74-OR8-1a	50	72%	36	0	36				
CM-462	CM-350(19)A X 74-OR8-1a	50	52%	26	0	26				
3rd. gen.										
CM-434	CM-422(11)a X CM-343(13)A	50	36%	18	0	18			0	18
CM-435	CM-422(12)A X 74-OR8-1a	50	52%	26	0	26			0	26

TABLE 5

LIST OF CROSSES (CONTINUED)

Cross No.		No. picked	% germination	No. tested	Segregation of mutant markers					
					gluc-2	gluc-2$^+$	leu	leu$^+$	cot	cot$^+$
CM-436	CM-422(13)A X 74-OR8-1a	50	40%	20	0	20			0	20
CM-438	CM-422(21)A X 74-OR8-1a	50	68%	34	17	17			19	15
		100	61%	61	28	33			28	33
CM-439	CM-422(28)a X CM-343(16)A	50	32%	16	9	7	13	3	6	10
CM-440	CM-422(36)a X CM-343(27)A	50	52%	26	10	16	16	10	12	14
		100	31%	31	0	31				
CM-445	CM-343(10)a X CM-422(21)A	50	46%	23	0	23	10	13	0	23
CM-468	CM-422(16)a X STA4	50	54%	27	0	27				
CM-469	CM-422(26)A X 74-OR8-1a	50	48%	24	0	24				
CM-470	CM-422(27)A X 74-OR8-1a	50	24%	12	0	12				
CM-475	CM-422(36)a X CM-350(18)A	50	55%	29	14	15	14	14	11	18
CM-477	CM-422(36)a X CM-108(64)A	50	76%	38	21	17	22	16	0	38
CM-478	CM-422(36)a X CM-356(40)A	50	50%	25	12	13	18	7		
CM-506	CM-422(21)A X 74-OR8-1a	30	94%	28	5	23	16	12		
CM-507	CM-343(10)a X CM-422(21)A	30	64%	19	0	19	8	11		
CM-508	CM-422(36)a X STA4									
CM-509	CM-422(28)a X STA4	50	60%	30	6	24	16	14		
CM-510	STA4 X CM-422(28)a	50	60%	30	4	26	17	13		
CM-511	STA4 X CM-422(36)a	50	14%	7	0	7	5	2		
CM-498	CM-422(36)a X 451A	50	72%	36	0	36				
CM-499	CM-422(28)a X 451A	50	28%	14	0	14				
		61	84%	51	0	51				
4th. gen.										
CM-502	CM-438(33)A X 74-OR8-1a	50	68%	34	0*	34	23	11		
CM-505	CM-440(21)a X 74-OR23-A	50	96%	48	10*	38	25	23		

* < normal wild-type response to Es-Fe test, but > gluc-2 response

although only one gluc-2 colony was recovered from a total of sixty. This did not strongly indicate heterocaryosis (Table 3). It was possible also, that the 3:1 ratio could have been due to a pseudo-wild-type strain (Pittinger, 1954).

Fincham and Day (1963) described these as aneuploids or disomics in ascospores (n+1). A cross is made between two auxotrophic strains mutant in distinct and complementary but closely linked loci. As a result haploid prototrophic recombinants will be formed with a frequency dependent on the amount of crossing over between the mutant sites. Secondly, any ascospore which carries homologous mutant chromosomes will be expected to be prototrophic if the mutations are in complementary loci. There was no evidence from vegetative reisolation of individual conidia that the gluc-2, su-1 strains were pseudo-wild-types. See Table 3. Pseudo-wild-types resemble heterocaryons in the vegetative life cycle of N. crassa (Pittinger, 1954).

Ordered asci were not dissected from crosses, CM-350 or CM-356, as only random ascospores were thought necessary at the time. In an attempt to obtain tetratype asci, as well as parental ditype asci, a new cross, CM-370, was made between CM-343(10)a and 12-2-68FA (Table 5). Only two complete asci were obtained, and all linear spore isolates were gluc-2^+ phenotypes.

Crosses CM-397 and 417 were made in an effort to obtain large numbers of unordered asci and random ascospores, respectively. It was observed upon analysis of these crosses that gluc-2 phenotypes were no longer being produced by crosses of gluc-2, su-1 to wild-type strains.

One complete ascus was dissected from CM-365. Results showed a 1:1 segregation of gluc-2 to gluc-2^+ progeny (Table 5).

Several unordered asci were collected from a cross, CM-400, having the identical parental strains used in CM-356. Results are shown in Table 5. The tetratype ascus was selected for further genetic studies. One ascospore isolate representing each of the four types was crossed to a wild-type strain. There seemed to be intermediate ranges of aryl-β-glucosidase activity as determined by the qualitative "Es-Fe" test in two of the three wild-type phenotypes isolated from the tetratype ascus from the cross CM-400. Vegetative reisolation of individual conidia from each phenotype was done, and results show no mixture of phenotypes in any of the four types isolated from the unordered ascus (Table 3).

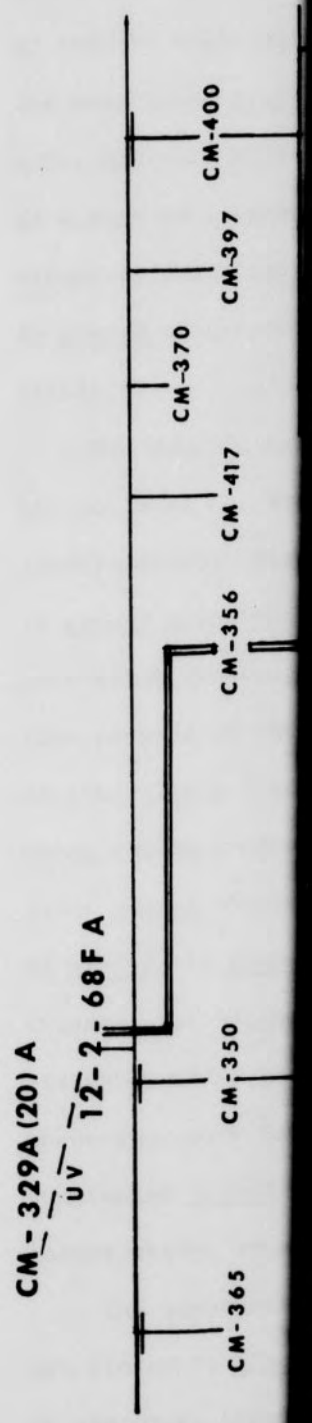
It should be noted that one phenotypic gluc-2 vegetative reisolate was found using conidia from strain CM-400(7). It is not known whether the finding is significant to the problem, or whether the gluc-2 strain was due to a contaminant. The other unordered ascospore isolates gave no unusual or unexpected phenotypes in conidial reisolates.

