GENETIC STUDY OF SEX AND CULTURAL CHARACTERS IN NEUROSPORA CRASSA

Thesis by

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CONTENTS

INTRODUCTION

The importance of genetic study of the ascomycetes results from the following considerations:

Genetic study of higher plants and animals indicates (1) that a reassortment of genes takes place in meiosis and (2) that the change of linkage known as crossing-over results from an interchange between homologous chromosomes in meiosis. Cytological study shows that meiosis provides a mechanism for the reassortment of genes and indicates in an unconvincing way that crossing-over might be the result of a meiotic mechanism. The genetic evidence that meiosis effects reassortment and crossing-over is completely convincing despite the fact that it is purely inferential. It is based on the assumption that two classes of gametes result from meiosis of a single diploid nucleus heterozygous for one gene and are equally numerous. Furthermore, it is assumed, that when crossovers occur, they are the result of a <u>single</u> diploid nucleus having produced two types

of exceptional gametes. However, the technical difficulties of isolating the gametes resulting from meiosis of a single diploid nucleus in the higher plants and animals have prevented any experiments.

In the ascomycetes the eight ascospores usually produced in a sac are the result of meiosis of a single nucleus. Moreover, the sacs in some species are long and narrow and the products of division are not jumbled together but are arranged in a row so that their positions indicate their relationships. Micromanipulation and aseptic culture technique enable one to isolate these ascospores in the proper order and to study the genetic characters of the mycelia resulting from their germination. If the history of the chromosomes were known, the clear cut genetic characters found in the fungi would soon settle the questions raised. But it is probable that no sex phenomenon has received so many contradictory interpretations at the hands of different writers as that of the ascomycetes. A single form has been described as being parthenogenetic, or having a single sexual fusion, or a double sexual fusion. Scarcely less divergent are the interpretations of the divisions in the ascus. There seems to be a general agreement that the chromosomes undergo what resembles

a meiotic division but the smallness of the chromosomes and the difficulty of counting has led to a variety of interpretations. The results presented here show that in N. crassa a double sexual fusion best explains the data.

An analytical knowledge of the genetics of an organism depends upon coordinating the nuclear history with the genetic behavior. This means that before the mechanism of meiosis can be studied by means of the arrangement of the ascospores in the long ascus of N. crassa, its nuclear history and sex phenomena must be known accurately. Once they are understood, it will provide an excellent object for studying the immediate effect of meiosis. The object of this study is to investigate meiosis directly rather than inferentially, and to attain this object understandingly, the nuclear history of the organism must first be described. The present paper contains the results of a genetic and cytological study of Neurospora crassa.

store are sufficiently accurate. A needle, inserted in a short glass rod, is stuck to the brass tube with modelling clay so that it just clears the surface of the microscope stage. Movement of the needle in an arc about the steel rod is obtained by a wooden lever pointing away from the operator. The base of the brass tube fits solidly in this wooden lever and the direction of motion is changed by a one-half centimeter wooden rod placed so that it is conveniently situated at the right hand of the operator. This rod is fitted solidly in an upright wooden handle which rests upon the table. The wooden rod must have enough spring so that it will allow the handle to rest on the table while the brass rod is being moved upward and downward. Sensitivity of the movement in the arc about the steel rod depends upon the length of the needle and the length of the wooden lever by the principle of similar triangles. For moderate magnifications one to five sensitivity is sufficient using a ten centimeter needle and a fifty centimeter lever.

A large cork is fitted firmly on the upper end of the brass tube. A two centimeter test tube which has been cut off about two centimeters from the base is fitted solidly in a hole in the base of the cork.

A fifty by two by two centimeter wooden bar slides in the grooved base. At its upper end a one centimeter piece of wooden rod about five centimeters long is tightly fitted. The tip of the rod is carefully rounded to rub against the cup formed by the base of the test tube. Up and down movement of the needle is obtained by sliding the fifty centimeter bar back and forth in the groove. To determine the sensitivity of this movement, let the length of the bar form the constant hypotenuse a of a variable right triangle. Let the horizontal side (groove) equal x and the vertical side (brass tube and steel rod) equal y.

Then $x^2 \quad y^2 = a^2$

By implicit differentiation with respect to x

$$\frac{dy}{dx} = \frac{-x}{y}$$

The sensitivity depends upon the ratio of the variable horizontal side to the variable vertical side of the triangle. Therefore, the upper angle must be acute to minimize the movement. It is possible to work with a horizontal side of about one centimeter and a vertical side of about fifty centimeters, thus minimizing the manual movement about fifty times.

Chromel needles were used. They were made by hammering a piece of wire very thin and cutting to a fine point with a scissors. After the last cut the wire was hammered to produce a hoe-like end rather than a pointed one. The dissections were performed on clear three percent agar.

Perithecia were crushed between flamed microscope slides and a drop of water was added from a sterile lip pipette. The asci emerge from the perithecia in groups and the clusters can be easily picked up with the pipette and placed on the agar. These asci still attached to the ascogenous hyphae spread out radially on the agar. Then individual asci are dissected as soon as the drop has dried out. The location of the spores in the ascus is indicated by numbering from one to eight; the spore at the outer end of the ascus being number one. The thin but broad hoe-like end of the needle is dropped between the first and second spore and the plate pulled away by hand, thus breaking the ascus wall. A mechanical stage is not necessary to hold and move the plate although some individuals might desire it. Most people soon become skillful at manipulating by hand and in this way they can work more rapidly. All eight spores are dissected, "hoed" out, one by one and placed in a

row. Then they are transferred to a second plate set up under a second binocular so that there is no difficulty in returning to the row of ascospores in the first plate. The position which a spore occupied in the ascus is indicated by a number marked in the agar along side it.

Heat treatment is used to germinate the spores and they are transferred to agar in small tubes by cutting out small pieces of agar containing the germinated spore under a binocular microscope. The heat treatment kills all of the conidia which might have been transferred with the spores into the agar plate. The transfer of the germinated spores to small tubes is made in a small glass chamber which has been liberally sprayed with dilute alcohol and wiped down with mercuric bichloride solution.

The small tubes (9 by 100 mm. or 3/8" by 4") are kept in racks made of pressed cork strips. This pressed cork is three millimeters thick and is cut in strips two by thirty centimeters. About sixteen holes, each nine millimeters in diameter, are punched in the strips with a cork borer. The cultures are kept in the same position in which they occur in the ascus and are held tightly enough in place so that they cannot fall out. These strips of tubes are mounted in wooden racks.

II. Heat Treatment Methods

Dodge (18) has shown that the ascospores of certain species of the Ascobolaceae, which do not ordinarily germinate in the laboratory, can be induced to germinate by heating. He places the spores on agar in petri plates and puts the plates in a gas oven, which is then raised to about 70°C. in about twenty to thirty minutes, and then removes the plates. In some species some spores fail to germinate even after this treatment and a few spores are always encountered which germinate without heating. He has since shown in other papers that this method may be employed successfully in inducing ascospores of certain species of Aleuria, Lachnea, and Neurospora to germinate. This method has also been employed by Ramlow(55), Betts (2), Wilcox (62), and others. The writer has conducted a series of experiments on ascospore germination and describes a more refined but not necessarily infallible method of heat treating spores of Neurospora. This method and a discussion of the variability of ascospores of Neurospora in their response to heat treatment follows:

1. Experiments to determine an optimum heat treatment.

In studying heat treatment spores of N. tetrasperma were placed on agar in small petri dishes. The depth of the agar varied from three to seven millimeters. The dishes were placed on a one-eighth inch copper plate set on top of a Columbia paraffin oven. The copper was allowed to come to a stable temperature (73° to 77°C.) before the petri dishes were placed on it. A few drops of water under each dish insured a good contact. The temperature at the surface of the agar gradually rose to nearly 70°C. Temperatures were determined by the melting points of various organic compounds. These were placed on thin cover glasses which were laid on agar in petri plates containing no spores. After many trials, the compounds shown in Table I were selected. The table also shows the time at which the compounds melted in two blank plates, giving a record of the approximate temperatures of the ascospores on the surface of the agar in the other plates. Fig. 3 shows the smooth curve plotted among these values. The main errors of this method are: (1) raising the cover of the blank plate to wipe off the moisture film for observation obviously cools the plate, (2) the lowering of the melting point of the

Table I. Compounds used as thermometers.

Compound	Melting Point	Time of Melting in Minutes Plate I	Plate II
Benzo-phenone	47.5°-48.5°C.	6.5 - 9.0	8.0 - 11.0
Betabrom-naphthalene	53.0°-55.0°C.	32.0 -34.0	38.0 - 42.5
Betachlor-naphthalene	56.5°-57.0°C.	39.0 -40.0	49.0 - 51.0
Palmitic acid	61.0°-61.5°C.	61 - 100	75 - 105
Stearic acid	69.0°-69.5°C.	240	240

organic compounds due to distillation into each other and water vapor, (3) the irregular heating of different plates due to differences in size, agar depth, position, poor contact, etc. It is questionable if a better method for determining the temperature would be worth the trouble because the variation among the several plates is probably considerable. A series of experiments were performed to determine the optimum time of heat treatment. Sixty-seven petri dishes containing spores of N. tetrasperma were treated for different lengths of time on the copper plate. Blanks were used with the organic compounds as thermometers. The results corresponded roughly with those shown in Table I and Fig. 3, and were not included in the data presented. When all the spores had germinated a count was made. Each percentage recorded is the final count from a single plate containing from forty to several hundred spores but rarely less than a hundred. Seven loadings of the copper plate were made and treatments of from eleven to eighty-six minutes were used. Table II shows the data and Fig. 4 is a graph showing the relation between the time of heat treatment by the copper plate method and the percentage of germination. These are the total data from sixty-seven petri dishes each containing on the average well over a hundred ascospores.

Table II. Germination counts on sixty-seven petri dishes containing Neurospora tetrasperma ascospores heat treated by the copper plate method for various lengths of time.

Time Min.	Per cent	Time Min.	Per cent
11	2	44	98
12	0	46	98
13	0	48	99
14	7	50	97
15	1	52	85
16	3	54	93
17	0	54	98
18	2	56	56
18	4	58	78
19	2	59	94
19	30	62	88
20	81	66	81
21	88	68	92
22	63	70	97
23	77	74	93
24	84	78	97
25	86	82	93
25	100	86	97
26	92	90	95
26	97	100	97
27	100	110	92
28	96	120	93
29	95	130	82
30	98	140	94
30	100	150	78
31	97	160	0
32	98	170	35
33	93	180	11
34	98	190	8
34	95	200	0
38	99	210	1
40	98	220	1
42	80	230	2
		240	3

In analyzing Fig. 4 it can be seen that the optimum heat treatment has the rather long range of from twenty-five to forty minutes. It can be concluded that a heat treatment of from twenty-five to forty minutes, with the temperature rising from 54° to 58°C., if the treatment takes twenty-five minutes to attain 54°C., will give very high percentages of germination. There is evidence to show that lower temperatures for longer times will give the same results but no effort has been made to determine a critical temperature.

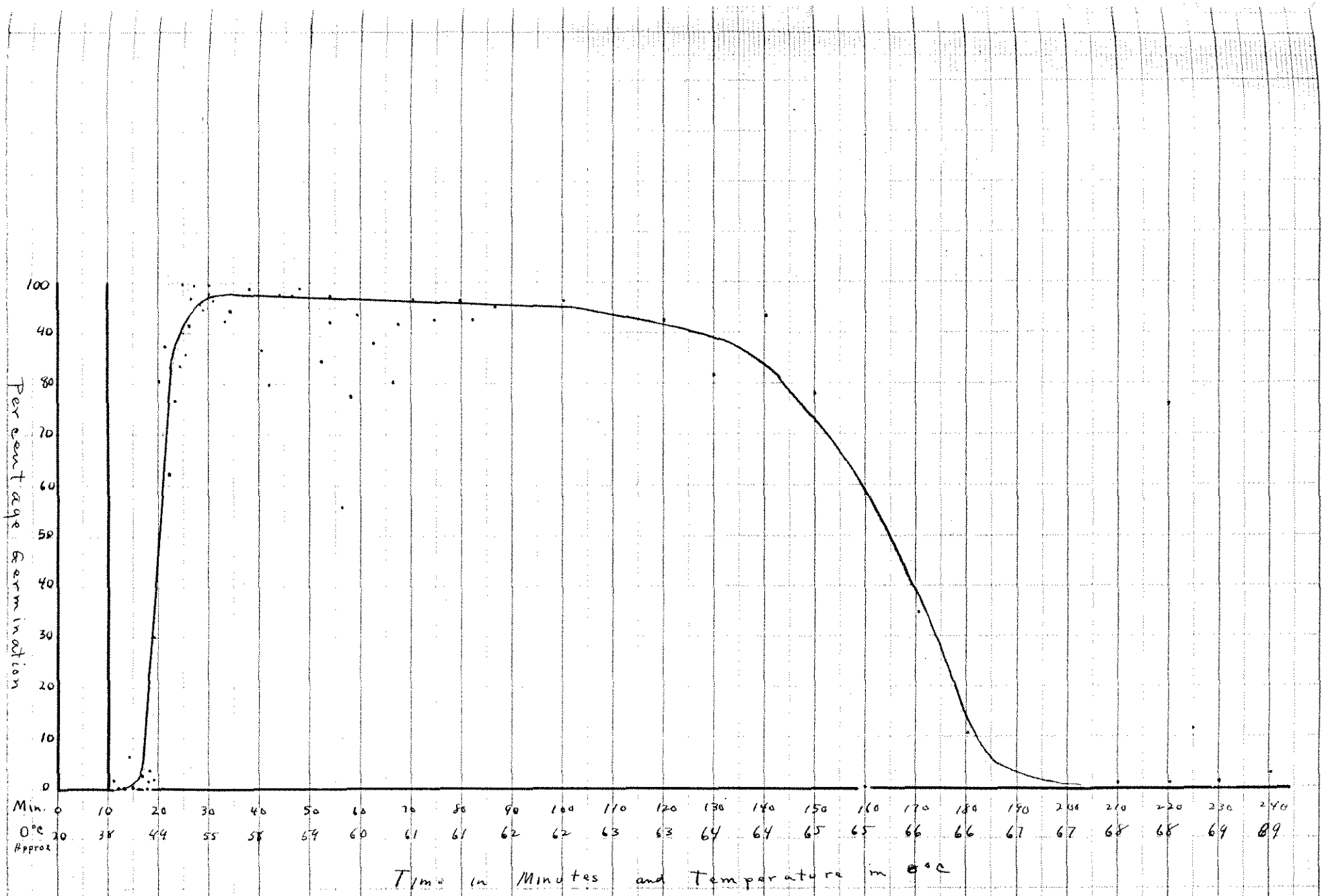


Fig.3. Graph showing the percentages of germination obtained in 67 different agar plates placed on the copper plate for various lengths of time. The temperature of the agar during the time the dish was on the copper plate is also shown. Note the flat maximum and the rapid increase beginning at about 20 minutes as well as the rapid fall after 140 minutes.

2. Experiments on killing of conidia.

The peculiar advantage of using Neurospora for genetic studies lies in the fact that the conidia and mycelia, which are of parental origin and may be transferred with the ascospore, are killed by the heat treatment which induces the ascospore to germinate. After the ascospores have germinated, fifteen to eighteen minute heat treatments by the copper plate method are sufficient to kill the mycelium of the germinated spores. In these experiments it required about eighteen minutes to raise the cultures from room temperature to 48°C. After changing from the copper plate method to the thermostat for inducing germination, it was necessary to discover if this treatment was sufficient to destroy the conidia. Fresh N. tetrasperma conidia were selected. These showed one hundred percent germination on cornmeal agar. Ten minutes in the thermostat at 58°C. delayed germination for a few hours, while forty minutes delayed germination for as long as two days but practically all of the conidia germinated. From 45 to 70 minutes heating at 58°C. killed all the conidia in thirteen of the small plates used and no later growth was produced although they were held for ten days.

In two cases, however, (one petri plate held for sixty minutes and the other for sixty-five minutes) the plates were overgrown when examined five days later. It is probable that only a small number of conidia survived in these plates. In general the asci are carefully washed and no conida are carried over. The chances of any one which might accidently be carried over surviving the sixty minute treatment are evidently not great.

Random single ascospore cultures of N. crassa were often made by sowing conidia and ascospores on an agar plate and allowing the conidia to germinate. Then the plate was heat treated and the ascospores transplanted to tubes. As a control many of the germinated and subsequently heat treated conidia were also transplanted but none ever grew.

3. Experiments on the inheritance of response to heat treatment.

In the present method of heat-treating ascospores, the copper plate is no longer used. It was thought that lower temperatures with longer treatments gave greater reliability with less injury and hence fewer killed spores. A small thermostat was built for this low temperature treatment. In the early part of the work it was thought that cooling the petri plates containing the spores down to about 10° C. for 12 to 24 hours before heating increased the certainty of germination. Recent experiments show that cooling has no such beneficial effect.

It was determined that the optimum heat treatment for a cooled petri plate in the thermostat held at 58° C. was one hour. The thermostat is so small that its temperature drops to about 45° C. on introducing the cooled plates and does not attain 58° C. until about 20 minutes later. It may be that the ascospores on the surface of the agar never quite reach the temperature which the thermometer in the thermostat indicates.

In the course of this study the spores from over 500 individual asci were isolated in order, one by one,

Table III. Fifty-two exceptional asci of N. crassa from a total of over 500 studied. These are exceptional in containing some ascospores which do not respond to heat treatment in the ordinary manner.

Ascospore	1	2	3	4	5	6	7	8
Ascus								
217	1	1	1	1	2	2	X	2
222	1	X	1	1	1	2	1	1
257	1	1	1	1	1	1	2	1
286	1	0	0	1	X	1	1	1
312	1	1	X	2	X	X	X	1
322	X	2	1	1	X	1	1	1
330	1	1	1	1	0	0	0	0
332	1	2	X	2	2	2	X	2
336	2	1	2	2	1	1	X	2
340	X	1	1	2	1	1	1	2
341	3	2	1	2	1	1	1	1
342	ab	ab	ab	ab	1	2	2	2
361	1	1	X	1	0	0	X	X
498	X	1	1	1	0	X	0	1
500	1	1	1	1	1	1	0	1
550	1	1	2	2	2	1	1	1
551	2	2	X	X	1	1	1	1
552	1	2	2	2	2	2	1	1
553	1	1	1	1	2	2	2	2
554	1	1	1	1	2	2	2	2
555	1	1	2	2	1	1	1	1
556	1	1	2	2	2	2	2	1
557	1	1	2	1	1	1	1	1
562	1	2	1	X	1	1	1	1
563	2	X	1	0	X	0	2	2
641	2	2	3	3	1	X	3	3

Ascospore	1	2	3	4	5	6	7	8
Ascus								
642	1	2	X	1	3	3	2	1
643	1	1	1	1	1	2	1	1
644	2	2	2	2	X	X	2	2
646	2	X	1	1	1	1	2	1
650	1	2	1	2	1	X	1	1
651	2	2	2	2	1	1	1	2
654	2	1	1	1	1	1	1	1
655	1	2	2	2	1	1	1	1
657	1	1	1	1	1	1	1	2
659	1	1	1	1	2	1	1	1
676	1	1	1	1	1	1	2	1
677	1	2	1	1	1	2	1	1
678	1	2	1	1	1	1	1	2
683	X	1	2	1	1	1	1	1
688	1	2	1	1	1	1	1	1
715	2	2	1	X	1	1	1	1
718	1	2	1	1	1	1	1	1
724	1	1	1	1	1	2	1	2
725	1	1	1	1	1	1	2	1
730	1	1	1	1	1	1	1	2
731	1	1	1	1	1	2	2	1
732	1	1	1	2	1	1	1	1
741	2	3	2	2	3	1	2	2
744	1	2	2	2	3	1	1	1
745	2	3	3	X	X	X	2	2
690	1	1	1	1	2	2	2	2

Legend

1 - spore germinated after first hour heat treatment
2 - spore germinated after second hour heat treatment
3 - spore germinated after third hour heat treatment
0 - spore germinated without heat treatment
X - spore does not germinate
ab - spore aborted

so that their positions in the particular asci were known. Only asci containing eight ripe spores were used. These spores were incubated on agar over night at room temperature to determine if any germinated without heating, and if any conidia had been carried over with them. At first all plates were cooled as above described before heat-treating, but later the cooling was abandoned. The plates were then treated in the thermostat held at 58° C. for one hour. About ten hours later the germinated spores were removed to culture tubes and those which had not germinated were treated for a second and sometimes for a third hour. Table III gives a record of the asci (from the 500 asci of N. crassa) in which ascospores were found that germinated without heating or that responded to heat treatment of longer than one hour. Less than one percent of the total number of spores germinated without heat treatment; about four-fifths of the total number germinated following the first hour of treatment, about two percent of the spores yielded to the subsequent heat treatments and about one-fifth of the spores failed to germinate. The ratio in which the different types of spores occurred and their distribution in the ascus suggest genetic differences but these supposed differences have not been adequately

Table IV. Nine asci of *N. sitophila* showing the position of the ascospore and the manner in which they respond to heat treatment.

Ascospore / Ascus	1	2	3	4	5	6	7	8
202	X	X	2	2	2	2	X	X
203	2	2	2	2	2	2	2	2
204	1	1	1	1	1	1	1	1
205	1	1	1	1	1	1	1	1
206	2	2	2	2	1	1	1	1
207	1	1	1	1	2	2	2	2
208	3	X	3	X	2	2	X	X
209	1	2	2	2	2	2	2	X
210	1	2	1	2	1	2	1	1

Legend

1 - spore germinated after first hour heat treatment
2 - spore germinated after second hour heat treatment
3 - spore germinated after third hour heat treatment
X - spore does not germinate

tested.

In order to test this point selection experiments and subsequent crosses would have to be made. Only one experiment was performed. A mating was made between the mycelia from two of the ascospores that germinated without heating. Fifty-nine ripe ascospores were selected from the offspring. They were incubated on agar at 27° C. for nearly two days, but none germinated. A one hour heat-treatment induced fifty-five of them to germinate indicating that they were mature. At first this was supposed to indicate that variations from response to one hour heat-treatment were not inherited. But subsequent experiments with crosses of definitely inherited types gave similar first generation results. Further generations, which in this case were not raised, would be required to prove this point.

It must also be noted that this group of asci were the inbred descendents from an ascus in which the spores germinated following a treatment of one hour and it is possible that other races of N. crassa may exist whose spores require heat treatment for different lengths of time.

Nine asci of N. sitophila, described in Table IV, showed a striking contrast to the case of N. crassa.

It must be noted that all the asci of N. sitophila studied appear in Table IV, while Table III includes fifty-two aberrant asci selected from a total number of over 500. Therefore, in the case of N. sitophila about one half of the spores required two hours heat-treatment, while less than half of them germinated after one hour treatment.

In the case of asci 202, 206 and 207, the arrangement of the spores seems to point to a segregation of a factor determining the response to heat treatment, but no further breeding tests were made.

That the type of treatment necessary for germination depends on genetic factors is indicated clearly by the following experiments, which were started, for another purpose, namely, to test the nature of a supposedly hybrid strain produced by Dr. B. O. Dodge. By crossing a non-conidial mutant of N. sitophila with typical conidial N. tetrasperma and selecting and back crossing for several generations he had first secured a non-conidial 4-spored race. Then from this non-conidial, 4-spored type he grew a bisexual, hermaphroditic, non-conidial mycelium, which he designated 3C. He mated this with a unisexual, conidial mycelium of N. tetrasperma, producing thereby perithecia which were supposedly hybrid.

Table V. Ascospores dissected from sixteen asci obtained from hybrid perithecia referred to in the text. It is also indicated whether or not the mycelia produce conidia and whether or not the spore needed heat treatment to induce germination.

Spore No.	1	2	3	4	5	6	7
Ascus 402	L H + C A+B	L WO +C A+B	S H +C B?	L H +C A+B?	S WO -C A		
Ascus 406	L H +C A+B	L H +C A+B	S H +C B?	L H +C A+B	S WO -C A		
Ascus 411	L H + C A+B	L H +C A+B	S H +C B?	L H +C A+B	S WO -C A		
Ascus 412	L WO +C A+B	L H +C A+B	S H +C B?	L WO +C A+B	S WO -C A		
Ascus 413	L H +C A+B	L not ger- minated	S H +C B?	L H +C A+B	S WO -C A		
Ascus 404	L H +C A+B	L H +C A+B	S H +C B	L H +C A+B	S WO -C A		
Ascus 410	L H +C A+B	L H +C A+B	S H +C B	L H +C A+B	S WO -C A		
Ascus 405	L WO +C A+B	L WO +C A+B	S WO -C B?	L H +C A+B	S H +C A		
Ascus 407	L H +C A+B	L H +C A+B	S WO -C B?	L H +C A+B	S H +C A		
Ascus 415	L H +C A+B	L H +C A+B	S WO -C A	L H +C A+B	S H +C B?		
Ascus 417	L H +C A+B	L H +C A+B	S WO -C A	L H +C A+B?	S H +C B?		
Ascus 403	L H +C A+B	L H +C A+B	L H +C A+B?	S WO -C A	S H +C B?		
Ascus 416	S WO -C A	L H +C A+B	S H +C B	L H +C A+B	L H +C A+B		
Ascus 408	L WO -C A	L WO -C A	S H +C B	L H +C B	S H +C B		
Ascus 414	S WO -C ?	S WO -C ?	S WO -C A	S WO -C A	S H +C B	S H +C B	S H +C ?
Ascus 401	L H +C A+B	L WO +C A+B	Med.WO -C B?	Med WO -C B?	S H +C A	S H +C A	

Legend:

L means large,)
S means small,) all refer to the sizes of the spores
Med means medium)

H means the spore needed heat treatment to induce germination
WO means that the spore germinated without heating
+C means the mycelium produced conidia
-C means that the mycelium failed to produce conidia
A and B mean sexes A and B
A+B means hermaphroditic
B? means sex B incompatible
A+B? means hermaphroditic but producing no perithecia

The manner of producing these perithecia is described in detail by Dodge in a paper that has recently appeared (Dodge '31).

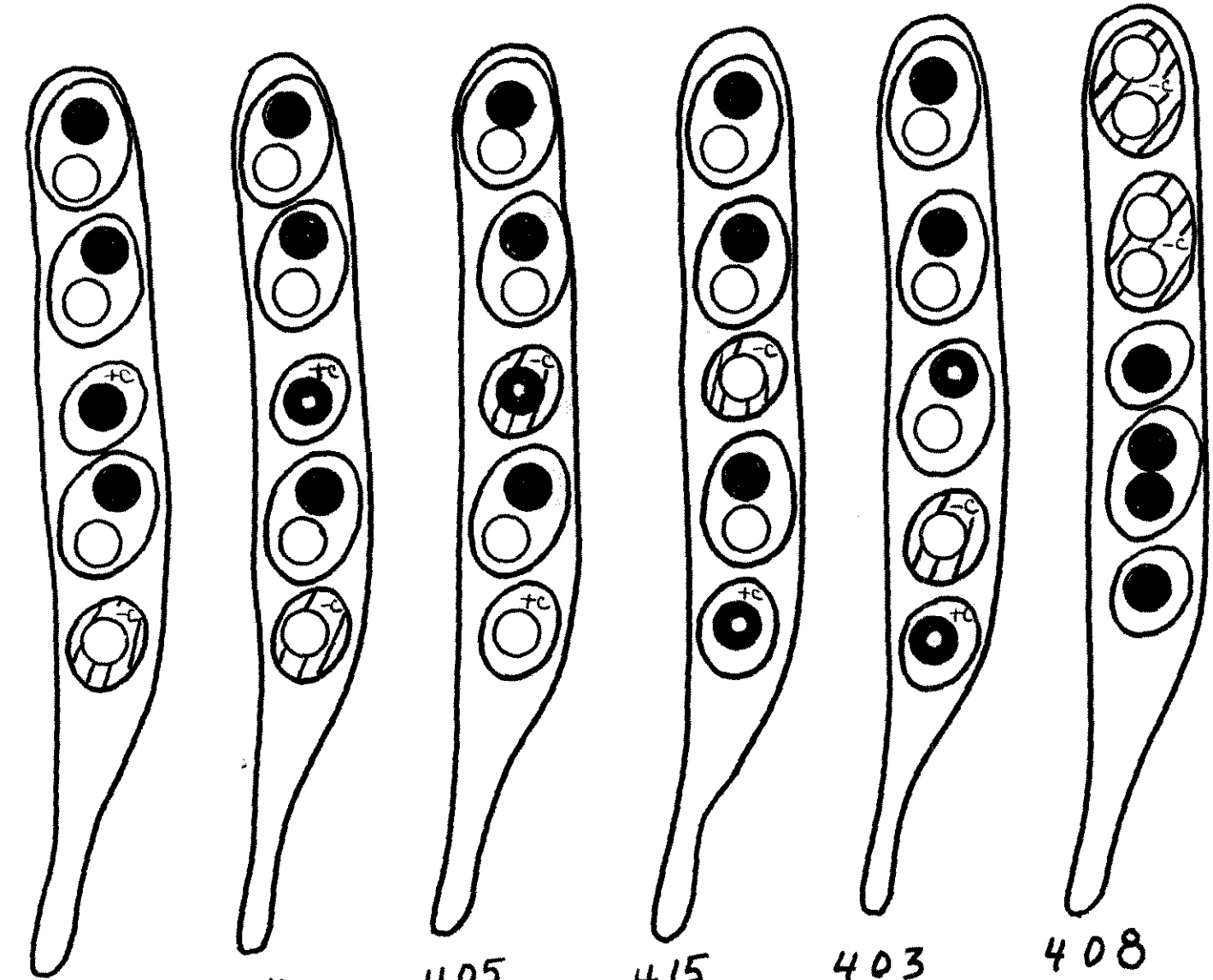
Proof that a hybrid had been produced between the hermaphroditic and the unisexual mycelium would be furnished if both conidial and non-conidial ascospores were found in the same ascus. The writer accordingly dissected sixteen exceptional 5-spored asci from this 4-spored hybrid. Since one parent was non-conidial and the other was conidial, 5-spored asci were selected in the expection that, if a cross had been made, one of the two small unisexual spores from each of these asci would show the character of one parent while the other would show the character of the second parent. In each of the three other standard, bisexual, hermaphroditic spores, containing two genetically different nuclei, one from each parent, masking of the non-conidial character might be expected. As Table V shows, all mycleia from the bisexual ascospores did produce conidia, indicating that the non-conidial character, if present, was masked. That the non-conidial characters were actually present was shown by its manifestation in about half of the mycelia from the small spores. The presence of the conidial and the non-conidial nuclei proves that the supposed hybrid had

actually been produced by the cross.

The data in Table V are represented diagramatically in Fig. 4. The 5-spored asci are grouped in classes according to the arrangement of the two small spores and the types of mycelia produced. The nuclei are shown as white, black, and black with a white center. The white nuclei indicate sex A; the black, sex B; the black nuclei with white centers were probably sex B but were incompatible with the tester strains used so cannot be definitely determined in every case. Incompatibility is sterility with tester strains of opposite sex, a phenomenon resembling self sterility. The large, i.e., standard-sized, spores were assumed to be binucleate in origin and the small spores uninucleate. Table V shows that the nuclei in most of the large spores contained two compatible nuclei, their sexes being indicated by A+B. A few of them carried incompatible nuclei, for the mycelia failed to produce perithecia. In such cases the sex is indicated by A+B?. The sign +C indicates that the mycelium from the spore produced conidia while -C indicates that it was non-conidial. The mycelia from all of the large spores (except from two in ascus 408, discussed in detail below) produced conidia. The two small spores in each ascus were one conidial and one

Fig. 4. Diagram of thirteen 5-spored asci dissected from a hybrid obtained by Dodge. The size, arrangement in the ascus, sex and incompatibility of the spores is shown. The presence or absence of conidia on the mycelium and whether or not the spores require heat treatment to induce germination is also shown.

White nuclei are sex A;
Black nuclei are sex B;
Black nuclei with white centers are Sex B incompatible;
Cross-lined spores germinate without heat treatment
open spores require one hour heat treatment;
-C = means that the mycelium develops no conidia;
+C means that it develops normal conidia.



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403

408

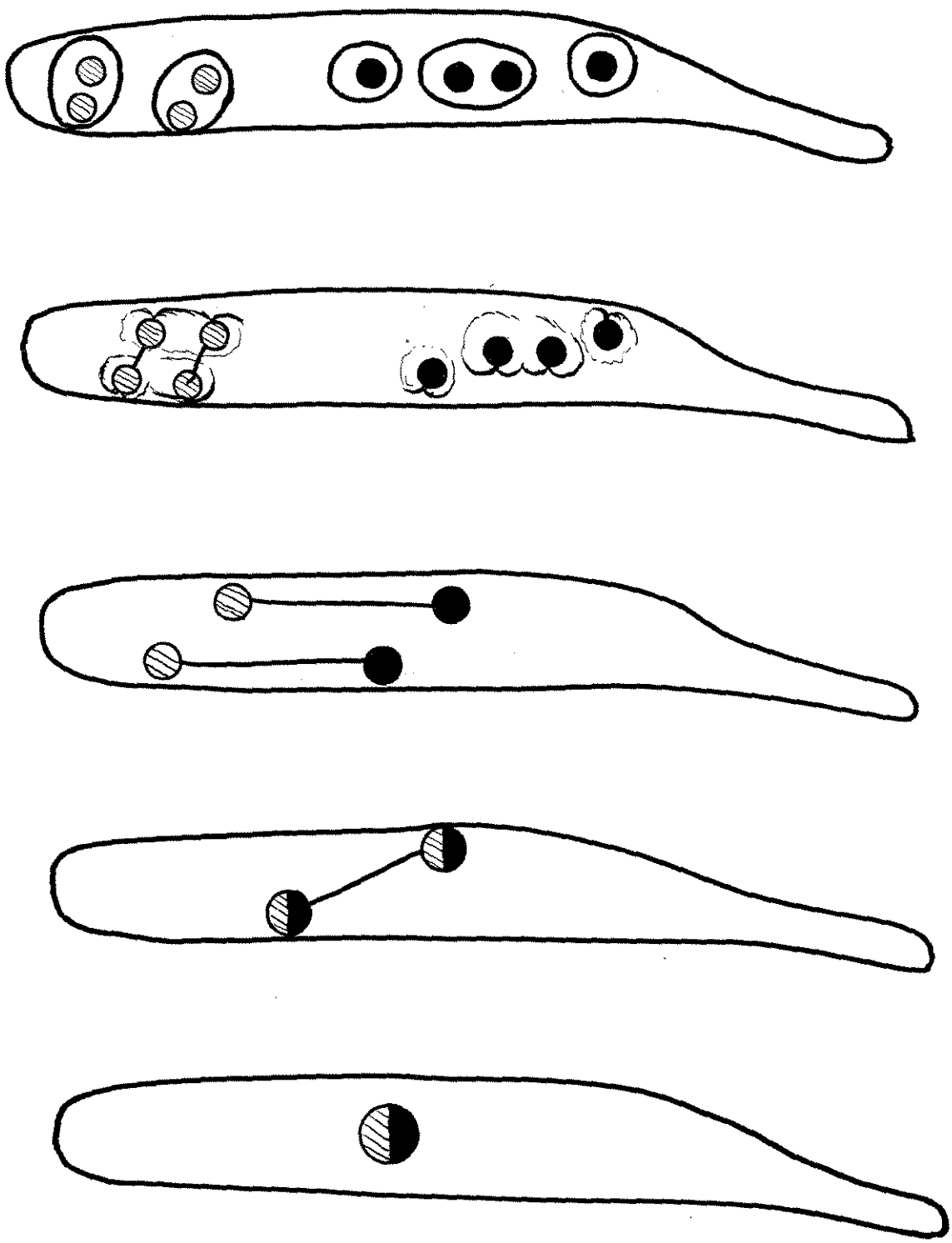
non-conidial (with the exception again of ascus 408 where both were +C). It is obvious from Fig. 4 that the perithecia were true hybrids since half of the nuclei in each ascus contained the conidial factor and half the non-conidial factor. Moreover, when a non-conidial and a conidial nucleus were present in the same ascospore, the mycelium produced conidia.