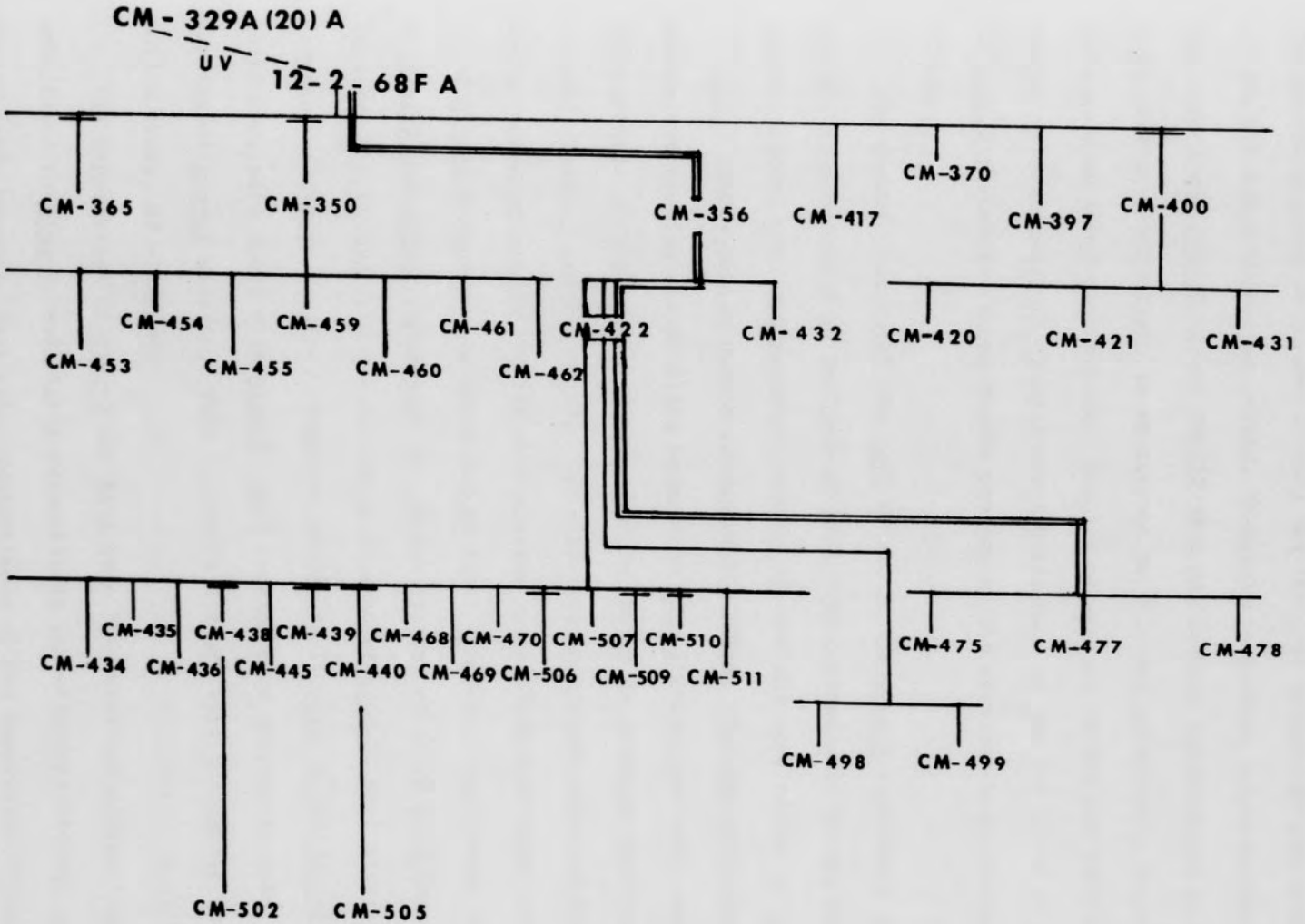
An interesting observation was that while wild-type progeny from random ascospore isolates from the cross CM-422 in 10 out of 15 crosses made yielded one-quarter gluc-2 phenotypes, the wild-type progeny from the cross, CM-350, when crossed to a standard wild-type produced no gluc-2 phenotypes (Table 5 and Figure 3). Attempts to recover gluc-2 progeny from fourth generation crosses of phenotypic gluc-2^{+} strains isolated from CM-422 to standard wild-type strains were unsuccessful (Table 5 and Figure 3).

Throughout this genetic study only Oak Ridge or St. Lawrence wild-type strains, or progeny of CM-343 were used to be crossed to the gluc-2, su-1 mutant strains. This was done in order to maintain heterocaryon compatibility in Neurospora stock strains (Garnjobst and Wilson, 1956).

Figure 3. GENETICS OF su-1

In this diagram are shown all crosses made in this study. Beginning with the ultraviolet irradiation of CM-329A(20)A and the isolation of 12-2-68FA, four generations are shown. The long double lines denote a cross of a gluc-2, su-1 strain to a gluc-2, su-1^{+} strain. The short double line above each cross number means that gluc-2 phenotypic progeny were isolated from a gluc-2, su-1 strain crossed to a standard wild-type.




CM-329A(20)A
UV
12-2-68FA
CM-365
CM-350
CM-356
CM-417
CM-370
CM-397
CM-400
CM-453
CM-454
CM-455
CM-459
CM-460
CM-461
CM-462
CM-422
CM-432
CM-420
CM-421
CM-431
CM-434
CM-435
CM-436
CM-438
CM-445
CM-439
CM-440
CM-468
CM-469
CM-470
CM-506
CM-507
CM-509
CM-510
CM-511
CM-475
CM-477
CM-478
CM-498
CM-499
CM-502
CM-505

Reciprocal crosses were made in only one instance because of the inability of the Oak Ridge wild-type strain, 74-OR8-1a to produce protoperithecia. The suppressed gluc-2 strain CM-422(28)a was crossed reciprocally to STA4, a St. Lawrence wild-type, in an attempt to rule out cytoplasmic inheritance as a mode of suppressor action. Results of crosses CM-509 and CM-510 are strong evidence against cytoplasmic inheritance, as the 3:1 ratio of gluc-2^+ to gluc-2 phenotypes among random progeny is the same for both crosses (Table 5).

The genetic nature of the su-1 effect on the gluc-2 regulatory gene has not been answered by analysis of data from crosses made during this genetic study. First generation crosses yielded expected ratios of gluc-2^+ to gluc-2 phenotypes in random ascospore isolation. Results of second generation crosses are puzzling because no gluc-2 phenotypes were recovered from crosses of the wild-type progeny from CM-350 to standard wild-type strains (Table 5 and Figure 3). The wild-type phenotypes selected from among random progeny of CM-356 when crosses to standard wild-types produced gluc-2 phenotypes in three out of eight crosses. Segregation ratios of gluc-2^+ to gluc-2 phenotypes for random progeny of third generation crosses, but varied from 3:1 to 1:1 in crosses of gluc-2, su-1 strains to standard wild-type strains. Expected ratios of 1:1 gluc-2^+ to gluc-2 phenotypes were found when gluc-2, su-1 strains were crosses to non-suppressed gluc-2 strains. This 1:1 ratio did not vary throughout the entire study, as expected.

The suppression of gluc-2 may have been the result of a single gene not linked to gluc-2, and highly recessive to its wild-type allele, su-1^+. It appeared, however, that other explanations of the suppressor effect

should be explored because of the complex interaction among several mutant markers segregating in the strains of N. crassa used in this study. Several modes of suppressor action are hypothesized in Table 6. The behavior of other mutant markers is observed throughout the remainder of this section of the thesis.

Forced Heterocaryons for Dominance Studies

The dominance of gluc-1 over its wild-type allele had already been established (Mahadevan and Eberhart, 1962). Since the dominance of gluc-2 was not known, heterocaryons were forced between certain gluc-2 and gluc-2^+ strains having the same mating type, but different nutritional requirements (Materials and Methods, p. 14).

In all three combinations of mutant strains that formed heterocaryons, the gluc-2 phenotype on "Es-Fe" media was clearly dominant (Table 7). The individual strains used as controls gave their expected responses. It was concluded that the resulting gluc-2 phenotype in all three heterocaryons formed was evidence that gluc-2 is dominant to its wild-type allele gluc-2^+.

Dominance studies of su-1 and its allele, su-1^+ (assuming that su-1 is a single gene) indicate that su-1 is recessive to su-1^+ because the gluc-2 phenotype results from heterocaryon formation between a gluc-2, su-1 strain with a wild-type response to "Es-Fe" and a gluc-2^+, su-1^+ strain with a wild-type response to "Es-Fe", also. These results are shown in combinations 1 and 2 of Table 8. It should be emphasized that both strains are phenotypic wild-types. In combinations 3 and 4 of Table 8 heterocaryons were formed between gluc-2, su-1 strains and gluc-2, su-1^+ strains. These individual strains are wild-type and gluc-2 phenotypically. The gluc-2 phenotype was observed after heterocaryon formation. It was therefore concluded that su-1 is recessive to su-1^+.