The special interest of this hybrid for the writer lay in the fact that Dodge had found that a large proportion of the spores germinated without heat-treatment. This characteristic appeared clear-cut in the spores dissected from the asci just described. In Fig. 4 the diagonally lined spores are those which germinated without heating. The figure shows also that the spores which germinated without heating were all non-conidial. In all but two asci (405 and 407) these non-conidial spores that germinated without heating were moreover sex A. This three-character linkage was very striking.

Ascus 408 was exceptional in two respects; first, the small spores were alike (B, +C, H), second, the three large spores were unisexual instead of hermaphroditic, and were -C. This anomolous situation can be accounted for in terms of the known peculiarities

of the spindles leading to the formation of the spores, provided the segregation for these characters occurs at the second division. Dodge (23) has described in de- the arrangement of the spindles in N. tetrasperma, showing that first-division segregation of factors for sex would always produce four hermaphroditic spores in each ascus. Since Dodge found no binucleate unisexual spores he was of the opinion that segregation of sex always occurred at the first division, or that if segregation occurred at the second division the orientation of the nuclei was such that unlike sexes came together. Without this peculiar orientation, second division segregation would produce binucleate unisexual spores. Ascus 408 is of particular interest, since all three of the binucleate spores were unisexual, and hence presumably lacked this special orientation of the nuclei. Following Dodge's study of spindle arrangement, and assuming that this hybrid has the same spindle mechanism as N. tetrasperma, we can conclude that ascus 408 shows second-division segregation of the sex factors as well as those for conidia and those for germinating without heating. Fig. 5 is a diagram showing the type of ascus behavior necessary to produce this rare kind of ascus. The straight lines are the axes of the spindles as they would lie if they followed the arrangements found

Fig. 5. Diagram showing how second division of the factors determining sex, response to heat-treatment and the presence or absence of conidia could give rise to a 5-spored ascus such as ascus 408, containing no hermaphroditic spores. Diagonally lined nuclei contain the factors for sex A, absence of conidia and ability to germinate without heat treatment. Black nuclei contain the factors determining sex B, production of conidia and inability to germinate without heat treatment.



for N. tetrasperma. This differs from Dodge's diagram for second-division segregation in only one feature, namely, that the orientation of one of the nuclei is reversed in the binucleate state of the ascus. (Fig.6).

The failure to produce perithecia in the case of the mycelia from spores from ascus 408 cannot be ascribed to incompatibility, since it was only sex B that showed incompatibility in this hybrid and in ascus 408 the sex B strain was tested and found compatible with the tester strain. That the failure to produce perithecia was actually incompatibility in the case of some of the mycelia of other asci was shown by the fact that the sex B strain from ascus 407, which failed to fruit with tester strains, fruited readily when mated with the sex A strain from ascus 406. This cross (406-5 x 407-3) was the mating of two mycelia both of which were non-conidial and were produced by ascospores which germinated without heating.

Unfortunately nothing is known of the reaction of the parents of this hybrid to heat treatment. Crossing a clone derived from non-heating ascospores by another of the same type, since both were supposed to be haplonts and presumably pure for any character which they show, should produce only offspring showing the character.

TABLE VI. Twenty-one asci dissected from a cross of two non-conidial ascospores that germinated without heating. The size and arrangement of the spores in the ascus are shown and whether or not germinated without heating. Abbreviations same as those used in Table V.

Asco-spores	1	2	3	4	5	6	
Ascus 1	L	L	S	L W.O	S W.O		
Ascus 3	S	S W.O	L	L	L		
Ascus 4	L W.O	S W.O	S	L	L W.O		
Ascus 5	L W.O	L W.O	S	L	S W.O		
Ascus 6	S W.O	L W.O	S	L	L		
Ascus 7	L W.O	L	S W.O	L	S		
Ascus 9	L	L W.O	S W.O	L	S		
Ascus 10	L	L	S	L	S W.O		
Ascus 14	L	L	S W.O	L W.O	S		
Ascus 15	L	L	S W.O	L	S		
Ascus 17	S	S W.O	L	L W.O	L		
Ascus 18	L W.O	L W.O	S	L W.O	S W.O		
Ascus 20	S W.O	L	L	L	S W.O		
Ascus 16	S	S	S W.O	S W.O	L	L W.O	
Ascus 2	L W.O	L W.O	L W.O	L W.O			
Ascus 8	L W.O	L	L	L			
Ascus 11	L	L W.O	L	L			
Ascus 12	L	L W.O	L W.O	L W.O			
Ascus 13	L	L W.O	L W.O	L			
Ascus 19	L W.O	L W.O	L W.O	L W.O			
Ascus 21	L W.O	Very large	L W.O	S W.O			

To test this point the spores from twenty-one 4, 5, and 6-spored asci from the cross 406-5 x 407-3, were dissected out and incubated at room temperature for several days. Table VI shows the ascospores from this group which germinated without heat-treatment. Forty-five percent of the large spores germinated without heating in this cross as contrasted with only twenty percent of the large spores in the parent hybrid. It is remarkable that in only one case (20) did both small spores germinate without treatment. This may have been a case like ascus 408.

We can conclude from this experiment that the factor causing ascospores to germinate without heating is hereditary and it is usually carried by nuclei of sex A. That half of the offspring, both binucleate and uninucleate spores, failed to germinate without heat-treatment negatives one of the assumptions made - namely, that the nuclei were haploid. This peculiarity of behavior is typical of the hereditary strains and will be discussed in detail later.

4. Summary of the experiments on the heat-treatment of ascospores.

These experiments showed that it was possible to determine, by appropriate tests, the optimum heat-treatment which causes the largest percentage (in some cases 100 percent) of the ascospores of Neurospora to germinate. Furthermore, it was shown that the heat-treatment commonly used killed the asexual spores, thus simplifying the separation of sexual from asexual offspring.

The ascospores from the race of N. tetrasperma seemed to be highly uniform in their response to heat-treatment. There was evidence that part of the small percentage of variation from one hour heat-treatment in N. crassa was due to genetic factors. Dodge's hybrid and the race of N. sitophila produced two types of ascospores in regard to their response to heat-treatment. In both races the differences were the result of genetic constitution, but there was evidence that the ability of a spore to respond to heat-treatment was varied by the environment. This was best shown by the fact that although all of the large spores in the hybrid had one nucleus containing a factor for germinating without heat-treatment, and one containing a factor determining response to one hour heat-treatment, some germinated without heating while others did not. In Dodge's hybrid, the unisexual

ascospores fell into two genetic groups; in one group all of the ascospores germinated without heat-treatment. In the other group the ascospores required one hour heat-treatment to initiate germination. The race of N. sitophila contained two genetic groups as well. One kind of ascospore required one hour heat-treatment, and the other required two.

Despite the evidence that response to heat-treatment is a genetic character, in these cases, it is improbable that it can be used extensively in a study of the genetics of Neurospora, because of its variability. In a closely inbred race, such as the race of N. crassa, which seemed to be rather uniform in its response to a one-hour heat-treatment, ascospores were encountered which germinated without heating and others which required a three-hour treatment. Although in a few cases, the order of the spores in the ascus indicated that factors determining response to heat-treatment had been segregated, it was obvious in most cases that the identical twin ascospores sometimes responded differently indicating that environmental factors were also effecting the variation.

Faull (32) concluded, on the basis of some experiments on heat-treatment of the ascospores of a strain of N. crassa, that heat-treatment does not increase

the percentage of germination. The data which she presents do not justify this conclusion. She found that 4 to 25 percent of the ascospores in various random samples germinated without heat-treatment. Ascospores in various random samples which she heat-treated at 51.5° C. for one to four hours showed 5 to 94 percent germination. Obviously she was working with a mixed strain producing at least two genetically different kinds of ascospores.

III. Sex and Arrangement of Spores in the Ascus of *N. crassa*.

Dodge (23) has pointed out the advantage of the ascomycetes for studying meiosis. The zygote nucleus is formed by the fusion of two nuclei. The cell containing this nucleus gives rise to the long, tube-like ascus. This nucleus then divides three times to form eight nuclei. Each of the eight spores receives one of these eight nuclei. In the species of *Neurospora crassa* and *N. sitophila*, an ascospore on germination gives rise to a mycleium which may be either one of the two sexes. Such a mycelium, usually called the "haploid" mycelium, is sterile when grown alone or with another of the same sex, but growing the mycelia of two opposite sexes together produces zygotes and ascospores again.

In the species mentioned, the tube-like ascus is long and narrow. Wilcox (62) has studied the spindles in the ascus of *N. sitophila* and has found that in the first two divisions they are oriented parallel to the sides of the ascus and do not overlap. The nuclei maintain their relative order and positions. This means that it is possible, as shown in Fig. 6, to determine at which division segregation of factors has taken place.

Table VII. All Possible permutations of ascospores in the ascus

Asymmetrical permutations 2/70 chance								
Spore	1	2	3	4	5	6	7	8
	+	+	+	+	-	-	-	-
	+	+	+	-	+	-	-	-
	+	+	+	-	-	+	-	-
	+	+	+	-	-	-	+	-
	+	+	+	-	-	-	-	+
	+	+	-	+	+	-	-	-
	+	+	-	-	+	+	-	-
	+	+	-	-	-	+	+	-
	+	+	-	+	-	+	-	-
	+	+	-	-	+	-	+	-
	+	+	-	-	-	+	-	+
	+	+	-	+	-	-	+	-
	+	+	-	-	+	-	-	+
	+	+	-	+	-	-	-	+
	+	-	+	+	-	-	-	+
	+	-	-	+	-	+	-	+
	+	-	-	-	+	+	-	+
	+	-	+	-	+	-	-	+
	+	-	+	-	+	-	+	-
	+	-	-	-	+	+	+	-
	+	-	-	+	+	+	-	-
	+	-	+	-	-	+	+	-
	+	-	-	+	-	+	+	-
	+	-	+	+	-	+	-	-
	+	-	+	+	+	-	-	-
	+	-	+	-	+	+	-	-
	+	-	-	+	+	+	-	-
	+	-	+	+	-	-	+	-
	-	-	+	+	-	+	+	-
	-	-	+	+	+	-	+	-
	-	-	+	-	+	+	+	-
	-	-	-	+	+	+	+	-

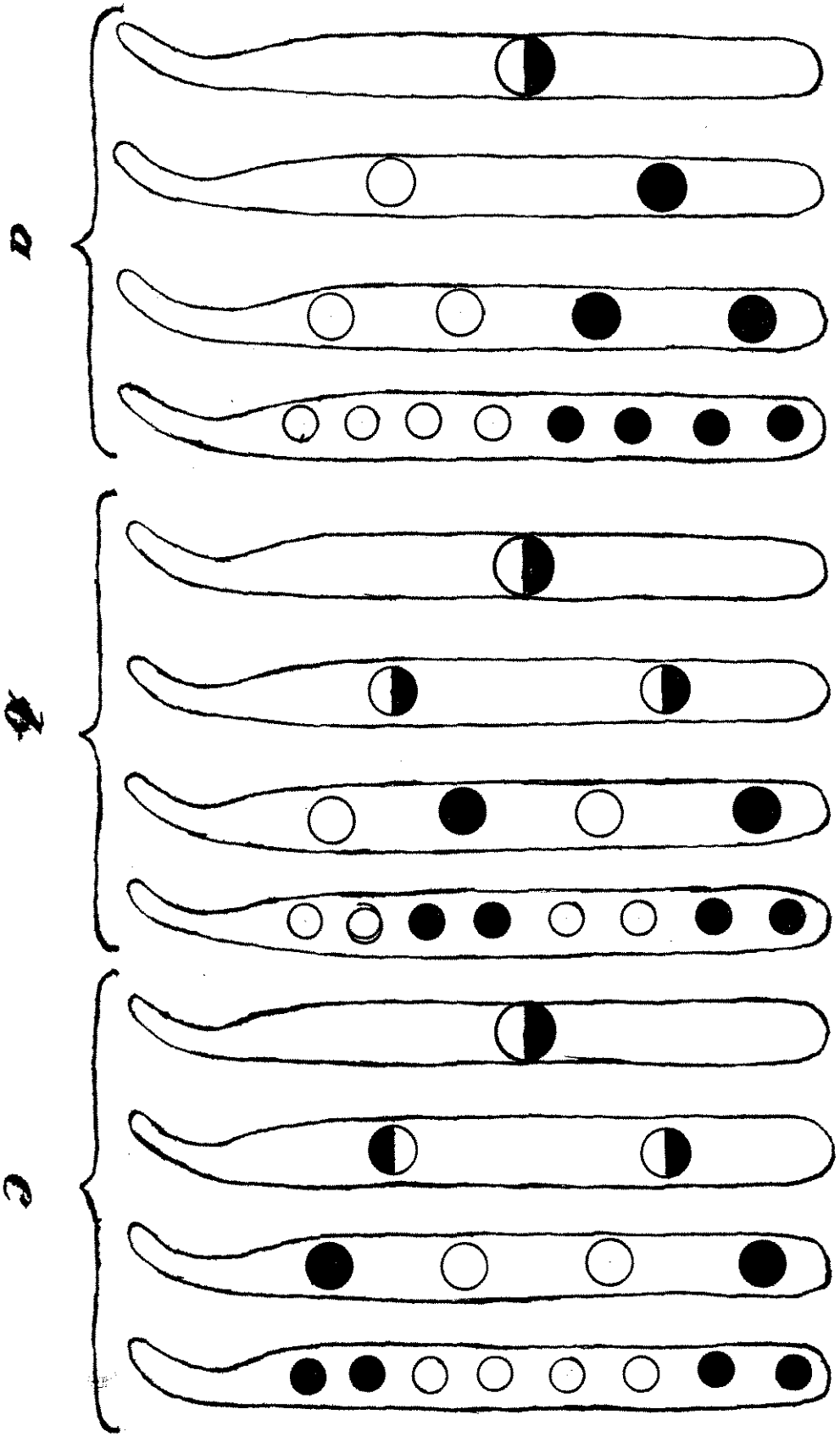
Symmetrical permutations 1/70 chance							
+	+	-	-	-	-	+	+
-	-	+	+	+	+	-	-
+	-	+	-	-	+	-	+
-	+	-	+	+	-	+	-
+	-	-	+	+	-	-	+
-	+	+	-	-	+	+	-

Fig. 6. In group a, is shown a diagram of the arrangement of spores of opposite sex in the ascus of N. crassa that would result from segregation of sex in the first division in the ascus, provided there was no passage of nuclei in the ascus. Black and white are used to designate the opposite sexes. The only shift that occurs in this arrangement that has been detected is a shift in the fourth and fifth place, giving three black, one white, one black and three white nuclei, but this shift is rather infrequent.

The second obvious possibility of a first-division segregation would result from the opposite orientation of the tetrad in the first division. The second figure would then show a white nucleus above and a black one below and the final arrangement in the ascus would be reversed.

In group b, is a diagram showing the arrangement of spores of opposite sex in the ascus that would result from a segregation of the factor for sex in the second division in the ascus and a failure to segregate in the first division. A second possibility would result from changing the orientation of both nuclei in the nucleate stage. In this case the last figure would show, from top to bottom, two white, two black, two white and two black. Ascus 348 is an example of a shift in positions 2 and 3, following this type of segregation.

In group c, is shown a diagram of another arrangement of the spores of diverse sex in the ascus of N. crassa which would result from a second division segregation of the factor for sex. If both nuclei in the binucleate stage were reversed, another possibility would be obtained with the four black nuclei in the center and two white at each end. Ascus 301 shows the change in this arrangement resulting from a one place shift.


b
a
c

This is done by studying the characters of the cultures grown from the respective ascospores of a single ascus, since the position of the spores in the ascus is known. Fig. 6 shows three of the six possibilities for first- versus second-division segregation. The other three are represented by reversing the positions of the two varieties of nuclei. In the case of the figure (6a) showing first-division segregation, the upper four nuclei would be "white" and the lower four "black" in the other equally probable orientations.