TABLE 6

HYPOTHESES CONCERNING SU-1

Hypothesis	Results Consistent with Cross	Results Consistent with Other	Results Inconsistent with Cross	Results Inconsistent with Other
1. *su-1* is a single gene suppressor for *gluc-2*	CM-350, 422, 438, 440, 506, 509, 510, 439		CM-370, 397, 417, 432	
2. *su-1* is very unstable	CM-370, 397, 417, 432, 498, 499, 440			
3. *su-1* alters the *gluc-2* phenotype to *gluc-2*$^+$		all conidial reisolates are *gluc-2* phenotypes		
4. *su-1* effect is due to pseudo-wild-type strains or to heterocaryosis	CM-350, 396			conidial reisolates of CM-356-2A, CM-422(21)A and CM-422(28)a
5. *su-1* is maternally inherited	CM-370, 397, 417, 420, 421, 445, 507		CM-422, 509, 510	
6. *su-1* strains suppress *cot*	CM-350, 370, 400, 422,	conidial reisolates of CM-329A(20)A, 12-2-68FA, CM-422(21)A, CM-422(28)a, and CM-422(36)a	CM-356	conidial reisolates of CM-356(2)A

TABLE 6

HYPOTHESES CONCERNING SU-1 (CONTINUED)

Hypothesis	Results Consistent with Cross	Results Consistent with Other	Results Inconsistent with Cross	Results Inconsistent with Other
7. *su-1* and the suppressor of *cot* are different genes	CM-356, 475			
8. CM-329A(20)A carries both *su-1* and *cot* suppressors	CM-396, 480	conidial reisolates of CM-329A(20)A	CM-507	
9. *leu* is a tag for *su-1*	CM-350, 422, 432, 438, 439, 506, 478, 356	all *su-1* strains crossed are *leu* requiring, or carry a modified *leu* marker		
10. no *leu* progeny when *su-1* strains no longer produce *gluc-2* progeny when crossed to a wild-type even though strain requires leucine	CM-432			
11. *su-1* is modified by certain wild-type strains	CM-370, 397 417, 420, 421, 445, 507		CM-356, 422	
12. *su-1* is epistatic		conidial reisolates of 12-2-68FA, CM-356(2)A, CM-422(21)A, CM-422(28)a, CM-422(36)a *gluc-2*$^{+}$ phenotype in all suppressed *gluc-2* strains		

TABLE 6

HYPOTHESES CONCERNING SU-1 (CONTINUED)

Hypothesis	Results Consistent with: Cross	Results Consistent with: Other	Results Inconsistent with: Cross	Results Inconsistent with: Other
13. su-1 is a spontaneous mutation	CM-396, 480	conidial reisolates of CM-329A(20)A	CM-387	
14. su-1 is a super suppressor	CM-422, 432		CM-356, 475	
15. su-1 acts at the tRNA level		wild-type levels of aryl-β-glucosidase produced		no change in electrophoretic mobility of aryl-β-glucosidase from normal wild-type
16. su-1 is the result of a chromosomal aberration		loss of su-1 in vegetative transfer, loss of gluc-2 phenotype. Meiotic chromosome studies		

TABLE 7

FORCED HETEROCARYONS FOR DOMINANCE STUDIES OF *Gluc-2* AND *Gluc-2*$^{+}$

Strain No.	Genotype	Growth on Minimal Noble agar	"Es-Fe" Reaction
1. A. CM-163(14)a	*gluc-2*$^{+}$, *pan-1*, *al-2*, *a*	--	+
B. CM-348(44)a	*gluc-2*, *leu*, *cot*, *a*	--	--
C. (A + B)		++	--
2. A. CM-350(18)A	*gluc-2*, *inos*, *cot*, *A*	--	--
B. CM-158(4)A	*gluc-2*$^{+}$, *nt*, *cot*, *A*	--	+
C. (A + B)		+	--
3. A. CM-158(6)a	*gluc-2*, *nt*, *cot*, *a*	--+	--
B. CM-163(14)a	*gluc-2*$^{+}$, *pan-1*, *al-2*, *a*	--	+
C. (A + B)		+++	--

Strains A and B were inoculated separately as controls and also together on a Vogel's minimal Noble agar medium containing 1% glucose, Esculin (20 mg/100 ml), and ferric ammonium citrate (100 mg/100 ml). The "+" sign indicates growth or a positive "Es-Fe" reaction. The "-" sign indicates no growth or a negative "Es-Fe" reaction. The "-+" sign indicates slight growth.

TABLE 8

FORCED HETEROCARYONS FOR DOMINANCE STUDIES
OF *su-1* and *su-1*$^{+}$

Strain No.	Genotype	Growth on Minimal Noble agar	"Es-Fe" Reaction
1. A. CM-163(14)a	*gluc-2*$^{+}$, *su-1*$^{+}$, *a1-2*, *a*	--+	+
B. CM-356(13)a[a]	*gluc-2*, (*su-1*$^{?}$), *nt*, *cot*, *a*	--	+
C. (A + B)		++	--
2. A. 12-2-68FA	*gluc-2*, (*su-1*), *leu*, *inos*, *A*	--	++
B. CM-158(4)A	*gluc-2*$^{+}$, *su-1*$^{+}$, *nt*, *cot*, *A*	--	+
C. (A + B)		+	--
3. A. CM-356(10)A[b]	*gluc-2*, (*su-1*$^{?}$), *nt*, *cot*, *A*	--	+
B. CM-350(18)A	*gluc-2*, *su-1*$^{+}$, *inos*, *cot*, *A*	--	--
C. (A + B)		+	--
4. A. CM-356(10)A	*gluc-2*, (*su-1*$^{?}$), *nt*, *cot*, *A*	--	+
B. CM-348(22)A	*gluc-2*, *su-1*$^{+}$, *inos*, *cot*, *A*	--	--
C. (A + B)		+	--

Strains A and B were inoculated separately as controls and also together on a Vogel's minimal Noble agar medium containing 1% glucose, Esculin (20 mg/100 ml), and ferric ammonium citrate (100 mg/100 ml). The "+" sign indicates growth or a positive "Es-Fe" reaction. The "-" sign indicates no growth or a negative "Es-Fe" reaction. The "-+" sign indicates slight growth.

[a] Proof of *su-1* not established. Homocaryotic for *gluc-2*$^{+}$ phenotype.

[b] Produced *gluc-2* progeny when crossed to a wild-type. Heterocaryotic nature of *gluc-2*$^{+}$ and *gluc-2* types uncertain. (See Table 3)

The dominance of *cot*$^{+}$, the wild-type allele of the colonial temperature sensitive mutant marker, *cot*, was established (Table 9).

The dominance relationships of *leu* and *leu*$^{+}$ were not investigated, and therefore have not been established.

Genetic Evidence for the Involvement of the leu Mutation in the Suppression of gluc-2

The original strain that was irradiated, CM-329A(20)A, whose genome included **gluc-2**, *inos*, and *cot*, responded well to inositol supplemented minimal medium, but there was no indication of other nutritional requirements. After irradiation of CM-329A(20)A, (see Tables 1 and 5), the selected mutant, **12-2-68FA**, was found to require leucine. When it was crossed to a wild-type (Cross CM-350), the progeny showed a 1:1 ratio of *leu* to *leu*$^{+}$ types.

All strains selected as suppressed **gluc-2** strains, based on their ability to produce one-quarter **gluc-2** progeny when crossed to a wild-type were found to require leucine, with one exception. The second generation strain, CM-356(2)A, did not require leucine for growth, but when crossed to a wild-type, produced one-quarter *leu* progeny, indicating that the leucine requirement of CM-356(2)A had been modified.

The involvement of the *leu* mutation in the suppression of **gluc-2** seemed apparent. There was evidence that as a **gluc-2**, *su-1* strain changes and becomes unable to produce **gluc-2** phenotypes, its ability to produce *leu* progeny disappeared as well (Table 5, Crosses CM-422 and CM-432). It should be emphasized, however, that the suppressed **gluc-2** strains retained their original leucine requirement, but no leucine requiring progeny were recovered from a cross to a wild-type strain (Table 5, Cross CM-432). Minimal test results were incomplete for most crosses.

TABLE 9

FORCED HETEROCARYONS FOR DOMINANCE STUDIES OF *cot* AND *cot*$^+$

Strain No.	Genotype	24 hr. growth on GCP with "Es-Fe" added			Heterocaryon formation after 24 hr. growth on minimal agar with "Es-Fe" added.	
		Enzyme activity		Colonial growth	Enzyme activity	Colonial growth
		25 C	33 C	33 C	33 C	33 C
A. CM-163(14)	*gluc-2*$^+$, *pan-1*, *al-2*, *cot*$^+$, *a*	+	+	*cot*$^+$	+	*cot*$^+$
B. CM-158(6)	*gluc-2*, *nt*, *cot*, *a*	-	-	*cot*	-	*cot*
C. (A + B)					-	*cot*$^+$

Strains A and B were inoculated separately and also together on a Vogel's minimal Noble agar medium containing 1% glucose, esculin (20 mg/100 ml), and ferric ammonium citrate (100 mg/100 ml) in a petri dish and placed in a 33 C incubator for 24 hours.

Strains A and B were also tested on glycerol complete medium containing esculin (20 mg/100 ml) and ferric ammonium citrate (100 mg/100 ml) in a culture tube at 25 C, and simultaneously at 33 C.

Results of crosses made by Isley (unpublished) indicate that CM-356(2)A had a modified or suppressed leucine requirement. Isley has also shown that the suppressed *gluc-2* strain, CM-422(21)A, after losing its ability to produce *gluc-2* progeny also lost its ability to produce *leu* progeny, although it retains its leucine requirement.

The Detection of a Gene Modifying the leu Mutation

Results of the cross CM-422 show a 3:1 ratio of leu^+ to leu mutant progeny as well as the 3:1 ratio of $gluc\text{-}2^+$ to gluc-2 phenotypic progeny. These results indicate suppressor or gene modifier action, or heterocaryosis of the parental strain, CM-356(2)A. Vegetative reisolation of fifty individual conidia of each parental strain was made, and each individual strain was tested on Vogel's minimal media. There were no nutritional mutants found among reisolates of either strain, strongly suggesting that the leucine requirement had been modified, or more specifically, suppressed (Table 3).

Further results indicated that as the gluc-2, su-1 strains change and are unable to produce gluc-2 progeny but retain their gluc-2 phenotype, that these strains also no longer produce leu mutants among their progeny (Table 5, Crosses CM-422 and 432). This observation strongly suggests the same mode of suppressor action for genes that seem to be physiologically unrelated.

Nutritional tests are incomplete for most of the crosses for several reasons. At the beginning of this genetic study the gluc-2 locus and the apparent suppression of the gluc-2 phenotype on "Es-Fe" media were the main concern. The involvement of the leu marker became apparent only as the study progressed. Therefore the observations are based on incomplete data, and the explanations are mostly speculative. It was beyond the scope of this thesis to investigate all questions posed by this investigation.

Since the research for this genetic study ended, Isley found that the irradiation strain, CM-329A(20)A, had a suppressed leucine requirement,

that is, it produced one-quarter leu mutants among random progeny from a cross to a wild-type. She also has shown that the irradiation strain is now a phenotypic wild-type, or gluc-2^+.

The genetics of the irradiation strain will be discussed later in this paper.

The Detection of a Gene Modifying the cot Mutation

It was stated earlier that the irradiation strain, CM-329A(20)A may have been heterocaryotic for the cot mutation, and its allele, cot^+, or that it carried in its genome a modifier of cot. It was also established that cot^+ is dominant to its cot mutant allele (Table 9). The results of the segregation of the cot and cot^+ markers are shown in Table 10. Cross CM-422 shows a 2:1 ratio of cot^+ to cot phenotypes, indicating that the cot mutation was modified in the parental gluc-2, su-1, leu strain (Table 1). This is evidence that a modifier of cot did segregate genetically. What relationship this modifier has to the modifiers of gluc-2 and leu remains unclear.

Genetic Evidence for the Modification of the Suppressor Gene by Wild-Type Strains

The complex interaction of genes that seem to be physiologically unrelated was obvious from the analysis of the crosses made during this genetic study. It would appear also by observing segregation ratios of gluc-2^+ to gluc-2 phenotypes recovered when a gluc-2, su-1 strain was crossed to a standard wild-type that certain of these wild-types, such as CM-343(10)a, might contribute modifiers that affect the behavior of su-1. In Table 11 evidence for this hypothesis is summarized. In Table 2 are found all crosses made in the study.

TABLE 10

INDIRECT EVIDENCE FOR THE SUPPRESSION OF *cot*

su-1 strain crossed to a wild-type (*cot*$^+$)	*cot* phenotype of the *su-1* strain at 33 C	Test for *cot* of individual reisolated conidia at 33 C		Cross number	Test for *cot* of individual random ascospore isolates at 33 C	
		cot	*cot*$^+$		*cot*	*cot*$^+$
1st. gen.						
12-2-68F	*cot*$^+$	Test 1 1	47	CM-350	18	22
		Test 2 0	60	CM-417		
2nd. gen.						
CM-356(2)A	*cot*	60	0	CM-422	12	24
				CM-432	27	33
3rd. gen.						
CM-422(21)A	*cot*$^+$	0	40	CM-438	47	48
CM-422(28)a	*cot*$^+$	0	35	CM-439	6	10
CM-422(36)a	*cot*$^+$	0	35	CM-440	12	14
su-1 strain crossed to a *gluc-2*						
1st. gen.						
12-2-68F	*cot*$^+$	(same as above)		CM-356	17	16

TABLE 10

INDIRECT EVIDENCE FOR THE SUPPRESSION OF cot (CONTINUED)

su-1 strain crossed to a wild-type (cot^+)	*cot* phenotype of the *su-1* strain at 33 C	Test for *cot* of individual reisolated conidia at 33 C	Cross number	Test for *cot* of individual random ascospore isolates at 33 C	
3rd. gen.					
CM-422(36)a	cot^+	(same as above)	CM-475[a]	11	18
			CM-477[b]	0	38

See Tables 1 and 5 for strains and crosses.
See Table 3.

[a] *su-1* crossed to a *gluc-2*, *cot*
[b] *su-1* crossed to a *gluc-2*, cot^+

TABLE 11

INDIRECT EVIDENCE FOR THE MODIFICATION OF *su-1* BY WILD-TYPE STRAINS

Cross No.	Parents	No. picked	% germination	No. tested	Es-Fe at 25 C	
					gluc-2	gluc-2$^+$
1st. gen.						
CM-370	CM-343(10)a X 12-2-68FA	2 linear asci			0	16
CM-397	CM-343(10)a X 12-2-68FA	5 unordered asci			0	40
CM-417	CM-343(10)a X 12-2-68FA	100	57%	57	0	57
2nd. gen.						
CM-420	CM-343(10)a X CM-400-9(7)	100	73%	73	1	72
CM-421	CM-343(10)a X CM-400-9(5)	100	25%	25	0	25
3rd. gen.						
CM-445	CM-343(10)a X CM-422(21)A	50	46%	23	0	23
CM-507	CM-343(10)a X CM-422(21)A	30	64%	19	0	19
CM-422*	CM-343(10)a X CM-356(2)A	100	36%	36	10	26

* This cross is the only exception to the observation that CM-343(10)a may modify *su-1*.