Dodge (27) and Wilcox (62) have shown in N. sitophila that the factors for cultural characters and the factors determining the two sexes can be segregated at either the first or at the second-division in the ascus. Dodge's (23) cytological evidence shows that in N. tetrasperma, where there is a very complex series of changes in spindle orientation, there is a greater probability of the sex factors being segregated at the first-division than at the second. The sex factors might be segregated at any one of the three divisions and still give rise to four hermaphroditic spores. He has also studied the segregation of the sex factors in N. crassa, and has inferred that they are segregated at the first division in this species. By

means of the arrangement of the spores in the long narrow ascus, the writer has studied the segregation of the factors for the two sexes and for various pairs of cultural characters in N. crassa. Segregation was found not to occur at the third division, since for all characters studied the spores were found grouped as four pairs of identical twins. Dodge's study of the orientation of the spindle in this species shows that it is probably identical with that of N. sitophila. All of the asci from which eight spores were germinated showed four spores of one sex and four of the opposite sex. The a priori possible arrangements in an ascus for eight spores, four of one sex and four of the opposite sex, are seventy. This is given by the formula:

$$\frac{8!}{4! \times 4!} = 70$$

Table VII shows 32 of the asymmetrical and the 6 symmetrical permutations. The remaining 32 asymmetrical permutations are obtained by an end to end reversion of the 32 asymmetrical ones shown. If the arrangement of spores in the ascus is determined by pure chance, there is one chance in 70 of each such permutation occurring.

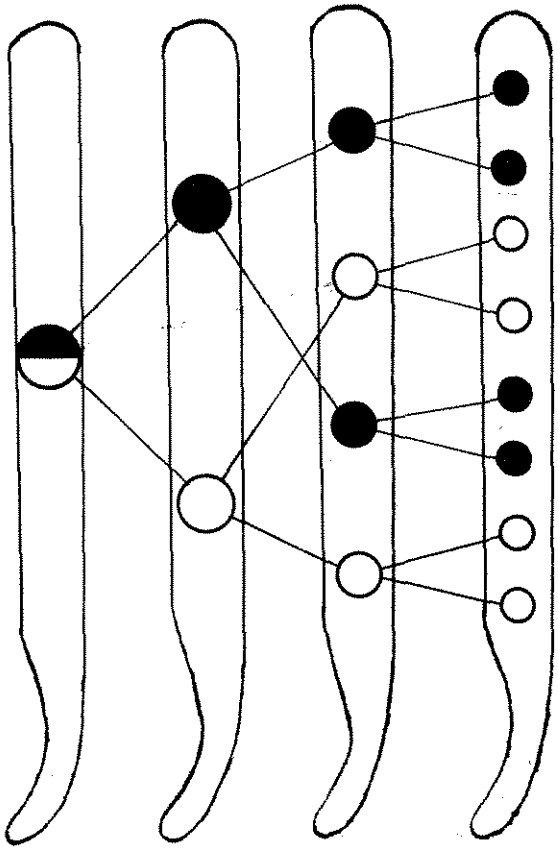


Fig. 7. A diagram showing how passing of the second and third nuclei in the four nucleate stage might result in an actual first division segregation producing the arrangement of spores ascribed to a second division segregation and vice versa. The cytological evidence of Wilcox and Dodge show this to be highly improbable.

Table IX. Sex and arrangement of ascospores in 275 asci of N. crassa=

P_3 Generation

Ascus								
0	-	-	-	-	+	+	+	+

P_2 Generation (0-1 x 0-8) (I selfed)

Ascus								
(1)	-	-	+	+	+	-	-	+
2	-	-	+	+	+	⊕	-	-
4	-	-	-	⊖	+	+	+	+
5	+	+	+	⊕	-	-	⊖	-

6	+	+	+	+	-	-	-	-
7	⊕	+	+	+	-	-	-	-
(10)	0	-	-	+	+	0	-	0

4 I : 2 II : 1 (?)

Back Cross Generation $P_2 \times P_3$

(0-1 x 6-2) I x I

ascus								
113	-	⊖	+	⊕	+	+	-	-
114	-	-	-	-	+	+	+	+
116	+	+	-	-	-	-	+	+
118	+	+	+	⊕	-	-	-	-
120	+	+	-	-	+	+	-	-
122	-	-	-	-	+	+	+	+
123	-	-	-	-	+	+	+	⊕
(124	⊕	+	+	+	-	-	-	-
(125	-	-	-	-	+	+	+	+
(126	+	+	+	+	-	-	-	-
(127	+	+	+	+	-	-	-	-
124(128	+	+	+	+	-	-	-	-
(129	+	+	+	+	-	-	-	-
(130	+	+	+	+	-	-	-	-
(131	-	-	-	-	+	+	+	+
(132	+	+	+	+	-	-	-	-
(133	+	+	+	+	-	-	-	⊖
(134	+	+	+	+	-	-	-	-
(136	+	+	-	-	-	-	+	+

15 I : 4 II

(0-1 x 7-3) I x I

ascus								
137(137	-	-	-	-	+	+	⊕	+
(139	-	-	-	-	+	+	+	+
141	-	-	-	-	+	+	+	+
142	+	+	-	-	-	-	+	⊕
144	⊖	-	⊖	-	+	+	+	+

4I : 1 II

(0-1 x 10-4) I x II(?)

ascus								
106	+	+	+	+	-	-	-	-
107	-	-	-	-	+	+	+	+
(109)	-	-	-	⊕	-	+	+	+
110	-	-	+	+	-	-	+	⊕
112	+	+	+	+	-	-	-	-

4I : 1 II

(0-1 x 1-3) I x II (?)

ascus								
100	⊖	-	-	-	+	⊕	+	+
101	-	-	+	+	+	+	⊖	⊖
103	-	-	-	-	⊕	+	+	+
104	+	⊕	⊕	+	-	-	-	-

3 I : 1 II

P_1 Generation (4-5 x 6-5) I x I

Ascus

299 ⊕ + + + − − − −
(301)+ − + − − − + +
302 − ⊖ − − + + + +
303 + + + + − − − −
306 − − − − + + + +
308 + + + + − − − −
314 + + + ⊕ − − − −
316 − − − − + + + +
317 + + + + − − − −
318 + + + + − − − −
320 − − − − + + + +
322 − − − − + + + +
323 + + − − − − + +
(324)− − − + − + + +
325 − − − − + + + +
329 − − − − + + + +

14 I : 3 II

F_1 Generation (Ascus 114 selfed) I selfed

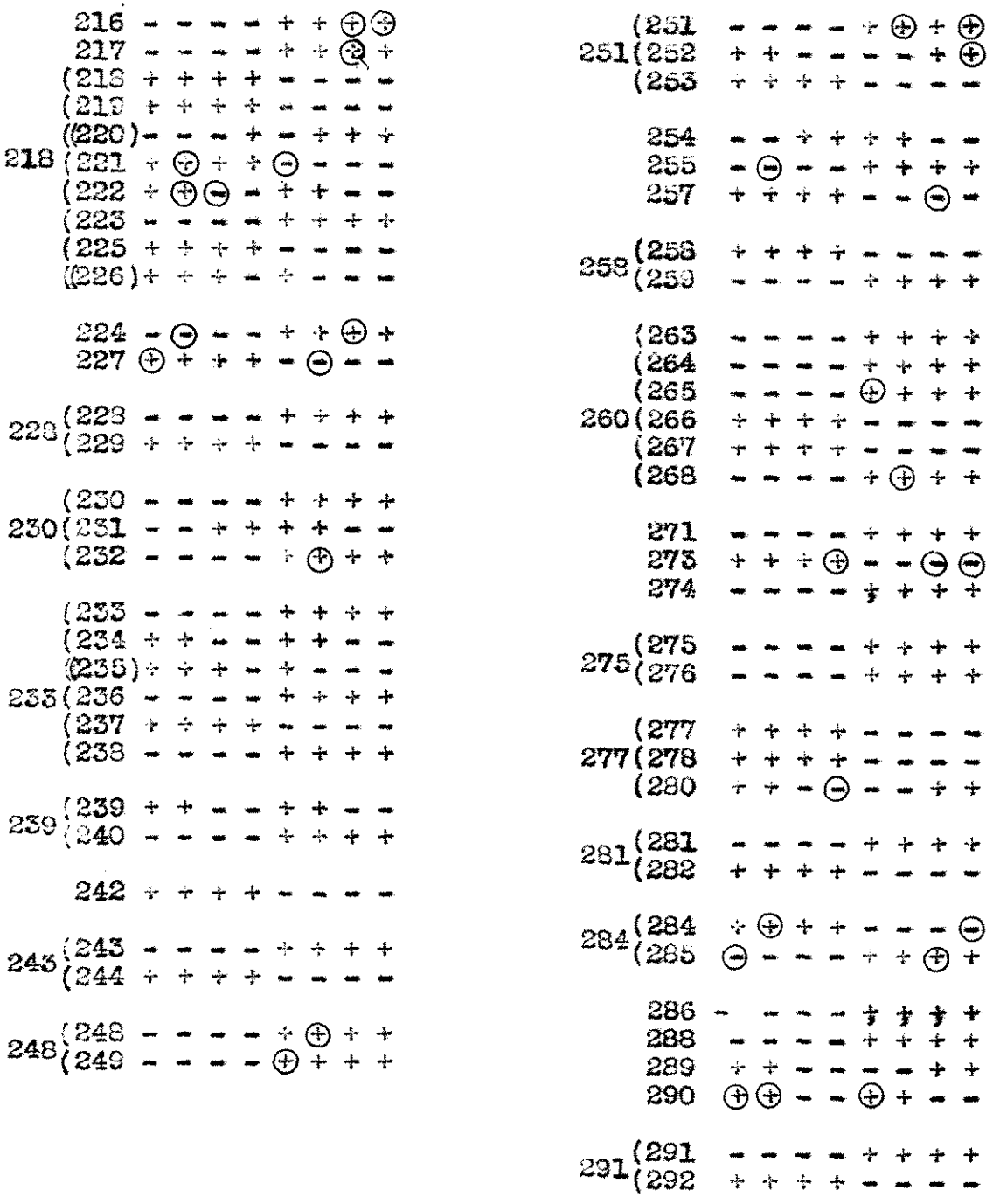
114- 1x3(I selfed)

153 + + + + − − ⊖ −
159 + + + + − − ⊖ −
160 + + + + − − − −
164 ⊕⊕ + + − − − −

4 I : 0 II

F_1 Generation (Ascus 114 selfed) I selfed

114-1x7



53 I : 9 II

F_2 Generation

(223-1 x 230-6) I x I

	ascus								
	331	-	-	-	-	+	⊕	+	+
	332	+	+	⊕	+	-	-	⊖	-
	333	+	+	⊕	+	-	⊖	-	-
	(335	-	-	-	-	+	+	+	+
	(336	-	-	+	+	+	+	⊖	-
	(337	+	+	-	-	-	-	+	+
	(338	-	-	+	+	+	+	-	-
335	(339	-	-	-	⊖	+	+	⊕	⊕
	(340	⊕	+	+	+	-	-	-	-
	(341	+	+	+	+	-	-	-	-
	(343	⊕	+	+	+	-	-	-	-

8 I : 3 II

(223-7 x 229-8) I x I

	ascus								
	345	-	-	-	-	+	+	+	+
	346	-	-	-	-	+	+	+	+
	347	+	+	+	+	⊖	-	-	-
	((348)	+	-	+	-	+	+	-	-
349	((349)	-	-	⊖	+	-	+	+	+
	(350	+	+	+	+	-	-	-	-
	351	+	+	-	-	-	-	+	+
	352	-	-	-	-	+	+	+	+
	353	-	-	-	-	+	+	+	+
	354	⊖	-	⊖	-	+	+	+	+
	355	+	+	+	+	-	-	-	-
	356	-	-	⊖	-	+	+	+	+
	357	-	-	-	-	+	+	+	+
	358	⊕	+	+	+	-	-	-	-
	359	-	-	-	-	+	+	+	+
	360	-	-	+	+	+	+	-	-
	362	-	-	-	-	+	⊕	+	⊕

14 I : 3 II

(160-1 x 8) I selfed

	ascus								
	(493)	⊖	-	-	+	-	+	+	+
	494	-	-	-	-	*	+	+	⊕
	496	-	-	⊖	⊖	⊕	+	+	+
	498	⊖	-	-	-	+	⊕	+	+
	500	-	-	-	-	+	+	+	+
	502	+	+	⊕	+	-	-	-	-
	503	+	+	+	+	-	-	-	-
	(504	+	+	+	+	-	-	-	-
	(505	-	-	-	-	+	+	+	+
504	(506	⊕	+	-	-	+	+	-	-
	(507	-	-	-	-	+	⊕	+	+
	(508	-	-	-	-	+	+	+	+
	(509	+	+	+	+	-	-	⊖	-
509	(510	+	+	+	+	-	-	-	⊖
	(511	⊖	-	⊖	-	+	+	+	+
	(512	-	-	-	-	+	+	+	+
	513	+	+	+	+	-	-	-	-
	514	+	+	+	+	-	-	-	-
	515	+	+	+	+	-	-	-	-
517	(518	⊕	+	+	+	-	-	-	⊖
	(519	-	-	-	-	+	+	+	+
	520	-	-	-	-	⊕	+	+	+

21 I : 1 II

(226-1 x 6) I selfed

	ascus								
	450	+	⊕	+	+	-	-	-	-
	452	+	+	+	+	⊖	⊖	-	-
	453	+	+	+	+	-	-	-	-
	459	+	+	+	⊕	⊖	-	-	-
	466	+	+	+	+	-	⊖	-	-
	467	+	+	+	+	-	-	-	⊖

6 I : 0 II

F_2 Generation

(243 - 2x5) I selfed)

ascus								
	(364	-	-	-	-	+	+	+	+
	(365	+	+	+	+	-	-	-	-
	(366	-	-	-	-	+	+	+	+
	(367	-	-	-	-	+	+	+	+
364	(368	+	+	-	-	+	+	-	-
	(369	-	-	-	-	+	+	+	+
	(370	-	-	+	+	-	-	+	+
	(372	+	+	+	+	-	-	-	-
	(373	-	-	-	-	+	+	+	+
373	(374	+	+	+	+	-	-	-	-
	(375	-	-	-	-	+	+	+	+
	376	-	-	-	-	+	⊕	+	+
	377	-	-	-	-	+	+	+	+
	(378)	-	-	-	+	-	+	+	+

12 I : 2 II

(273-3 x 290-3) I x II

569	(569	-	-	-	-	⊕	+	⊕	+
	(570	+	+	+	+	-	-	-	-
571	(571	+	+	-	-	+	+	-	-
	(572	+	+	+	+	-	⊖	-	-
573	(573	-	-	-	-	+	⊕	⊕	+
	(574	⊖	⊖	⊕	+	-	-	+	⊕
	(575	⊕	+	+	⊕	⊖	⊖	-	-
	576	+	+	+	+	-	-	-	-
577	(577	+	+	+	+	-	-	-	-
	(578	-	-	-	-	+	+	+	+
	(579	+	+	+	+	-	-	-	-
	(580	-	-	-	-	+	+	+	+
581	(581	-	-	-	-	+	+	+	+
	(582	-	-	-	-	+	+	+	+
583	(583	-	-	-	-	+	+	+	+
	(584	+	+	+	+	-	-	-	⊖
	(585	-	-	-	-	+	+	+	+

15 I : 2 II

(288-5 x 290-7) I x II

546	(548	-	-	-	-	⊕	+	+	+
	(549	-	-	⊖	-	⊕	+	+	⊕
	(550	-	-	+	+	+	+	-	-
551	(551	-	-	⊖	⊖	+	+	+	+
	(552	+	+	+	+	-	-	-	-
553	(553	+	+	+	+	-	-	-	-
	(554	+	+	+	+	-	-	-	-
555	(555	-	-	-	-	+	+	+	+
	(556	-	-	-	-	+	+	+	⊕
	(557	-	-	-	-	+	+	+	+
558	(558	+	+	+	+	-	-	-	-
	(559	+	+	+	+	-	-	-	-
560	(561	-	-	-	-	+	+	+	+
	(562	-	-	-	⊖	+	+	+	+
	(564)	⊕	⊕	+	-	+	-	-	-
	567	-	-	-	-	+	+	+	+

15 I : 1 II

F_3 Generation

(337 - 6x7) II selfed

ascus								
379	+	+	+	+	−	−	−	−
380	−	−	−	−	+	+	+	+
381	−	−	−	−	+	+	+	+
382	+	+	−	−	−	−	+	+
383	+	+	+	+	−	−	−	−
384	−	−	−	−	+	+	+	+

5 I : 1 II

(338 - 6 x 7) II selfed

ascus								
(470	+	+	+	+	−	−	−	⊖
470 (471	−	−	−	⊖	+	+	+	+
(473	−	−	−	⊖	+	+	+	+
(474	−	−	−	⊖	+	+	+	+
473 (475	+	+	⊕	+	⊖	−	−	−
(476	+	+	+	+	−	−	⊖	−
(477	−	−	−	−	+	+	+	⊕
477 (478	−	−	+	+	+	+	−	−
(479	⊕	⊕	+	+	−	−	−	−
(480	−	−	−	−	+	+	+	+
(481	−	−	−	−	+	+	+	+
480 (482	−	−	+	+	+	+	−	−
(483	−	−	−	−	+	+	⊕	+
(484	−	−	⊖	−	+	+	+	+
(486	−	−	−	−	+	+	+	+
486 (487	+	+	⊕	+	−	−	−	−
(488	−	−	−	−	+	+	+	+
488 (489	−	−	−	⊖	+	+	+	+
(490	−	−	−	−	+	+	+	+

17 I : 2 II

(337 - 2 x 3) II selfed

ascus								
386	+	+	+	+	⊖	−	−	−
387	−	−	−	−	+	+	+	+
388	−	−	+	+	+	+	−	−
389	−	−	−	−	+	+	+	+
390	−	−	−	−	+	⊕	+	+

4 I : 2 II

(338-2x3) II selfed

ascus								
521	−	−	−	−	+	+	+	+
(525	−	−	+	+	−	−	+	+
525 (526	+	+	+	+	−	−	−	−
(527	+	+	+	+	−	−	−	−
(528	−	−	−	−	+	+	+	+
(529	−	−	−	−	+	+	+	+
528 (530	+	+	+	+	−	−	−	−
(531	+	+	+	+	−	−	−	−
532 (532	⊖	−	+	+	−	−	+	+
(533	+	+	+	+	−	−	−	−
(534	−	−	−	−	+	+	+	+
534 (535)	+	+	+	+	−	−	−	−
(536	−	−	⊖	−	+	+	+	+
537	+	+	+	+	−	−	−	−
540 (540	+	+	+	⊕	−	−	−	−
(541	−	−	−	−	+	+	+	+
543	⊖	−	⊕	+	+	+	−	⊖
544	+	+	+	+	−	−	−	−

15 I : 3 II

The 6 arrangements of which 3 are shown in Fig. 6 could all be the result of either first-or second-division segregation, provided the spindles in the second division were long enough and the ascus wide enough to permit the two middle nuclei to pass each other in the four nucleate stage. Fig. 7 is a diagram showing such a shift in position. With such a shift, a first-division would produce the arrangement usually ascribed to a second-division segregation and vice versa. If such a shifting occurred, it might be objected that the data do not show evidence of more than one type of segregation. But Wilcox (62) in her cytological study of a closely related species, N. sitophila, shows that no such shifting takes place. Moreover, ascus 114 (Fig.8) proves that both types of segregation occur. A character which was called tan, appeared in the mycelia grown from the ascospores of this ascus. The conidia of tan were produced more sparsely than in the wild-type, and were white or yellow, while those of the wild-type were orange or light orange. In Table VIII it can be seen that the sex factors were segregated in the first division, while wild and tan were segregated in the second. Even if it were assumed that the second and third nuclei exchanged positions in the second division, the results show that both first-

and second-division segregation must have taken place. Fig. 8 shows a diagram of ascus 114.