This observation of modification of *su-1* by a wild-type strain may not be a valid observation because *su-1* appeared to be highly labile or recessive, and the failure to recover one-quarter *gluc-2* phenotypes from a cross of *gluc-2*, *su-1* to a standard wild-type could have been due simply to the lability of *su-1*.

Genetics of the Irradiation Strain

There was sufficient evidence to show that the irradiation strain, CM-329(20)A, with the genome, *gluc-2*, *inos*, *cot*, was heterocaryotic for *cot* and cot^+, and/or carried a suppressor of *cot* at the time of vegetative reisolation of conidia (Table 3). It has already been shown that this strain whose origin is shown in Figure 2 included a suppressed *leu* mutation in its genome (Isley, unpublished).

In Table 12 several crosses are listed having the original strain that was irradiated, CM-329A(20)A, as a parent. The cross, CM-329, from which it was isolated is listed at the beginning of the table. The segregation of *cot* and cot^+ alleles among random progeny suggested either heterocaryosis or a *cot* modifier. The approximate ratio of cot^+ to *cot* was 2:1.

Results of CM-387 indicated a 3:1 ratio of cot^+ to *cot* among random progeny. The $gluc\text{-}2^+$ to *gluc-2* ratio was as expected. See Table 12. The results of CM-396 reveal a change in the cot^+ : *cot* ratio between the first and second random ascospore isolations. See Table 12. The percentage of germination remained the same. The *cot* mutation appeared to be changing even in the ascospore stage. The *gluc-2* : $gluc\text{-}2^+$ ratio remained the same, approximately 2:1, indicating a modifier or modifiers.

TABLE 12

GENETICS OF THE IRRADIATION STRAIN

Cross no.	Date of cross	Parents	No. picked	% germination	No. tested	Es-Fe at 25 C		Growth at 33 C		Growth on minimal at 25 C	
						gluc-2	gluc-2$^+$	cot	cot$^+$	leu	leu$^+$
1st. gen.											
CM-329	Sept., 1968	CM-75(9-5)a X 89601A*	37	59%	22	10	12	7	15		
2nd. gen.											
CM-387	Apr., 1969	CM-329(20)A CM-343(10)a	50	52%	26	13	13	6	20		
CM-396	Apr., 1969	CM-329(20)A CM-75(9-5)a	100	63%	63	44	19	45	18		
	May, 1970	(2nd. picking)	50	62%	31	20	11	5	26	10	21
CM-480	Sept., 1969	CM-422(36)a CM-329A(20)A	50	72%	36	0	36	0	36		

* See Tables 1 and 5 for genotypes of parental strains.

There were no gluc-2 phenotypes among random ascospore isolates of CM-480. The maternal strain was one of the gluc-2, su-1 strains from CM-422, CM-422(36)a. Whether the absence of gluc-2 phenotypes was su-1 action or whether CM-329A(20)A behaved as a normal wild-type at the time the cross was made is unknown. According to Isley (unpublished), however, CM-329A(20)A transferred from silica gel culture and allowed to conidiate gave a typical wild-type response to "Es-Fe" media. The irradiation strain had changed in its gluc-2 phenotype, its cot phenotype, and carried a suppressed leu marker.

Linkage Studies of the Suppressor Gene

The regulatory genes, gluc-1 and gluc-2, have never been successfully linked to other genes. There is some evidence that gluc-1 and its allele gluc-2, occupy a locus at the extreme end of the left arm of Linkage Group III in N. crassa (Eberhart and Miller, unpublished). This was based on 35 to 40% recombination with thi-4.

The suppressor (su-1) of gluc-2 has shown twenty-eight to thirty percent recombination with ylo in Linkage Group VI of the linkage may (Table 13 and Figure 4). The su-1 marker showed 42% recombination with tryp-2 on the right arm of Linkage Group VI.

Because of the complexity of the genetic interaction and the lability of the suppressor effect further linkage studies seem inadvisable.

TABLE 13

LINKAGE DATA

Cross No.	No. randoms picked	No. randoms germinated	% germ.	% recombination of *su-1* with segregating genes					
				cot	leu	inos	tryp-2	ylo	nt
I. CM-475	50	29	55%	48%	54%	43%			
gluc-2, *su-1*, *leu* X				P-15	P-13	P-16			
gluc-2, *su-1*$^{+}$, *inos*, *cot*				R-14	R-15	R-12			
J. CM-477	50	38	76%		50%		42%		
gluc-2, *su-1*, *leu* X					P-19		P-22		
gluc-2, *su-1*$^{+}$, *tryp-2*					P-19		R-16		
C. CM-356	103	70*	68%		a			30%	
gluc-2, *su-1*$^{+}$, *nt*, *cot*								P-27	
ylo X *gluc-2*, *su-1*, *leu*,								R-12	
inos									
E. CM-422	100	36	36%	b				28%	
gluc-2^{+}, *su-1*$^{+}$ X					c			P-26	
gluc-2, *su-1*, *cot*, *ylo*								R-10	

[a] Only 39 morphological wild-types were tested for linkage. No nut. linkage data could be obtained because of a morphological mutation segregating.

[b] No linkage data on *su-1* and *cot* because of the inability to distinguish *su-1* types from wild-types.

[c] 25% *leu* types recovered among progeny that were *gluc-2*$^{+}$ phenotypes. All *gluc-2*'s were *leu*$^{+}$. No evidence of linkage of *su-1* to *leu*, but not sure that CM-475 and 477 carry *su-1*.

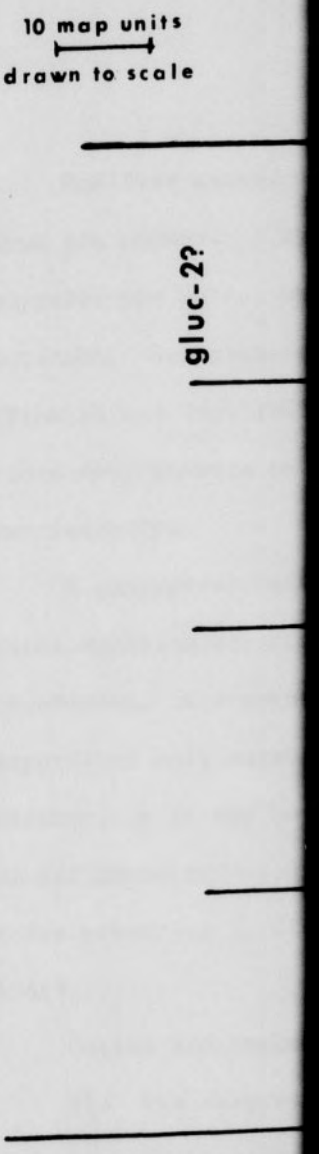
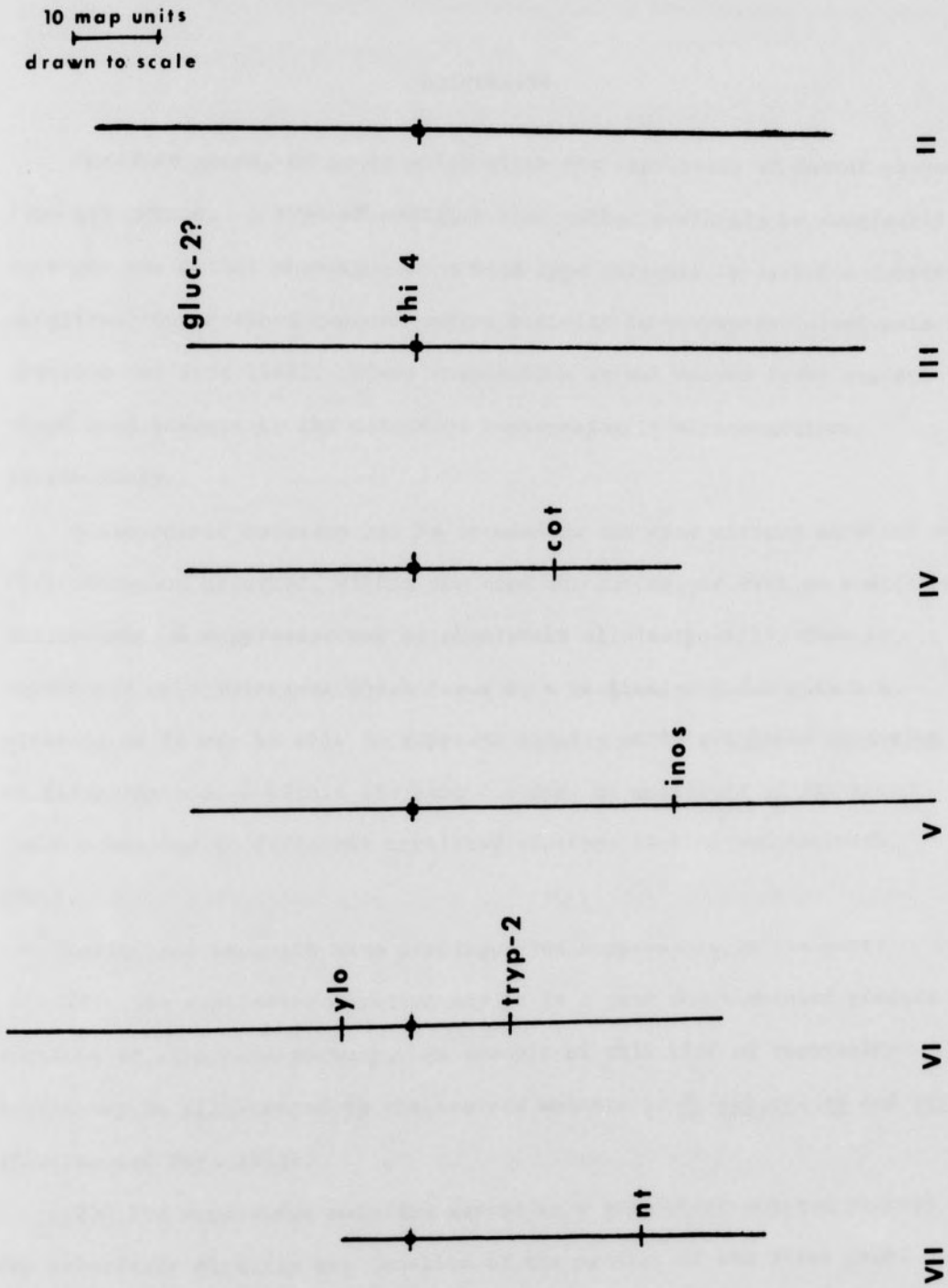


Figure 4. LINKAGE MAP OF NEUROSPORA CRASSA

Six of the seven linkage groups of Neurospora crassa are shown in this diagram. The genetic markers involved in the linkage of su-1 are shown in their proper relation to the centromere. The linkage of gluc-1, and its allele gluc-2 has not been established definitely.


10 map units
drawn to scale
II
gluc-2?
thi 4
III
cot
IV
inos
V
ylo
tryp-2
VI
nt
VII

DISCUSSION

Modifier genes, or genes which alter the expression of mutant phenotype are common. A type of modifier that either partially or completely reverses the mutant phenotype to a wild-type response is called a suppressor mutation. Suppressors restore enzyme activity to enzyme-deficient mutants (Fincham and Day, 1963). Close examination at the enzyme level has provided many answers to the nature of suppression in microorganisms, particularly.

A suppressor mutation may be located in the same cistron in which the first mutation occurred, within the same chromosome, or even in a different chromosome. A suppressor may be absolutely allele-specific, that is, it suppresses only mutations which occur at a particular codon within a cistron, or it may be able to suppress equally well, mutations occurring at different codons within the same cistron, or mutations of the same codon occurring in different unrelated cistrons (Gorini and Beckwith, 1966).

Gorini and Beckwith have distinguished suppressors in six ways:

(1) The suppressor mutation may be in a gene whose mutated product opens up an alternate pathway. An example of this kind of suppressor action may be illustrated by the acetate mutants in N. crassa, sp and car, (Fincham and Day, 1963).

(2) The suppressor mutation may be in a gene whose mutated product may substitute directly the function of the product of the first gene. The pyr and prol mutants in N. crassa are able to grow on minimal media

as the result of a suppressor which relieves the nutritional requirements of these mutants (Davis, 1962).

(3) The suppressor mutation provides cytoplasmic conditions which affect the structure of the finished product of the first mutated gene. The td24 mutant in N. crassa produces normal td activity when suppressed, but the level of activity is lower than in wild-type. The suppressor gene acts by altering the cellular environment so that the altered td protein may become active. This type of suppressor action is based on control at the functional rather than at the enzyme forming level (Yanofsky and Bonner, 1955).

(4) The suppressor mutation introduces an amino acid substitution within the same peptide chain, which reintegrates completely or partially, the protein function, with reappearance of the wild-type or wild-type like phenotype. Missense mutations in E. coli are good examples of this mode of action. The td mutants have been thoroughly investigated by numerous investigators, and these suppressors have elucidated the translation process in protein synthesis (Yanofsky, Helsinki, and Maling, 1962; Brody and Yanofsky, 1964; Brody and Yanofsky, 1963; Gupta and Khorana, 1966; Yanofsky and Bonner, 1955; Carbon, Berg, and Yanofsky, 1966; and Yanofsky, 1960).

(5) The suppressor reintroduces the correct code meaning by means of a frame shift within the same cistron in which the codon reading frame was altered by addition or deletion of a base pair. Nonsense mutations in N. crassa, yeast, and E. coli are examples of this mode of action (Terry, 1966; Seale, 1969; Case and Giles, 1968; Hawthorne and Mortimer, 1963; Gilmore, 1967; Magni and Puglisi, 1966; and Garen and Siddiqi, 1962).

(6) The suppressor mutation concerns one of the factors controlling the mechanism of transfer of genetic information from DNA to protein. "Ambivalent" mutations in the rII locus of phage T4 whose phenotypes can be reversed by suppressor mutations in its bacterial host, E. coli, suggest that a suppressor mutation in the bacterium can result in adding to the cell a new sensible coding unit, constituting a change in the genetic code (Benzer and Champe, 1962). In vitro studies of the mechanism of a nonsense mutation demonstrate that tRNA is the component of the protein synthesizing machinery responsible for genetic suppression (Englehart et al., 1965, and Capecchi and Gussin, 1965).

The first three modes of suppressor action may be termed "indirect suppressions". The second three types of action are a direct correction of the gene product altered by the original mutations. Types four and five may be called intragenic suppression, that is, both mutations are transcribed into the gene product. Type six is an alteration of a codon, and may be called an "informational" suppressor, either inherited or cytoplasmic.

The isolation of a suppressor for the gluc-2 regulation gene in N. crassa raised many questions about the nature of the mechanism of restoring the wild-type like phenotype to a mutant (gluc-2) that regulates production of less than one-percent of normal wild-type levels of aryl-β-glucosidase. For a rather short period of time after recovery of the suppressed gluc-2 mutant, 12-2-68FA, the su-1 mutation allowed production of aryl-β-glucosidase from two to three times the normal levels found in wild-types, as determined by both qualitative and quantitative enzyme tests. See Table 4. Electrophoretic patterns of the gluc-2, su-1 mutant

strains failed to show any new or altered protein from the normal wild-type (Madden, unpublished). These results are perhaps the best evidence for the regulatory function of su-1. It is possible that other physical properties of the enzyme were altered, such as K_m, pH, thermal stability, etc., but these properties were not investigated. Quantitative enzyme assays made several months after the isolation of the suppressed gluc-2 mutant revealed that aryl-β-glucosidase activity seemed identical to that of a wild-type strain (Madden, unpublished).