Table IX is a record of the sex and arrangement of the ascospores in 275 asci of N. crassa, comprising six generations. These asci are all descended from a single ascus. In each column the numbers at the left are the numbers of the individual asci. Plus and minus signs are used to indicate whether or not the mycleium of an individual ascospore produced fertile perithecia when mated to a tester strain. The p lus sign denotes one sex and the minus sign the other. The eight spaces from left to right correspond to the positions of the ascospores in the ascus. The first position indicates the ascospore located at the tip of the ascus and the eightth position indicates the ascospore located at the base. A bracket at the left of the numbers of the asci indicates that all of the asci bracketed come from one perithecium and the number at the left of the bracket is the number of the perithecium. Above each group is shown the mating which produced the corresponding asci. For example, O-1 x 6-2 means mating the mycelium from

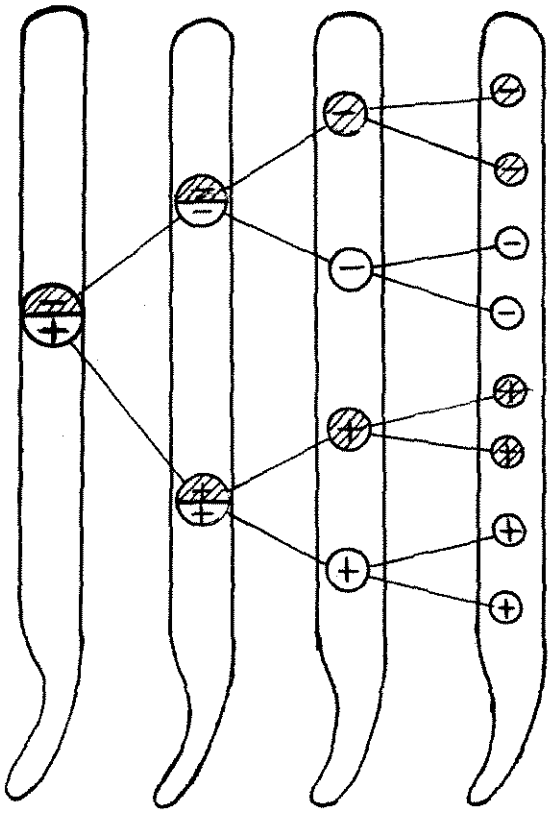


Fig. 8. A diagram of the arrangement of spores of opposite sex and producing tan and wild type mycelia in ascus 114. The arrangement of the spores proves that even if a shift as described in Fig. 7 had taken place, both first-and second-division segregation occurred.

the first ascospore in ascus O to the mycelium from the second ascospore in ascus 6. The symbols I x I following indicate that ascus O was an ascus in which sex was segregated in the first division in the ascus and ascus 6 was an ascus in which sex was also segregated in the first division. Roman numberal II is the symbol used to indicate second division segregation of sex.

When ~~every~~ spores did not germinate, the position of an ungerminated spore is indicated by a ring. When it is possible from the position and sex of the other spores to determine the sex of the missing spore a plus or minus sign is placed within the ring. From the fact that in every case where all eight spores germinated, there were four of one sex and four of the opposite sex in the ascus, this determination is easy to make.

The arrangement of the spores in the asci shows that segregation of factors determining sex may occur either in the first or the second division of the ascus nucleus. Exceptional asci are marked by parentheses. These undoubtedly are all due to slipping of adjacent nuclei past each other during or after the third division. Proof of this point rests on the correspondence between the arrangement of cultural characters as shown in Table XV and sex as shown in Table IX in

the case of asci containing such shifted nuclei. Ascus 226 is a case in point. The arrangement of the ascospores of different sex shown in Table IX (for ascus 226) indicates a shift in positions 4 and 5. The arrangement of the spores producing different types of mycelia as shown in Table XV confirms the fact that this is merely shifting of the nuclei. It is clear that tan and sex are independently segregated yet the same peculiar arrangement of the fourth and fifth spores in ascus 226 holds for both tan and sex as shown in Table X. Reversing the fourth and fifth spores gives a simple first division segregation of sex and second division segregation of tan and orange. Two other exceptions which cannot be readily interpreted on this basis are asci 1 and 10, but these were dissected before the writer became expert enough to be certain of his technique.

To date no evidence has been obtained of segregation of any factors in the third division in the ascus. Fig. 9 is a diagram of a hypothetical ascus which would be evidence for a third division segregation if encountered. None such ascus has been discovered.

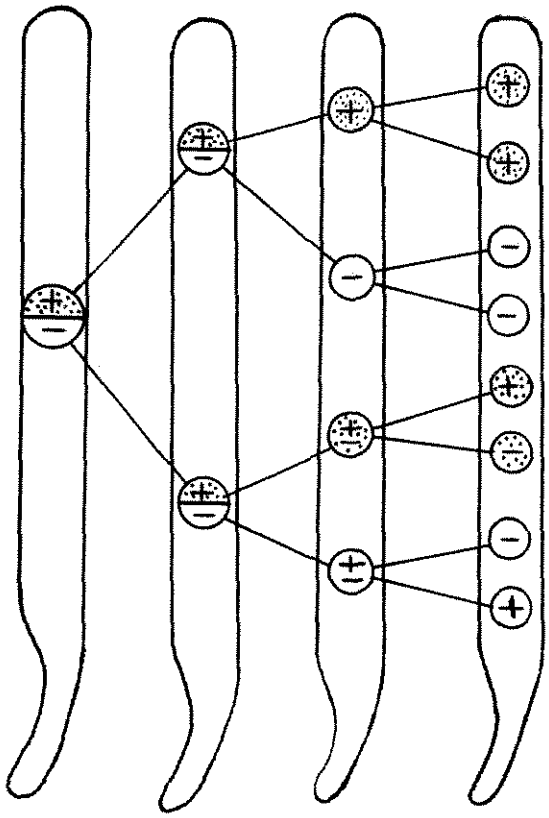


Fig. 9. Diagram of a hypothetical ascus showing an arrangement of spores of opposite sex and different cultural characters that would prove that third division segregation had occurred. No such ascus has been found.

In order to facilitate discussion from this point on, some abbreviations will be used. In making matings, individual ascospores are planted in separate tubes on nutrient agar until mycelia and conidia appear. Then some of the mycelia and conidia from two tubes is planted into a third tube. Strictly speaking matings are made between clones produced by ascospores and not between the ascospores. However, from now on, such matings as that which produced the P_2 generation will be described as follows: Ascospore 0-1 was mated to ascospore 0-8, or more briefly; 0-1 x 0-8 (I selfed). The expression in parentheses is a second convention indicating that the mating was made between mycelia produced by ascospores from the same ascus (selfed) and that segregation of sex in that ascus occurred in the first division (I).

Ascus 0 shown in Table IX is the parent ascus of all the N. crassa ascospores studied. From the P_2 generation which was produced by a I selfed mating (0-1 x 0-8), six asci were dissected. In four of these sex was segregated in the first division and in two of them it was segregated in the second division.

Stated briefly: the P_2 generation produced by a I selfed mating contained four I and two II. This is 67 percent I.

The four groups in the $P_2 \times P_3$ back cross generation contained 79, 80, 80 and 75 percent I respectively. The first two groups were produced by a I x I mating and the second two groups by a I x II mating.

It is evident in this and the succeeding generations (perithecia 124, 218, 230, 233, 239, 251 and 277) that first-and second-division segregation of sex may occur in asci side by side in the same perithecium.

Table XI summarizes the data in Table IX. It is apparent that the three types of matings (I x I, I x II, and II x II) give approximately the same percentage of I. The mean of the total 275 asci is 85 percent I, and the percentages obtained in the II x II matings vary no more from this mean than those obtained by the I x I matings.

Table XII illustrates this point. All of the matings are grouped into their three different classes. I x I produced 85 percent I; II x II produced 84 percent I; and I x II produced 88 percent I.

Table XI. Summary of Table IX showing the percentages of first division segregation produced by twenty matings in seven generations.

Generation	Total No. of Asci	Mating	%-I
P_3	1		100
P_2	6	I selfed	67
P_2x P_3	19	I x I	79
P_2x P_3	5	I x I	80
P_2x P_3	5	I x II?	80
P_2x P_3	4	I x II?	75
P_1	16	I x I	88
F_1	62	I selfed	86
F_1	4	I selfed	100
F_2	11	I x I	73
F_2	17	I x I	82
F_2	22	I selfed	96
F_2	14	I selfed	86
F_2	6	I selfed	100
F_2	16	I x II	94
F_2	17	I x II	88
F_3	6	II selfed	83
F_3	19	II selfed	90
F_3	6	II selfed	67
F_3	18	II selfed	83
		Mean	85% I

Table XII. Data in Table XI arranged to show the percentage of first division segregation produced by all the IxI, IxII and IIxII matings.

Mating	Number of asci showing first division segregation	Number of asci showing second division segregation	Percentage of first division segregation
I x I	155	27	85
I x II	37	5	88
II x II	41	8	84

The best test for the possibility of genic factors regulating the type of segregation is selection and subsequent crossing. Table XIII gives the results of six generations of selection for first division segregation. It may be seen from the table that even after six generations of I x I matings, the percentage of first division segregation is not increased.

Therefore, there is established this definitive numerical datum: Sex in Neurospora crassa is segregated in the first division in the ascus in 85 percent of the asci and in the second division in the ascus in 15 percent of the asci. These percentages are constant regardless of whether sex had been segregated in the first or second division in either or both parents. It is not possible by selection to increase or diminish these percentages. Thus the type of segregation obtained cannot be directly attributed to the effect of genic factors.

On the basis of pure chance, four types of II should occur equally frequently. Table XIV shows the frequency of each one of the four types encountered in the forty II obtained. The 26 cases in which four like nuclei are grouped in the center of the ascus as compared with the 14 cases in which the like nuclei are arranged by twos in the ascus indicates an attraction of like nuclei for each other, but the numbers obtained are too small to be conclusive.

Table XIII. Results of selection for first division segregation carried on through six generations.

Generation	Number of asci	Percentage of first division segregation	Mating
P_3	1		
P_2	6	67	(0-1x0-3)(I selfed)
P_{2x3}	19	79	(0-1x6-2)(I x I)
P_1	16	88	(4-5x6-5)(I x I)
F_1	62	86	(2[illegible]4-1x7)(I selfed)
F_2	17	82	(223-7x229-8)(I x I)

Table XIV= Frequency distribution of the four possible types of second division segregation.

Type of II	Frequency
- - + + + + - -	16
+ + - - - - + +	10
+ + - - + + - -	9
- - + + - - + +	5

IV. INHERITANCE OF CULTURAL CHARACTERS

1. Description of cultural characters.

Among the asci produced in the first two generations, no deviations from the wild-type cultural characters were noted. In a third generation from which over thirty asci were dissected, one ascus was different from the rest in that the mycelia produced by four of its ascospores were extreme variants from the characteristic wild type growth. This ascus (114) was one of the 19 others obtained from a single mating, but none of the other cultures from ascospores of the other asci showed any indication of this variation. This variant was called tan and, by using it as a parent, several subsequent variants were obtained. A description of the cultural characters of these variants as they appear on corn meal agar follows:

a. Wild Type. The wild type culture of N. crassa contains a dense growth of bright orange conidia in large clumps almost filling the diameter of the tube a short distance above the agar. The agar slope is covered with a fluffy growth of short grayish white aerial mycelium. At the upper rim of the agar, below the mass of conidia, there is a crescent of dense cottony white fluff. The substrate rarely has any color other than that of the

agar itself, but occasionally a small light or dark brown spot appears. Numerous bulbils appear in most wild-type cultures. In some cultures these are colorless and can only be seen with the microscope, but in others they turn dark brown and give the culture a specked appearance. Occasionally a few of these enlarge to the size of normal perithecia but crushing them always discloses the fact that they contain no ascospores. Although these sterile perithecia are encountered in both kinds of unisexual cultures, they are more frequent in those of minus sex. The abbreviation w is used to designate this type of culture.

b. Tan. The tan variant never produces the dense mass of conidia that characterizes the wild type cultures. The color of the conidia may be white, yellow or light orange. The substrate is smooth with no aerial mycelium and has a tan color. It is unusual to find the specking characteristic of the wild type cultures in a tan culture. Five classes of the tan variant are recognized: dark tan (dt), tan (t), light tan (lt), very light tan (vt), and extremely light tan (xt). Dark tan cultures (dt) produce extremely sessile conidiophores and white conidia. Extremely light tan cultures (xt) produce almost the same color and density of conidial growth as the wild-type

cultures. The other classes fall between these two extremes in the order named. That is to say, there seems to be a definite correlation, with few exceptions, between the density of the substrate color and the amount and color of the conidia.

c. Albinistic. Albinistic (a) cultures contain fluffy white mycelium growing two or three times higher in the tube than wild type conidiophores. The ends of the hyphae that touch the tube send out a radiate finger-like cluster of short hyphae tightly applied to the glass that are suggestive of appressoria. The name albinistic is used because of the similarity of this character to the albinistic strains developed by Dodge in the species N. sitophila. At the base of the white fluffy growth, conidia are produced in masses considerably less dense and lighter in color than in the wild type. Tan substrate sometimes occurs in albinistic cultures (ta) and when this happens, the height to which the fluffy mycelium grows in the tube is much reduced and conidium formation is almost entirely suppressed.

d. Even. The even (e) character gets its name from the fact that an even velvety yellow growth of mycelium fills the tube completely a short distance above the agar. The even character of the growth is very pronounced. It spreads entirely around the inner

circle of the tube with no prominences or depressions. The outer edge of this circle is made up of yellow clavate appressoria tightly applied to the glass. Much reduced sessile bunches of conidia are produced below the ring of even growth.

e. Pale. The pale (p) cultures differ from the wild type in the extremely light orange color of the conidia and the perfuse sparse growth of the conidiophores.

f. Black. Black (b) cultures resemble the wild type except for the appearance of an orange coloration of the substrate a week or so after the ascospore is planted which changes to dark brown in a few weeks. When this coloration is very intense the culture is called dark black (db) and when it is not very pronounced, the culture is called light black (lb).

g. Opalescent. Opalescent cultures (o) are characterized by a perfuse growth of conidiophores bearing a few light orange conidia and a pearly felted appearance of the substrate. This felted appearance of the substrate sometimes appears in tan cultures (to).

h. Orange. Orange (or) resembles wild type except for an orange coloration of the substrate.

2. Inheritance of tan.

Following the appearance of the tan variant in ascus 114, a series of breeding experiments were performed. Table XV gives a detailed record of these experiments and Fig. 11 is a condensed genealogical tree. Three matings were made from ascus 114: 1 x 7 (w x t), 1 x 8 (w x t), and 3 x 8 (t x t). The first mating was made in duplicate in two different tubes. Fifty-five asci were dissected from this cross and planted on corn meal agar. Table XV shows the position of the ascospores in the ascus and the various characters developed by them in culture. A first glance at the table shows the scarcity of opalescent cultures obtained from the mating made in Tube A and the abundance of them obtained from the mating made in Tube B. This indicated that accidental selection of one sector in transferring the cultures had resulted in hereditary transmission of the selected character.

The next thing that strikes the eye is that a single perithecium as, for example, perithecium 233 may produce (in this case) five different kinds of asci. Further inspection of the table reveals that four tan cultures were produced from almost every ascus, but the other four cultures instead of being wild type were

Table XV Cultural characters of five generations of N. crassa and position of ascospores in ascus.