If su-1 is indeed a regulatory gene it could possibly be a counter regulator or a co-regulator with gluc-2, acting in the cytoplasm.

Models for gene regulation, such as the Jacob-Monod lac operon theory in E. coli, cannot be applied easily to eucaryotes such as N. crassa Jacob-Monod, 1960). Control systems in fungi are obviously very complex, and in question of whether operons exist in fungi is still open. Fincham (1970) in reviewing fungal genetics discussed this problem. He cited several gene clusters in N. crassa that have been called operons. The arom region of N. crassa has been studied perhaps more extensively than the others (Case and Giles, 1968; Rines et al., 1969; Burgoyne et al., 1969). This arom cluster, or operon, determines five enzyme activities associated with a protein of a molecular weight approximately 230,000. There may be subunits of the polypeptide chain, but there is no indication of how many (Burgoyne et al., 1969). More detailed analysis of protein structure is necessary. There is a strong suggestion that the arom cluster is a unit of translation, and therefore, of transcription.

No operator segments have been identified in fungi, and therefore, the analogy with bacterial operons is questioned (Fincham, 1970).

The complex genetic region (arom) discussed immediately preceding this statement is very likely to be units of transcription, but whether they are also units through which control is exercised is still debatable (Fincham, 1970; Gross, 1969).

The molecular weight of the aryl-β-glucosidase is relatively high, about 168,000. There is no knowledge of subunits, but this is a possibility for uncovering a mechanism for regulatory control of enzyme production in N. crassa.

Initially, a 3:1 ratio of gluc-2$^+$ phenotypes to gluc-2 phenotypes was observed (Table 5). See Genetics of the Suppressor Mutation on p. 27). This result indicated a single gene not closely linked to gluc-2. Subsequent crossing of the phenotypic gluc-2$^+$ progeny to standard wild-type strains filed to produce this 3:1 ratio, but a 4:0 ratio of gluc-2$^+$ to gluc-2 phenotypes. See Figure 3 and Table 5, crosses CM-350 and 453 through 462. These results suggest the highly recessive nature of su-1, a heterocaryosis of the mutant, 12-2-68FA, or the pseudo-wild-type nature of 12-2-68FA (Table 6), or that su-1 is lethal in certain genetic combinations.

The recovery of gluc-2 phenotypes from among progeny of the cross, CM-422, however, suggested that crossing the gluc-2, su-1 mutant to a non-suppressed gluc-2 favored the selection of the gluc-2, su-1 strains, that is, strains that could produce one-quarter phenotypic gluc-2 progeny. In Figure 3 is shown the transmission of su-1 through three generations. The question was then asked: Is su-1 specific for gluc-2? It would appear so on the basis of the foregoing discussion, however, the involvement of seemingly physiologically unrelated genes was apparent as analysis

of genetic data in Table 5 and in Table 10. Evidence for the involvement of the genes, leu and cot, is shown in Table 6. The separate headings in the Results section which deal with this subject are: (1) Genetic Evidence for the Involvement of the leu Mutation in the Suppression of gluc-2; (2) The Detection of a Gene Modifying the leu Mutation; and (3) The Detection of a Gene Modifying the cot Mutation.

Since other genes seemingly not related physiologically to su-1 were involved and suppressed by unknown mechanisms, the idea of nonsense suppression was advanced. Nonsense suppressors have been studied recently in N. crassa (Seale, 1968; 1969). Seale discovered a non-linked suppressor of the CRM negative (glutamate dehydrogenase-deficient) mutant, am-17, that causes the formation of a distinctly abnormal form of glutamate dehydrogenase, and also suppresses a number of mutants of the arom series, and certain td mutants (Case and Giles, 1968). No real identification of the suppressor of gluc-2 as a nonsense suppressor could be made for several reasons: (1) The recessive or labile nature of su-1; (2) The lack of obvious abnormal structural properties of the aryl-β-glucosidase enzyme; (3) The lack of CRM tests; and (4) The lack of necessary amino acid substitution analysis in N. crassa.

Forced Heterocaryons for Dominance Studies (Results, p. 35) clearly revealed the dominance of gluc-2 to its wild-type allele, gluc-2^{+}. See Table 7. The suppressor, su-1, is recessive to su-1^{+} in heterocaryons (Table 8). The dominant regulatory role of gluc-2 seems to be completely overturned by the action of su-1 in suppressed gluc-2 strains whose wild phenotype is expressed even though these strains are no longer able to produce gluc-2 phenotypes among random progeny when crossed to a standard

wild-type. Enzyme assays and electrophoretic patterns reveal no obvious change from ordinary wild-type strains (Madden, unpublished). These observations are evidence for the epistatic nature of *su-1*. Is *su-1* expressed instead of *gluc-2* when both are present at the same time? Is *gluc-2* absent when *su-1* is present, or do both genes act together to regulate production of aryl-β-glucosidase? Has *gluc-2* disappeared from the suppressed mutants? It would be interesting to force heterocaryons between the changed *gluc-2*, *su-1* strains, such as the original mutant, 12-2-68FA, and a wild-type, $gluc\text{-}2^{+}$, $su\text{-}1^{+}$, and compare the results with original results. This could be accomplished by using the combination 2. shown in Table 8. Is the *gluc-2* phenotype expressed now?

The involvement of the *leu* mutation in the suppression of *gluc-2* seems clearly apparent. Suppressor action occurred only when the *leu* mutation was present. There was one apparent exception to this statement; CM-356(2)A did not require leucine for growth, but upon analysis of CM-422 it was shown that the suppressed strain carried a modified *leu* mutation (Tables 1 and 5, crosses CM-356 and CM-422).

These observations indicate an apparent necessary role of the *leu* mutation in the suppression of *gluc-2*. Results of minimal tests on random progeny from crosses CM-422 and CM-432 in Table 5 reveal that as a *su-1* strain changes and becomes unable to produce *gluc-2* phenotypic progeny its ability to produce *leu* progeny disappears as well. The presence of a modifier of the *leu* mutation is also indicated by the ratios of leu^{+} to *leu* progeny as listed in Table 5. The relationship between *su-1* and *leu* is unknown, but it would seem that the *leu* locus is an invaluable clue for further investigations of the mechanism of suppressor action.

The cot mutation has been widely used in the genetic studies of the gluc-2 locus (Miller, unpublished), particularly in the mutant screening techniques following untra-violet irradiation of conidia of N. crassa. It has already been shown that the mutant, 12-2-68FA, was phenotypically cot^{+}. Vegetative reisolation of conidia from CM-329A(20)A, the strain used for ultra-violet irradiation, indicated heterocaryosis for cot and and its allele, cot^{+}. There was not strong evidence, however, for heterocaryosis of the cot alleles in the gluc-2, su-1 strain, 12-2-68FA (Table 3). Segregation ratios of cot and cot^{+} in random progeny from all crosses tested were erratic (Table 5). Genotypes of the strains involved are found in Table 1. These results of variable ratios indicate a modifier of cot. The question must be asked: Is there a significant relationship between the suppression of gluc-2 and the suppression of cot?

Three seemingly physiologically unrelated genes, su-1, leu, and cot, have exhibited abnormal or erratic segregation ratios throughout the genetic investigation. Gene modifier action seems indicated.

The suppressor of gluc-2, su-1, may have been modified by certain wild-type strains such as CM-343(10)a, as shown in the tabulated results on page . Failure to recover gluc-2 phenotypes from progeny of crosses in which CM-343(10)a was used as the maternal parent in all but one cross (CM-422), may have been due to the reversion of su-1 or the instability of the suppressor effect rather than the action of modifiers contributed by the maternal wild-type strain.

Phenotypic changes have occurred in the strain used for ultra-violet irradiation, CM-329A(20)A, as shown in Table 12 on page , since this research was completed. These changes include an apparent reversal of

the gluc-2 phenotype (Isley, unpublished). The cross, CM-396, shows changes in the ratio of cot to cot$^+$ between the first and second random ascospore isolations. The ratios of gluc-2$^+$ to gluc-2 phenotypes among random progeny have changed from 1:1 as shown for cross CM-329 to 4:1 in CM-480 where CM-329A(20)A was crossed to a third generation strain believed to carry the suppressor mutation. The recovery of leu progeny in the second random ascospore isolation of CM-396 was strong evidence for a suppressed leu mutation transmitted by CM-329A(20)A. These results in Table 12 indicate that the suppressors of cot, gluc-2, and leu may have been introduced by CM-329A(20)A. According to Isley (unpublished), CM-329A(20)A exhibits a normal wild-type response to the "esculin and iron" test. How these modifier genes are interrelated remains unclear.

Another hypothesis concerning the mechanism of suppressor action is that su-1 is not a chromosomal gene, but an extrachromosomal element of undetermined nature. Maternal inheritance as a mode of suppressor action seems to be ruled out by data shown in Table 6. Results of genetic analysis of the crosses are tabulated in Table 5.

There is no real evidence that the suppressor effect is due to epigenetic or extrachromosomal factors, but the possibility exists. McClintock (1951) discovered controlling elements in maize that modify or suppress gene action and in some instances produce mutational effects. The most extensively analyzed is a pair called Ac-Ds. In the presence of Ac but not in its absence, Ds is associated with apparent chromosome breaks at the locus at which it is found. At this locus a dicentric and a corresponding acentric chromatid may be formed. When this occurs the acentric chromatid, which is composed of the chromosomal segment extending

from Ds to the end of the arm, is lost at mitotic anaphase. Ds, in the presence of Ac has other remarkable properties. It can change position, and can show different linkage relations. Ds can also modify other genes near its location. It can be observed that the presence of Ds results in phenotypic expression similar to that determined by some mutant form of the gene. Ac responds to Ds, but may also itself control gene action at a locus where it is situated, causing chromosome breaks, mutation-like events, and it can undergo transposition.

Changes in the chromosomes causing abnormal structure and behavior should be included in this discussion of the reversal of the gluc-2 phenotype to a normal gluc-2^{+} phenotype. Breakage-fusion-bridge cycles may cause duplications or deficiencies of genetic material. These cycles may be demonstrated cytologically in organisms having large chromosomes, but in N. crassa with its extremely small chromosomes, visual detection is difficult.

Duplications are extra parts of chromosomes caused by breakage-fusion-bridge cycles. Alleles may be present several times causing unusual effects. Genetic effects of deficiencies in chromosomes include phenotypic changes in an organism due to the loss of a gene or genes.

Translocation, an aberration in which a fragment of one chromosome becomes attached to a non-homologous chromosome, either reciprocally or non-reciprocally, alters linkage associations for genes contained in the exchanged chromosomal segments. Semisterility is another genetic effect of translocations. Translocated genes may not function normally.

Inversions also put genetic material into new associations. This can result in instability of gene action (Levine, 1970).

Any of the chromosomal aberrations mentioned could offer an explanation for the phenotypic changes observed in this genetic study. Absence of cytological proof makes this explanation of the change in the gluc-2 phenotype speculative.

In summary, several questions are raised about the nature of the suppressor, su-1. Is su-1 a single gene at a locus distinct from the gluc-2 locus that restores enzyme activity to the gluc-2 regulatory mutant gene? Is the mode of action of su-1 indirect or direct, as distinguished by Gorini and Beckwith (1966)? Is aryl-β-glucosidase activity restored by su-1 identical to normal wild-type activity? Is su-1 a counter-regulator or a co-regulator with gluc-2? Does su-1 act in the cytoplasm? Can su-1 be described as an operator gene as described in the Jacob-Monod lac operon model of gene regulation? Are there subunits of the aryl-β-glucosidase polypeptide chain, and if so, can they provide mechanisms of control for regulation of enzyme activity in N. crassa? Is su-1 allele-specific, or is it site-specific and locus non-specific, as nonsense suppressors are? Is su-1 epistatic to gluc-2? Is su-1 lethal in certain genetic combinations? Is su-1 expressed when gluc-2 is absent, or do both genes act together as regulators? Is leu always associated with su-1? Is the suppressor effect due to a chromosomal aberration, such as a breakage-fusion-bridge cycle with duplications and deficiencies, a translocation, or an inversion? Is su-1 due to extrachromosomal factors, such as controlling elements, Ac-Ds, in maize as described by McClintock (1951)?

SUMMARY

Studies of a new suppressor effect on the gluc-2 gene are reported here. It is believed this effect is due to the su-1 gene. Genetic studies of su-1 indicated initially single gene action at a locus other than gluc-2. The gluc-2 regulatory gene in turn may have a super-repressor effect, since it repressed aryl-β-glucosidase activity to less than one-percent of wild-type levels. The suppressed gluc-2 mutant appeared to be a revertant to wild-type, gluc-2^+.

The phenotypic wild-type mutant strain, 12-2-68FA, was selected by ultraviolet irradiation of conidia from a gluc-2, inos, cot, "A" strain, subsequent screening by a modified "inositol-less death" technique, and recovery of a phenotypic gluc-2^+ colony. The genotype of the mutant strain recovered was gluc-2, su-1, leu, inos, "A". The cot mutation had changed to cot^+. Vegetative reisolation of individual conidia revealed homocaryosis for leu, cot, inos, and gluc-2^+ phenotypes. Heterocaryon tests showed gluc-2 to be dominant to its wild-type allele, gluc-2^+. The wild-type allele, cot^+, was shown to be dominant to cot; su-1 was recessive to su-1^+ in a heterocaryon.

Aryl-β-glucosidase levels regulated by the mutant were initially greater than wild-type as shown by qualitative tests and quantitative enzyme assays. Preliminary electrophoretic studies revealed no different pattern from a normal wild-type strain of N. crassa.

Genetic studies of the mutant gluc-2, su-1 strain, 12-2-68FA, crossed to standard wild-type strains, gluc-2^+, su-1^+, resulted in a 3:1 ratio of

phenotypic wild-types to gluc-2 phenotypes among random progeny of the first generation. In crosses of gluc-2, su-1, to gluc-2, su-1^{+}, (gluc-2 phenotype), a 1:1 ratio of phenotypic gluc-2^{+} to gluc-2 types was observed among random progeny. An attempt was made to prove the genetic nature of su-1 by crossing second generation phenotypic gluc-2^{+} strains selected from a cross of a gluc-2, su-1 x gluc-2^{+}, su-1^{+} to standard wild-type strains and examining progeny for gluc-2 phenotypes. No gluc-2 phenotypes were observed. Phenotypic gluc-2 random progeny were observed, however, when gluc-2^{+} phenotypic progeny from a cross of a second generation gluc-2^{+} phenotypic strain to a gluc-2 phenotypic strain. Among progeny of the third generation, the ratio of gluc-2^{+} to gluc-2 phenotypes varied from 3:1 to 1:1. The presence of the suppressor could be detected for three generations, but could not be detected in fourth generation progeny.

The possible involvement of other mutant markers, leu and cot, was observed. The segregation ratios of these genes indicated a modifier or modifiers of both mutant markers. Because of the extreme lability of su-1 it was impossible to demonstrate the exact mechanism of suppressor action. Several hypotheses concerning the nature of the suppressor are advanced in this thesis.

The linkage of su-1 to other genes in Linkage Group VI was investigated. The genetic changes in the strain used for ultraviolet irradiation were noted throughout the course of this genetic investigation.

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