First Generation 114 - 1 x 7 (w x t)

Tube A

217	t	t	t	t	or	or	(or)	or
(218	t	t	t	t	lb	lb	lb	lb
(219	t	t	t	t	or	or	db	db
((220)	t	t	or	t	or	t	b	b
(221	w	(w)	t	t	(t)	t	b	b
218(222	b	b	t	t	b	b	t	t
(223	t	t	t	t	lb	lb	db	db
(225	t	t	lb	lb	or	or	t	t
((226)	or	or	t	or	t	or	t	t
224	lb	(lt)	t	t	t	t	(lt)	lb
(228	db	db	t	t	or	or	b	b
228(229	lb	lb	w	w	or	or	db	db
(230	lb	lb	w	w	lt	lt	dt	dt
230(231	or	or	lb	lb	t	t	t	t
(232	or	or	t	t	w	(t)	w	t
(233	t	t	or	or	db	db	or	or
(234	o	o	o	o	o	o	o	o
((235)	o	o	lt	dt	lt	dt	o	o
233(236	t	t	o	o	o	o	t	t
(237	or	or	lb	lb	t	t	t	t
(238	or	or	lb	lb	t	t	t	t
(239	t	t	or	or	db	db	t	t
240	or	or	t	t	or	or	t	t
242	t	t	db	db	or	or	t	t
(243	o	o	lt	lt	o	o	o	o
243(244	o	o	o	o	o	o	o	o
249	w	w	t	t	t	t	or	or

TUBE B

251(252	w	w	t	t	o	o	t	(t)
(253)	t	t	t	o	t	o	o	o
254	o	o	o	o	lt	lt	o	o
255	o	(o)	lt	lt	o	o	lt	lt
257	t	t	o	o	t	t	o	o
258(258	o	o	lt	lt	dt	dt	o	o
(259	o	o	dt	dt	lt	lt	o	o
(263	o	o	dt	dt	lt	lt	o	o
(264	o	o	lt	lt	lt	lt	o	o
(265	o	o	lt	dt	dt	dt	o	o
260(266	o	o	o	o	lt	lt	dt	dt
(267	lt	lt	o	o	dt	dt	o	o
(268	o	dt	o	dt	lt	(lt)	o	o
271	dt	dt	dt	dt	o	o	o	o
274	lt	lt	o	o	o	o	dt	dt

Tube B?

275(275	o	o	o	o	lt	lt	lt	lt
(276	o	o	o	o	dt	dt	lt	lt
(277	o	o	dt	dt	o	o	lt	lt
277(278	o	o	dt	dt	lt	lt	o	o
(280	o	o	lt	(lt)	o	o	dt	dt
281(281	lt	lt	dt	dt	o	o	o	o
(282	lt	lt	o	o	o	o	dt	dt
284(284	o	o	o	o	dt	dt	lt	(lt)
(285	(lt)	lt	o	o	o	o	(dt)	dt
286	o	o	o	o	(dt)	dt	lt	lt
289	lt	lt	o	o	dt	dt	o	o
291((291)	o	o	o	o	t	t	lt	lt
(292	w	w	t	t	t	t	w	w

Table XV - First Generation - continued.

114 - 3 x 8 (t x t)

627	-	w	→	w	lto	w	w	lto
630	o	w	o	o	vto	vto	vto	vto
631	o	o	o	(o)	to	to	(to)	to
(632	w	w	w	(w)	to	to	to	to
632(633	w	w	(w)	w	to	to	w	w
(634	lto	lto	vt	vt	(w)	w	w	w
635	w	w	to	to	w	to	to	w
636	to	to	to	to	w	w	w	w
637	xt	xt	w	(w)	to	to	w	w
638	w	w	w	(w)	lto	lto	w	w
639	w	w	w	w	to	to	lt	to
640	w	w	w	w	to	t	to	to
641(641	to	to	w	w	w	(w)	w	w
(642	w	w	(w)	w	w	w	to	to

114 - 1 x 8 (w x t)

158	w	w	t	t	w	t	(w)	t
159	w	w	w	w	t	t	t	t
164	o	o	t	t	w	w	w	w
165	t	t	w	o	o	w	t	t

Table XI - continued.

Second Generation

223-1 x 230-6 (t x t)

331 t t t t w (w) w w
332 tt t (lt) lt w w (w) w
333 t t t t t (t) vt vt

335
(335 o o t t t lt o o
(336 w w w w lt dt (dt) dt
(337 w w w w t t t t
(338 t t t t o o o o
(339 t t t t w w (w) (w)
(340 (w) w w w t t t t
(341 w w lt lt dt dt w w
(342 (w) w t t t t o o

228-5 x 290-7 (dt x dt)

546
(546 to to to (to) w w (w) (w)
(548 w w w w (t) t t t
(549 w w (w) w t t t (t)
(550 w w w w to to to to

551
(551 w w (w) (w) to to vlt to
(552 w w to to to to w w

553
(553 to to to to w w w w
(554 to to to to w w w w

555
(555 w w w w to to to to
(556 w w w w to to to (to)
(557 w w w w to to to to

558
(558 dtodtoto to w w w w
(559 to to to to w w w w

560
(561 w w w w to to to to
(562 w w w w to to to to
((564)(to) (to) to w to w w w
(565)(w) w (w) w to t lto (lto)

566 (to) to (to) to w w w w
567 w w w w dtodtodtodto

273-3 x 290-3 (o x o)

569
(569 w w w w (w) w (w) w
(570 w w w w w w w w

571
(571 w w w w w w w w
(572 w w w w w (w) w w

573 w w w w w (w) (w) w
576 w w w w w w w w

577
(577 w w w w w w w w
(578 w w w w w w w w
(579 w w w w w w w w
(580 w w w w w w w w

581
(581 w w w w w w w w
(582 w w w w w w w w

583
(583 w w w w w w w w
(584 w w w w w w w (w)
(585 w w w w w w w w

586 w w w w w w w w

223-7 x 229-8 (db x db)

345 b b b b b b b b
346 b b b b b b b b
347 b b b b (b) b b b
(348) b b b b b b b b
(349) b b (b) b b b b b
350 b b b b b b b b
351 b b b b b b b b
352 b b b b b b b b
353 b b b b b b b b
354 b b b b b b b b
355 b b b b b b b b
356 b b (b) b b b b b
357 b b b b b b b b
358 (b) b b b b b b b
359 b b b b b b b b
360 b b b b b b b b
361 b b (b) b b b (b) (b)
362 b b b b b (b) b (b)

Table XV - continued.
Second Generation - continued.

243-2x5 (o x o)

(364	w	w	db	db	w	w	b	b
(365	db	db	w	w	w	w	b	(b)
(366	w	w	b	b	b	b	w	w
(367	b	b	w	w	vb	vb	w	w
364(368	w	w	lb	lb	vb	vb	w	w
(369	b	b	w	w	w	w	b	b
(370	db	db	w	w	w	w	b	b
(372	db	db	db	db	w	w	w	w
(374	w	w	db	db	w	w	b	b
373(375	b	b	w	w	w	w	b	b
376	b	b	w	w	db	(db)	w	w
(378)	db	w	w	lb	w	lb	w	w

226-1x6 (or x or)

449	w	(w)	e	(e)	o	o	e	e
450	e	(e)	e	e	w	w	w	w
452	e	e	e	e	○	○	w	w
453	e	e	w	w	w	w	e	e
454	w	(w)	w	(w)	e	e	○	○
459	e	e	w	(w)	(w)	w	e	e
466	w	e	w	e	e	(e)	w	w
467	e	e	w	w	w	w	e	(e)

228-7 x 216-4 (lb x lb)

545a	p	(p)	p	p	w	w	to	to
545b	w	w	p	(p)	lt	lt	○	○
545c	t	t	p	p	p	p	w	w

Random ascospores

17 t : 17 w : 31 p

Offspring of selected clone
160-1t x 160-8t

(493)	(w)	w	lt	w	lt	lt	lt	lt
494	w	w	lt	lt	w	w	lt	(lt)
(495	(lt)	lt	○	○	w	w	w	w
(496	w	w	○	○	lt	lt	lt	lt
495(497	lt	lt	(w)	w	lt	(lt)	(w)	w
(498	○	lt?	lt	lt?	w	○	w	w
499	(w)	w	lt	lt	w	w	○	○
500(500	lt	lt	lt	lt	w	w	w	w
(501	○	○	w	w	lt	lt	lt	lt
502	w	w	lt	lt	lt	lt	w	w
503	lt	lt	w	w	w	w	lt	lt

160-1w x 160-8w

(504	lt	lt	lt	lt	w	w	w	w
(505	w	w	w	w	lt	lt	lt	lt
504(506	(lt)	lt	w	w	lt	lt	w	w
(507	w	w	w	w	lt	(lt)	lt	lt
(508	w	w	w	w	lt	lt	lt	lt
(509	lt	lt	lt	lt	w	w	(w)	w
(510	lt	lt	lt	lt	w	w	w	(w)
509(511	(w)	w	(w)	w	lt	lt	lt	lt
(512	w	w	w	w	lt	lt	lt	lt
513	t	w?	w?	t	w	w	w	w
514	lt	lt	lt	lt	w	w	w	w
515	lt	lt	lt	lt	w	w	w	w
517(518	(lt)	lt	lt	lt	w	w	w	(w)
(519	w	w	w	w	lt	lt	lt	t
520(520	w	w	w	w	w	w	w	w
(521	(w)	w	○	○	(w)	w	lt	lt

Table XV - continued

Third Generation

368-6 x 374-8 (lb x lb)

(724	lb	w	w	w	w	w	lb	w
(725	w	w	w	w	w	w	w	w
(726	w	w	w	w	w	w	w	w
(727	w	w	w	w	w	w	w	○
(728	w	ⓦ	w	w	w	w	w	w
726(729	w	w	w	w	w	w	w	w
(738	w	w	w	lb	w	w	w	w
(739	w	w	w	w	w	w	w	w
(740	w	w	w	w	w	w	w	w
(741	lb	lb	w	w	w	w	w	w
730	lb	w	w	w	w	lb	w	w
731	w	w	w	w	w	w	w	w
732	w	w	○	w	○	w	○	lb
733	w	w	lb	w	w	w	w	w
(734	w	w	○	w	w	w	w	lb
734(735	w	w	w	w	w	w	w	w
736	w	w	w	w	w	w	w	w
737	w	w	w	w	w	w	w	w

545c-4x5 (p x p)

776 - 25 single ascospore cultures

all p

453-1x8 (e x e)

742 - 150 ascospores all e

355-1x8 (b x b)

755 - 15 random ascospores

all b

Table XV - continued

Third Generation -continued

337-6x7 (t x t)

379	t	t	t	t	w	w	w	w
380	w	w	t	t	t	t	w	w
381	t	t	t	t	w	w	w	w
382	t?	t?	w	w	w	w	t?	t?
383	t	t	w	w	t	t	w	w
384	w	w	t?	t?	w	w	t?	t?

337-2x3 (w x w)

385	w	w	w	w	w	w	Ⓦ	w
386	w	w	w	w	w	w	w	w
387	w	w	w	w	w	w	w	w
388	w	w	w	w	w	w	w	w
389	w	w	w	w	w	w	w	w
390	w	w	w	w	w	Ⓦ	w	w

338-2x3 (t x t)

524	lt	xt	w	w	w	w	lt	xt
(532 Ⓦ	w	lt	lt	lt	lt	w	w	
532 (533	lto	vt	w	w	lt	lt	w	w
(534	w	w	to	to	w	w	lt	lt
534 (535)	vt	w	vt	w	lt	lt	w	w
(536	w	w	Ω	lt	lt	xt	w	w
537	to	vto	to	vt	w	w	w	w
544	w	w	w	w	t	lt	vt	lt
(525	w	w	w	w	w	w	w	w
525 (526	w	w	w	w	w	w	w	w
(527	w	w	w	w	w	w	w	w
(528	w	w	w	w	w	w	w	w
528 (529	w	w	w	w	w	w	w	w
(530	w	w	w	w	w	w	w	w
(531	w	w	w	w	w	w	w	w
(540	w	w	w	w	w	w	w	w
540 (541	w	w	w	w	w	w	w	w
543	w	w	Ⓦ	w	w	w	w	Ⓦ
(538	su	su	su	su	w	w	w	w
538 (539	su	su	su	su	Ⓦ	w	w	w

338-6x7 (o x o)

470	w	w	w	w	w	w	w	Ⓦ
471	w	w	w	Ⓦ	w	w	w	w
(473	w	w	w	Ⓦ	w	w	w	w
(474	w	w	w	Ⓦ	w	w	w	w
473 (475	w	w	Ⓦ	w	Ⓦ	w	w	w
(476	w	w	w	w	w	w	Ⓦ	w
(477	w	w	w	w	w	w	w	Ⓦ
477 (478	w	w	w	w	w	w	w	w
(479 Ⓦ	Ⓦ	w	w	w	w	w	w	w
(480	w	w	w	w	w	w	w	w
(481	w	w	w	w	w	w	w	w
(482	w	w	w	w	w	w	w	w
(483	w	w	w	w	w	w	Ⓦ	w
480 (484	w	w	Ⓦ	w	w	w	w	w
(485	w	w	w	w	Ⓦ	Ⓦ	w	Ⓦ
(486	w	w	w	w	w	w	w	w
486 (487	w	w	Ⓦ	w	w	w	w	w
(488	w	w	w	w	w	w	w	w
488 (489	w	w	w	Ⓦ	w	w	w	w
(490	w	w	w	w	w	w	w	w

Table XV - continued

Third generation continued

338- 1x4 (t x t)

All wild

(674 w w w w w w w w
(675 w w w w w Ⓦ w w
674(676 w w w w w w w w
(677 w w w w w xt w w
(678 w xt xt w w w w w
(679 w w w Ⓦ w w w w

all tan

696(696 lto to lto to to to to to
(697 vto vto to vto vt (vt) vt vt

71(716 lto lto vto vto lto lto vto vto
(717 vto vto vto vto lto lto lto lto

Extremely light tan and wild

(663 xto xto xto xto w w w w
(664 xto xto ○ xto w w w w
(665 w w w w xto xto xto xto
(666 w w w w xto xto xto xto
(667 w w w w xto xto xto xto
663(668 w w w w xto xto xto xto
(669 w w w w xto xto xto xto
(670 w w w w xt w xt xt
(671 xto xto xto xto w w w w
(672 xto xto xto xto w w w w
(673 xt w xt xt w w w w

685 xt xt xt xt w w w w

Mixed

(643 lto lto lto lto w w w w
(644 xt w xt xt ○ ○ w w
643(645 w w w w w w xt xt
(646 w Ⓦ w w w w w w
(647 w Ⓦ Ⓦ w w w Ⓦ w

648(648 lto lto lto lto lto to lt lt
(649 w w w w lto lto xto lto

Light tan and wild

(650 w w w w vto xto vto
(651 w w w w vto vto vto vto
650(652 w w w w lto lto lto ○
(653 (vt) vt vt vt w w w w
(654 w w w w lto lto lto lto

(655 w w w w lto lto lto lto
655(656 lto lto lto (lto) w w w
(657 w w dt dt vt dt w w
(659 t t t lto w w w w

Table XV - continued

Third generation - continued

338-1x4 (t x t) continued

tan and wild

661 (661 ○ ○ ○ to w w w w
(662 to to to to w w w w

(680 w w w w lto ○ ○ to
(681 to vto w Ⓦ to vto w w
(682 w w w w to to ○ lto
680 (683 w w w w to to to to
(684 w w w w xt xto xt lto

(686 to to to to w w w w
(687 lto to to vt w Ⓦ w w
686 (688 to to to to w Ⓦ w w
(689 w w w w to to to to
(690 Ⓦ w to to w w to to

691 (691 to to to to w w w w
(692 to to ○ ○ w w w w

(693 w w w Ⓦ to to to ○
693 (694 t t t t w w w w
(695 w w Ⓦ w ○ lto to to

(698 w w w w t t to xt
698 (699 vto lto lto xto w w w Ⓦ
(700 lto lto lto lto w w w w
(701 w w w w xto xto xto lto

(702 w w w w lto lto lto lto
(703 xto xto vto ○ w w w w
702 (704 w w w w lto lto lto lto
(705 lto ○ xto vto w w w w
(706 t ○ t ○ w w w w

(707 to to w Ⓦ w w to to
707 (708 xt lto lto lto w w w w
(709 w w w w lto vt ○ vt
(710 t t t t w w w w
(711 w w w w lto lto lto lto

(712 vto vto w w w w vto vto
(713 vto vto vto lto w w w w
712 (7141to lto w w w w lto lto
(715 w w lto ○ w w lto lto

(718) lto lto lto w lto w w w

(7191to lto lto lto w w w w
(720 w w w w lto xto xto lto
(721 lto ○ w w w w xto lto
718 (722 Ⓦ w w w lto lto lto lto
(723 lto ○ lto lto w w w w

TABLE XV - continued

Third generation - continued

Offspring of Selected Clones

338-1 w selected x 338-4 w selected

835	t	t	t	t	w	Ⓦ	w	w
836	w	w	w	w	t	t	w	w
837	w	w	w	w	w	w	w	w
838	w	w	w	w	w	w	t	t
839	w	w	w	w	w	w	w	w
840	w	w	Ⓦ	w	t	t	t	t
(841)	w	Ⓦ	t	w	t	w	Ⓦ	w
842	w	w	w	w	w	w	t	t
843	w	Ⓦ	w	w	w	w	w	w
844	w	w	w	Ⓦ	w	w	t	t
(845	t	t	w	w	w	w	w	w
845 (846	t	t	t	t	w	w	w	w
847	w	w	w	w	w	w	t	t
848	w	w	w	w	t	t	t	t
849	w	w?	t	t	w	Ⓦ	w	Ⓦ
850	w	w	Ⓦ	w	w	w	t	t
851	t	t	w	w	w	w	w	w
852	w	t	w	t	t	t	w	w
853	w	w	w	w	t	t	w	w
854	Ⓦ	w	w	Ⓦ	w	w	t	t
855	w	w	w	w	w	w	w	w
856	w	w	w	w	w	w	t	t
Total(5	w	w	w	w	w	w	w	w
(11	w	w	w	w	w	w	t	t
(5	w	w	w	w	t	t	t	t

Each ascus, except 845 and 846 are from an individual perithecium

TABLE XV - continued

Fourth Generation

381-8 x 379-6 (w x w)

593	((593)	a	w	a	w	a	a	w	w
	(594	xta	xta	lta	a	○	○	w	w
	595	w	w	w	○	a	vta	lta	lta
598	(598	a	a	w	w	⊕	t	a	a
	(599	w	w	to	○	a	a	a	a
	(600	w	w	w	w	○	○	toa	xta
603	(603	w	w	w	w	a	a	a	a
	((605)	w	w	w	ⓐ	w	a	ⓣa	ta
606	(606	w	w	a	a	a	a	t	lt
	(607	a	ⓐ	w	w	a	a	to	to
	((608)	a	w	a	w	○	○	to	○
	(609	a	a	a	a	w	w	w	w
	(610	t	lt	○	a	a	a	○	w
	(611	a	a	w	w	w	w	a	a
	(612	w	w	a	a	w	w	a	a
613	(613	w	ⓦ	a	a	w	w	xta	xta
	(614	a	a	w	w	w	w	a	a
	615	○	xta	ⓐ	a	w	w	w	w
616	(616	a	a	w	w	a	a	w	w
	(617	toa	toa	toa	toa	ⓦ	w	w	ⓦ
	618	to	⊕	a	a	w	ⓦ	○	a
	619	w	w	w	w	w	ⓦ	ⓦ	w
	620	a	a	w	w	toa	toa	w	w
	623	to	to	toa	toa	a	a	w	w

742 -(e x e)

743	(743	e	e	e	e	e	e	e	e
	(744	e	e	e	e	e	e	e	e
	(745	e	e	e	ⓔ	ⓔ	ⓔ	e	e

TABLE XV - continued

Fourth Generation - continued

775-1 x 8 (b x b)

831	W	W	W	W	W	W	W	W
832	Ⓦ	W	W	W	W	Ⓦ	W	W
833	W	W	W	W	W	W	W	W
834	W	W	W	W	W	W	W	W

Table XV - continued

Fifth Generation 610-1 x 606-7 (t x t)

748(749	w	w	Ⓦ	w	Ⓦ	w	vt	w
750(750	lt	lt	w	w	lt	lt	w	w
750(751	w	w	(lt)	lt	Ⓦ	w	lt	(lt)
(753	w	w	w	w	t	○	○	○
753(754	w	w	(vt)	vt	w	w	lt	lt
(755	lt	lt	lt	vt	w	w	w	w
(756	t	t	w	w	○	○	w	w
756(757	lt	lt	w	w	w	w	○	○
(758	lt	(lt)	w	w	(lt)	lt	w	w
760((764)	w	t	w	t	○	t	w	w
760(765	t	t	t	t	w	w	w	w

593-1 x 6 (a x a)

766	a	a	a	a	a	a	vta	vta
767(767	a	a	a	a	a	a	ta	ta
767(768	a	a	a	a	a	a	a	a
769	a	a	a	a	a	a	a	a
770	a	a	a	a	a			
771	a	a	a	a	a	a	ta	ta
772	a	a	a	a	a	a	a	a
773(773	ta	(ta)	ta	ta	a	a	a	a
773(774	Ⓐ	a	a	a	a	a	a	a

743 -1 x 6 (e x e)

(778	e	e	e	e	e	e	e	e
(779	e	e	e	e	e	e	e	e
778(780	e	e	e	e	e	e	e	e
(781	e	e	e	e	e	e	e	e

Also 28 random single ascospore cultures - all e

TABLE XV - continued

Sixth Generation - continued

772-1x6 (a x a)

782	782	ta	ta	ta	ta	a	a	a	a
	783	a	a	a	(a)	ta	ta	ta	ta
784	784	a	a	a	a	(a)	(a)	a	a
	785	a	a	a	a	lta	lta	a	a
	786	a	a	ta	ta	a	a	a	a
	787	ta	ta	a	a	a	a	(a)	a
	788	a	a	a	a	a	a	a	a

TABLE XV - continued

F_3 x F_5 (a x b)

772-8 x 775-1

809	a	a	w	w	w	w	a	a
816	a	a	w	w	w	w	a	○
822	a	a	w	w	w	w	a	a
826	ⓐ	a	w	w	ⓦ	ⓦ	a	a
812	w	w	a	a	a	a	w	w
814	w	w	a	a	a	a	w	w
321	○	○	a	a	a	a	w	w
330	w	w	a	a	a	a	w	w
813	a	a	w	w	a	a	w	w
815	a	a	w	w	a	○	w	w
819	a	a	w	w	a	a	w	w
820	a	○	w	w	a	a	w	w
828	a	a	w	w	a	a	w	w
810	w	w	a	a	w	w	a	a
811	w	w	a	a	w	w	a	a
817	w	w	a	a	w	w	a	a
818	w	w	a	a	w	w	a	a
823	w	w	a	a	w	w	a	a
(827)	w	w	a	a	w	a	w	a
824	w	w	w	○	a	a	a	a
829	w	w	w	w	a	a	a	a

mostly diverse variations from wild type. In seven asci the 4:4 ratio was not obtained. From four asci (228, 233, 243, and 254) there were produced two tan and six non-tan cultures. From three asci (229, 234, and 244) no tan cultures were produced. In many of the asci two different kinds of tan, light and dark, were obtained. This indicated the effect of another factor modifying tan.

Fig. 10 gives these data in condensed form. Instead of indicating the eight ascospores in an ascus, four symbols are used to indicate the four pairs of ascospores. When individual asci are indicated, the first symbol to the left indicates 1 and 2, the second symbol indicates 3 and 4, the third symbol indicates 5 and 6, and the fourth symbol indicates 7 and 8. When a number of asci are indicated, only the ratio is given by the symbols. All cultures other than tan are shown as wild type, because all of the non-tan variants resembled wild type in most of their characters.

From a second mating within the ascus, 114-3x8 (t x t); fourteen asci were dissected. Nine asci produced four tan and four variants resembling wild type, while five asci contained two tan producing and six non-tan producing ascospores. It is obvious from this test

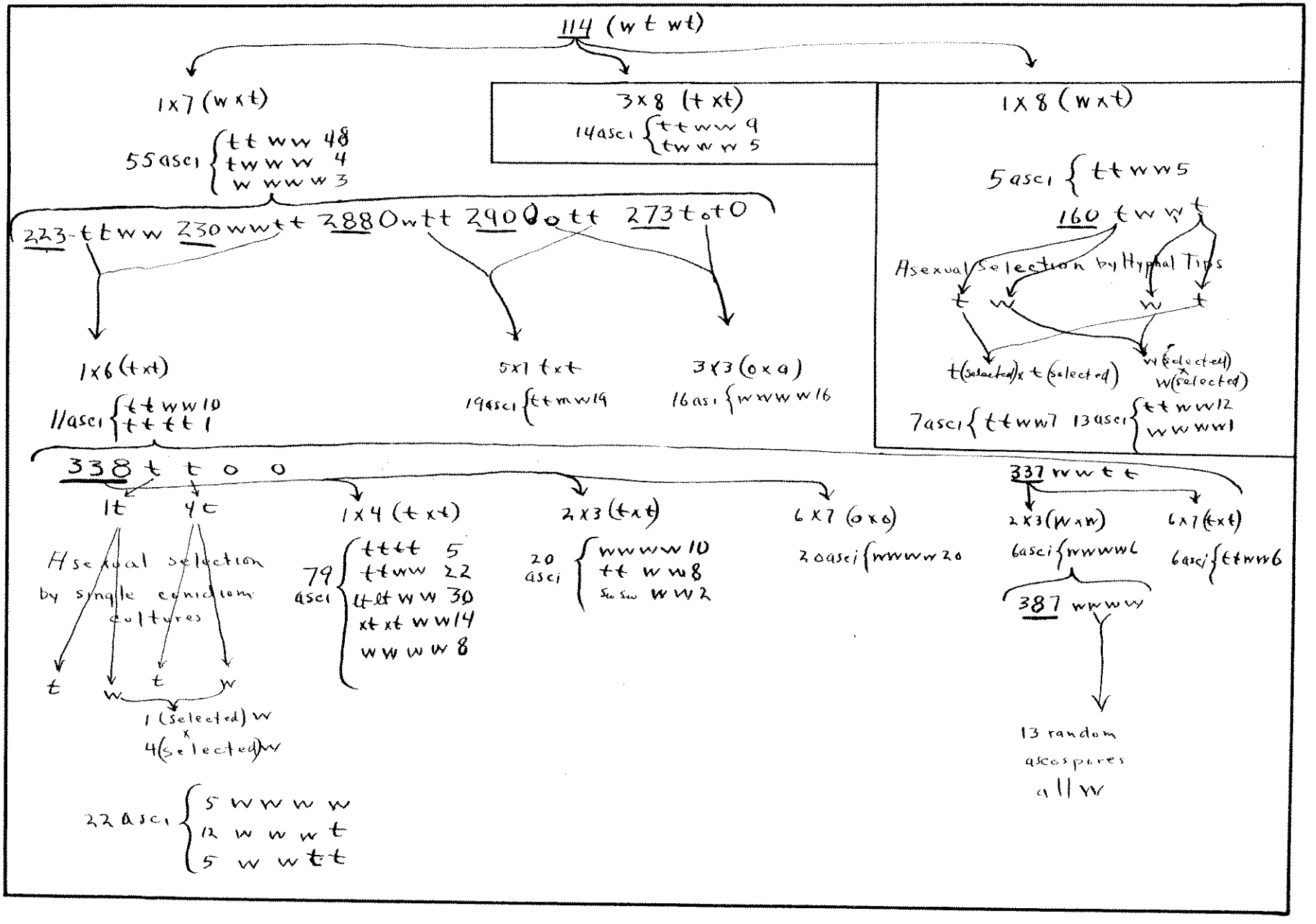


Fig. 10.

Genealogical Tree of Tan and Wild-Type

that the tan character does not breed true.

A third mating, 114-1x8 (w x t), was made. Five asci were dissected and these produced each four non-tan and four tan cultures. It was noted at this time that when conidia and mycelium from tan cultures were transplanted to other tubes, the tan character usually failed to appear, the culture reverting to wild type. Two tan cultures (160-1 and 160-8), offspring of this third mating, were chosen to study this phenomenon. Conidia were sowed on plates and subcultured by cutting off hyphal tips and transplanting them to small tubes. The subcultures from each original culture were selected for tan and wild type. About 400 subcultures were made in from three to six clonal generations and cultures of wild type were obtained which rarely produced any tan offspring. The tan lines, however, always showed some reversion to wild type.

One of the tan cultures selected from 160-1, was then mated to one of the tan cultures selected from 160-8 and seven of the resulting asci were analyzed. Each contained four tan and four wild type producing ascospores.

One of the wild type cultures selected from 160-1 was mated to one of the wild type cultures

selected from 160-8, and thirteen of the asci analyzed. Twelve contained four tan producing and four wild type producing ascospores. One of them contained eight wild type producing ascospores.

As shown in Fig. 10, two more tan by tan matings were made in the F_1. From the mating 223-1 x 230-6 (t x t), eleven asci were dissected. Ten of these produced asci containing four tan producing and four wild type producing ascospores while one of the asci produced eight tan cultures. From a second mating 288-5 x 290-7 (t x t), nineteen asci were dissected and each contained four tan producing and four wild type producing ascospores. It is evident that the tan character does not breed true any more readily in the F_2 than in the F_1, and that the attempt to stabilize the character by asexual selection had failed.

Fig. 10 shows a third mating of opalescent by opalescent among the sister asci of this group. The asci from which these opalescent cultures had been obtained had also produced ascospores from which tan mycelia grew. Sixteen asci were dissected from this mating of 290-3 x 273-3 (o x o) and every ascospore produced the wild type character. Opalescent resembles

wild type closely so that it is difficult to state absolutely that this was a case of reversion, but it is important to note that no tan appeared in the progeny of this opalescent x opalescent mating. Apparently tan was completely separated from its allelomorph in the second division in the ascus.

A second mating of opalescent by opalescent was made as is shown in Table XV. The parent ascus (243) had produced two tan and six opalescent cultures. From mating 243-2x5 (o x o), twelve asci were dissected. Each ascus produced four wild type and four black cultures. It is clear that one pair of opalescent cultures differed genetically from another pair.

The mating 223-7 x 229-8 (db x db) produced only black cultures. The black character bred true in this generation, but on later inbreeding of this line, as shown in Fig. 11, only wild type offspring were obtained.

It is interesting to note that variations of intensity of black were entirely different characters genetically although one looked like a dilution of the other. This fact was shown by the mating of the light black and black culture (228-7 x 216-4) (lb x b). Each ascus produced pale, wild-type, and tan cultures in the

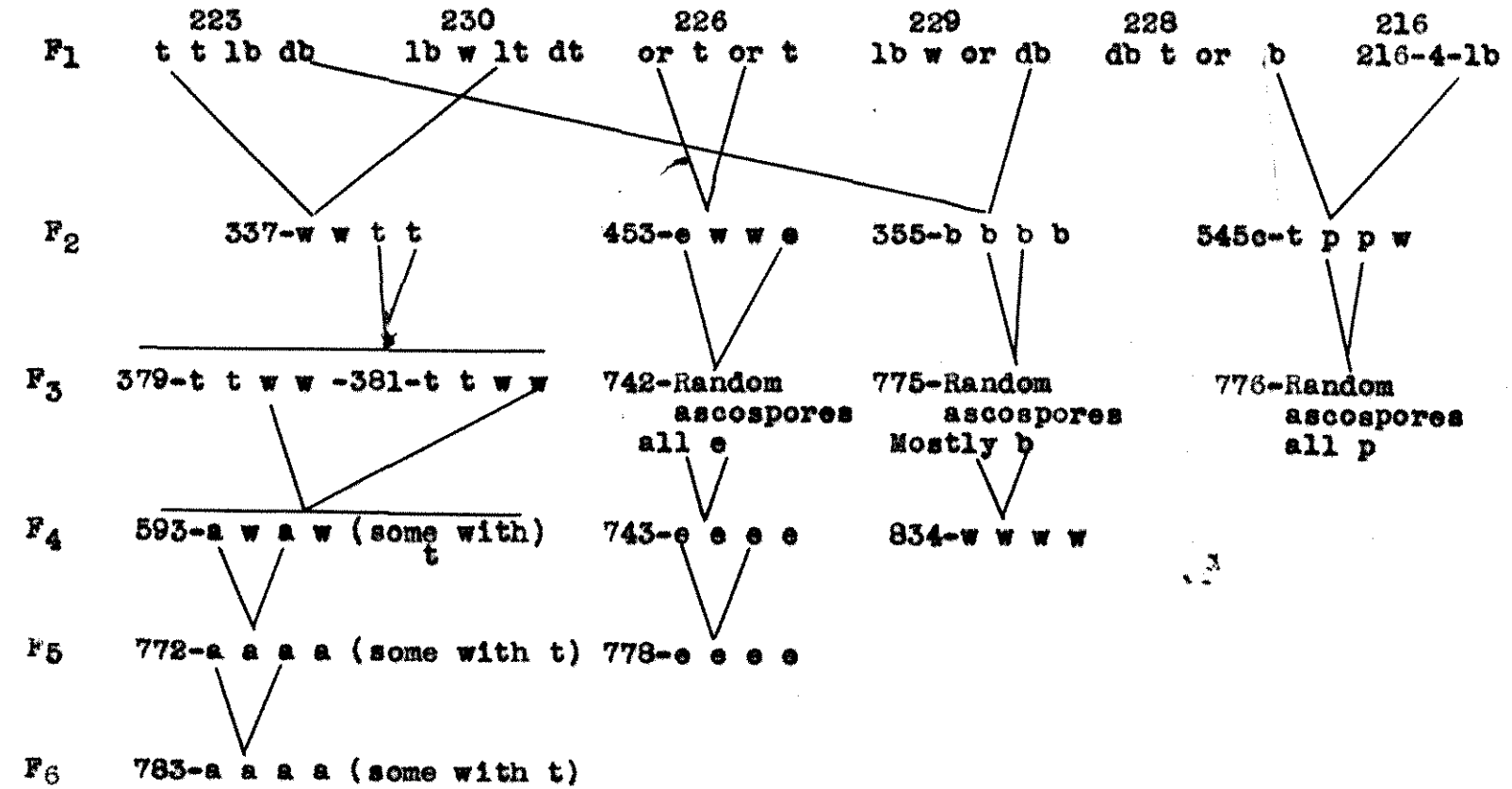
ratio of 2:1:1. Sixty-five random ascospore cultures verified this ratio. This showed that non-tan segregated from tan may again throw tan offspring. A second example of the same kind is the inbreeding of the albinistic line, shown in Fig. 11. Although the parents of the albinistic line were wild-type, when albinistic was produced, tan reappeared.

A striking example of the fact that the offspring obtained by mating two like mycelia may show entirely new characters, is in the mating of the two orange cultures 226-1 x 226-6. Each ascus produced four wild-type and four even cultures.

Asci 337 and 338 were selected from the second generation for further breeding tests. From mating 337-6x7 (t x t), six asci were dissected. Four tan and four wild type cultures were produced from each ascus. The parents, grand-parents and one of the great-grand-parents were tan but still the mating three half tan and half wild type offspring.

Two matings of the non-tan cultural characters appearing in the asci 337 and 338 were made. From mating 337-2x3 (w x w), six asci were dissected. Only wild-type offspring were produced. Two of these when mated

Fig. 11. Genealogical tree showing the development of pure breeding lines by inbreeding.



threw wild type in the next generation. From mating 338-6x7 (o x o), twenty asci were dissected. All of the offspring were wild type. This is the same result as that obtained in one of the selfings of opalescent in the first generation. Apparently the wild-type and opalescent cultures obtained in asci 337 and 338 do not throw any considerable number of tan offspring. It is interesting to note, on the other hand, that practically half of the offspring of the tan cultures from the same two asci were wild-type.

From mating 338-2x3 (t x t), twenty asci were dissected. These fell sharply into three classes. Ten asci produced only wild type; eight asci produced half wild type and half tan, and two asci produced half wild type and half submerged. (Submerged is shown by the hyphae from the germinated ascospore growing only a few centimeters through the agar before the culture dies. This is a true lethal character). More interesting than the fact that three kinds of asci were produced, is the fact that all of the asci from a single perithecium were alike. Referring to Table XV, perithecia 525, 528, and 540, produced only asci which contained eight wild type producing ascospores. Perithecia 532 and 534 produced only asci containing four wild type producing and

four tan producing ascospores. Perithecium 538 contained only asci producing four wild type producing and four submerged producing ascospores.

A second mating was made in ascus 338. The two tan sisters of 2 and 3, that is, 1 and 4, were mated. From this mating, 338-1x4 (t x t), 79 asci were dissected. Table XVI shows the numbers of perithecia which fell into the three different classes: (1) all wild type, (2) four ascospores wild type and four tan from each ascus, (3) all tan. Table XV gives the details of this mating and shows that the second class can be subdivided into at least three other classes. In two perithecia, 643 and 648, the asci were mixed. It will be later shown that the mixed perithecia can be logically explained. The similarity of the asci obtained from the same perithecium was much greater than can be indicated in the table. There was a striking uniformity of the cultures from each perithecium in the color of the conidia, the habit of growth, the spacing of the substrate, the production of fluff, etc. that distinguished them from the cultures from other perithecia. Space does not permit a sufficient description of these differences.

It is clear that the nature of the ascus is determined at the origin of the perithecium. This is a

genetic demonstration of the same fact that Harper (42) demonstrated cytologically.

3. Proof of nuclear interchange between thalli of opposite sex.

An experiment on the cross breeding of pure lines gives a clear proof of the fact that nuclear interchange must be effected between thalli of opposite sex. Fig. 11 shows the development and ancestry of four pure lines that were developed. The details of the data are given in Table XV. The even line bred perfectly true. The albinistic lines always threw a number of tan cultures, but all these tan cultures were also albinistic. From a mating 772-1 x 743-1 (a x e) several asci were obtained producing four albinistic and four even cultures. Some asci produced some wild type. This is expected since each pure line may contain the normal allelomorph of the other. The details of the result will be published in a later paper. It is important to note that the recovery from a single ascus of true breeding types so different from the wild type and of such divergent origin is clear proof that nuclear interchange between the two different thalli must have taken place.

4. Cytological and genetic evidence of the "double fusion" in N. crassa.

Harper (42) first proposed the idea that there were two sexual fusions in the ascomycetes. He demonstrated cytologically that one fusion takes place at the initiation of the perithecium. Dangeard (14) had previously shown that one fusion takes place at the initiation of the ascus, after the perithecium is formed. Cytological and genetic evidence will be presented to show that both these fusions occur in N. crassa. This phenomenon is called the "double fusion". It will also be shown that the first fusion in the case of N. crassa involves four nuclei, two from each parent. This means that three meioses must occur. The places where these meioses occur will also be shown.

Matings were made on a thin layer of agar spread on slides and the hyphae killed with Flemming's without acetic and stained with iron haemotoxylin. Plate I,(figs. 1, 2, and 3) show hyphae of N. crassa in the neighborhood of young perithecia. Each cell in these hyphae was a dicaryon except the initial of the perithecium which contained four nuclei. The nuclei were large and strikingly paired. These mycelial threads could be traced back through anastomoses to

unisexual hyphae such as those shown in fig. 5. These threads grew rapidly and the mycelia as well as the perithecium initial became multinucleate, as shown in fig. 4. The initials coiled as shown in figs. 4 and 6, and a growth of hyphae around the coil formed the perithecium.

Within the perithecium of N. crassa, typical croziers were found as described by the Moreaux. Fig. 7 shows five such ascus initials. The Moreaux have shown that the croziers, like the one shown in fig. 7d, arises from a uninucleate cell. It is on this fact that the complete proof of the "double fusion" rests. There is much objection on a priori grounds to the idea of a "double fusion". On such a basis it could very well be objected that the dicaryons in the perithecium initial were merely associated nuclei which divide conjugately until reaching the ascus initial and there fuse. The finding of the Moreaux that the ascus crozier arose from a mononucleate initial, combined with the genetic evidence collected by Dodge and the writer, firmly establish the fact that a "double fusion" did take place. The genetic evidence is clinched by cross breeding of the pure lines albinistic and even.

The genetic experiments already reviewed show that both a tan x tan and a tan x wild mating may produce tan and wild offspring. This is a strong argument against the use of characters that have not been inbred until there can be no question of their purity, in breeding experiments designed to solve the question of nuclear interchange between thalli of opposite sex. Furthermore, it is an argument against the use of wild-type in matings to study nuclear behavior because several of the variable types studied revert to wild type. However, a certain definite percentage of wild type must be expected on the basis of the assumption that each pure line contain the normal allelomorph of the other.

Albinistic and even were both carefully tested for purity of the two characters involved. A mating was made between them. The offspring in many asci contained four albinistic and four even producing ascospores. This is proof of the interchange of nuclei between two thalli of opposite sex.

There are two objections to the interchange of nuclei between thalli of opposite sex in Neurospora that have been recently raised in the literature. The first is that of the Moreaux.(52) They point out that in

both homothallic and heterothallic species of Neurospora, the perithecia originated from a coil such as that shown in Plate I. fig. 4, and that there was no evidence of the organ that they consider to be the trichogyne effecting a union with the coil. Fig. 6. shows a coil originating near two anastomoses. Coils always originate near anastomoses, but it is impossible as Koehler (49) has shown, to find a field in a total mount of a culture of Neurospora that does not contain some anastomoses. Furthermore, the septations in the larger hyphae are false as shown in fig. 5, resembling those found by Lehfeldt (51) in the basidiomycetes and should allow an easy exchange of nuclei. It is thus clear that an organ such as a trichogyne is unnecessary and that if it did exist, it would be at some stage previous to the initials, and not at a stage so late as the coil.

The second objection is that of Koehler (49). He pointed out that anastomoses were equally numerous between the hyphae of like and unlike sex. He also performed the following experiment. He grew conidia of unlike sex together and removed and transplanted the hyphal tips expecting to get some of them to produce perithecia. He failed to obtain perithecia in every one of 150 trials. The following experiments show that this would

be expected.

When matings were made in test tubes, it was necessary to plant the two inocula some distance from each other in order to obtain many perithecia. If the two inocula were planted in the same spot the crop of perithecia was small. Dodge (29) has shown that perithecia arise usually on only one side of the plate when two inocula of opposite sex are placed in the opposite sides of petri dishes. The writer spread a very thick suspension of mixed spores of opposite sex over the surface of a plate of corn-meal agar. No perithecia developed. In a second plate, half of the plate was covered with the thick suspension used above and on the half of the plate inoculated, no perithecia appeared while on the other half a few small perithecia developed. In a third plate, inocula from the same two cultures were placed at opposite ends of the plate and numerous perithecia were produced on one side after the mycelia met and later a sprinkling of perithecia appeared on the other side as well. This evidence showed that Koehler's experiment did not test the question of whether or not nuclear interchange took place at anastomoses.

There is ample proof that the ascus nucleus originates by the fusion of two nuclei. Since the

Moreaux have shown that these two nuclei are derived from a single nucleus and since the ascospores produced from the ascus nucleus show the characters of both parents, the only conclusion possible is that the single nucleus in the ascogenous hyphae is a fusion nucleus. Since pairing in the ascogenous hyphae did not occur, it is reasonable to believe that the paired nuclei found in the perithecium initial fuse. This is the first fusion, and the crozier fusion is the second.

5. Experiments on the inheritance of tan and wild-type by the use of selected clones in matings and the bearing of these data on the question of a tetraploid fusion in the perithecium initial of N. crassa.

The unsuccessful selection experiment cited in the case of the tan cultures from ascus 160, was repeated on the tan cultures 1 and 4 from ascus 338. Cytological study of hyphal tips, after the first experiment was completed, showed that the hyphal tips were more crowded with nuclei than any other part of the thallus, and since these hyphae arose from an anastomosed mycelium originating from a polysporous inoculation, it was decided to try asexual selection by single conidium cultures. A single conidium sometimes contained a single nucleus and often three nuclei, but in the majority of cases, two nuclei were found in each conidium. Single conidia were isolated from the tan cultures 338-1 and 338-4. The subcultures grown from these conidia fell into three classes: tan, wild type, and intermediate. Tan and wild-type were selected from both sources for further selection and at the end of the fourth asexual generation, all four clones were apparently pure. Selection was made for a fifth generation and all twenty cultures from each clone

maintained their purity. Then two of these purified wild-type clones were mated with the result shown in Table XV - Third Generation, Offspring of Selected Clones. A single ascus was selected from each perithecium since the previous results from unselected clones had shown that all of the asci from one perithecium were alike. The table shows that three types of perithecia were obtained; one in which all eight spores from the ascus produced wild-type cultures; one in which six wild-type and two tan spores were obtained from each ascus; and one in which four wild-type and four tan spores were obtained from each ascus. There were no perithecia found containing all tan ascospores and a new type of perithecium containing asci with a 3:1 ratio of wild to tan was discovered. Table XVI shows the kinds of perithecia obtained from the mating of the unselected clones 338-1x4.

Calculating the total percentage of wild-type found in all of the various kind of perithecia (not asci), the table shows that 47 percent wild-type were obtained. Table XVII shows comparable data obtained from the mating of selected clones. It is apparent that the ability to throw tan has not been lost entirely by selection, but the percentage of wild-type

Table XVI Classes of perithecia obtained from the mating of the unselected clones 338-1 x 338-4 (t x t)

Classes of Perithecia			
w w w w	w w t t	t t t t	Total percentage of w ascospore
1	14	2	47

Table XVII. Classes of perithecia obtained from mating of wild type clones selected from tan clones 338-1 x 338-4 through five conidial generations.

classes of perithecia			
w w w w	w w w t	w w t t	Total percentage of w ascospore
5	11	5	75

has been increased from 47 to 75 percent. The percentage of tan has been decreased from 53 to 25 percent. The new type of perithecium which was encountered, namely, one which contained asci with six wild-type and two tan ascospores, as well as the absence of the all tan asci, were further evidence of the positive effects of selection.

The selections were made just as soon as it was possible to recognize the cultural characters, that is, two or three days after planting the single conidium. No matings were made between the asexually selected tan cultures, but after they had grown for a few weeks, forty polysporous transfers were made from them. All of these subcultures developed wild-type or intermediate cultural characters. That this cannot be considered evidence for mutation of tan back to wild-type is shown by the fact that when the hyphae of a sister of a tan ascospore are mutilated in transferring the germinating ascospore, the cultural character developed by the mycelium is wild-type. The main reason why hyphal tip selection is so unsuccessful is because similar phenotypic variations are produced by the mutilation necessary to remove the hyphal tip. This is shown by the uniformity of hyphal tip cultures, as

compared with the diversity of single conidium cultures made from the same original culture.

These experiments show that thallus developed from a single ascospore contained more than one kind of nucleus. In this connection, it is important to recall that the ascospore of N. crassa is a dicaryon. Cytological investigation of Dodge, confirmed by the writer showed that as soon as the wall of the ascospore began to form the single nucleus inclosed within the wall divided forming two nuclei which took up their positions opposite each other on the short axis of the spore. Moreover, the writer has found that the single nucleus delimited within the ascospore, following the third division in the ascus, was very different from any of the other ascus nuclei. It enlarged to practically the same size as the ascus zygote nucleus and formed a long stringy spireme. These stages were very frequently found and apparently lasted longer than any of the other nuclear stages in the ascus except the ascus zygote nucleus. The two-nucleate and four-nucleate stages are extremely infrequent.

The experiments on selection show clearly that this dicaryon, which resulted from a nucleus resembling one preparing for maturation, contained different kinds of nuclei.

In the face of this evidence, it was unnecessary to assume mutation as the cause of these different kinds of nuclei, but much more reasonable to suppose that the nucleus delimited in the ascospore was a diploid nucleus (homozygous for sex) preparing for a meiotic division. From this meiosis, a maximum of four different kinds of nuclei could be formed by a combination of pre- and post-reduction of the various factors. It has been shown that the first two divisions in the ascus were reduction divisions, which constituted a single meiosis, and that the third division was mitotic. If the nucleus resulting from this third division were diploid, the ascus zygote nucleus would be tetraploid. If a single meiosis occurred in the crozier, as was indicated by the variety of asci produced in some perithecia, the single nucleus in the crozier initial must have been tetraploid. If this nucleus was derived by mitoses from a single fusion nucleus that was formed in the p rithecium initial, that fusion must have been tetraploid and involved four nuclei, presumably two from one thallus and two from the other thallus. It is noteworthy that four nuclei were found in the perithecium initials. This line of reasoning indicates that all four of these nuclei fused to form a single perithecial fusion nucleus, but this fusion has not been observed.

It is obvious that meiosis of a diploid heterozygous nucleus (if no other genes are involved) could not produce a 3:1 ratio. Moreover, a single meiosis of a tetraploid nucleus with one factor for tan and three for wild-type would produce four diploid nuclei, two of them heterozygous for tan and wild-type, and two of them homozygous for wild-type. A second meiosis would result in a 3:1 ratio, with the subsequent result that the reduced nuclei would be haploid. In order to obtain a 3:1 ratio with the production of diploid nuclei in the ascospore, double meiosis in the first two divisions in the ascus of an octoploid ascus zygote nucleus would be required. Another assumption, justified by the genetic data, is that a single meiosis of a tetraploid ascus zygote nucleus produces two nuclei heterozygous for tan and wild but one of them is phenotypically wild because of the independent segregation of a modifier. The variations found in the tan character, particularly in the 338-1 x 4 mating, are evidence for the existence of modifiers. The production by inbreeding albinistic and by selfing light black, (characters giving no evidence of tan) with the subsequent production of tan in their progeny showed complete suppression of the expression of tan in mycelia containing the factor.

6. Percentages of first-division segregation of the factors for various cultural characters and the bearing of these data on four-strand crossing-over in N. crassa.

Fig. 10 shows that in the attempt to inbreed black, the character reverted to wild-type. Since black resembled wild-type in every respect except substrate color, these reverted cultures were used as pure inbred wild-type. A mating with inbred albinistic is interesting in its bearing on the segregation of cultural characters. This mating, 772-8 x 775-1 (a x w), produced 21 asci containing four albinistic and four wild-type producing ascospores. In nineteen of them, albinistic was segregated from wild-type in the second division in the ascus. This, is therefore, 10 percent first division segregation. Table XVIII shows the percentages with which other cultural characters segregate in the first division.

It has been shown that sex is segregated in the first division in 85 percent of the asci and that this relation is constant. This must mean that the different factors determining sex were separated from each other in this division in 85 percent of the asci. Two mechanisms are known which may effect this result. Carothers (8) has shown that segregation of different

TABLE XVIII. Percentage of first division segregation of cultural characters in several different matings.

Mating	No. of Asci	Percentage of I
114-1x7	48	35
228-5 x 290-7	15	93
243-2x5	12	8
338-1x4	79	86
772-8 x (axa) 775-1	21	10

heteromorphic chromosomes may occur always in the first reduction division, or always in the second reduction division, or , in other cases, a certain definite percentage of first division segregation is found. Bridges (3) has shown that four strand crossing-over best explains the production of "equational" exceptional individuals in Drosophila matings. In four-strand crossing-over and reduction in Drosophila, segregation occurs at the second division for all genes lying beyond a crossing-over point and at the first division for all genes lying between the spindle fiber and a crossing-over point.

A definite percentage of crossing-over in the four-strand stage can explain the constant first-division segregation of factors in the ascus, provided that the percentage of recombinations due to crossing-over between the spindle fiber and the locus of the given character is not more than 50 percent. In the case of 10 percent of first-division segregation obtained in the mating of albinistic by wild-type, this explanation fails because then 90 percent recombination would have to be assumed, and in the event of such a high percentage of crossing-over, there would be an even chance of an odd or an even number of breaks occurring between the two factors involved and the percentage of first-division segregation

resulting could not be more than 50 percent. This demonstrates that four-strand crossing-over does not account for all the second-division segregations obtained and, furthermore, that the spindle-fiber attachments of the homologous pairs of tetrads are not always segregated in the first division in *N. crassa*.

7. Summary of the experiments of the inheritance of cultural characters, and an explanation of the genetic behavior of N. crassa which best fits the known facts.

The following scheme for the genetic behavior of N. crassa which best explains the data accumulated is proposed:

(1) That a tetraploid fusion takes place in the perithecium initial.

(2) That two nuclei of opposite sex from each gamete are involved in this fusion.

(3) That these nuclei are selected at random from the nuclei in the two thalli of opposite sex.

(4) That clonal selection is effective in separating these nuclei.

(5) That this tetraploid nucleus divides mitotically until the mononucleate initial of the crozier is attained.

(6) That the two divisions in the crozier constitute a meiosis.

(7) That reduction of a gene may take place either in the first or second division of the crozier meiosis.

(8) That the reduced nuclei formed in the crozier are both diploid.

(9) That the tetraploid fusion nucleus in the ascus reduces in two divisions.

(10)That a gene may be reduced in either the first or second division in the ascus, the first two divisions constituting a meiosis.

(11) That the factors for sex are segregated in the first division in 85 percent of the asci.

(12) That the first two divisions in the ascus result in the production of diploid nuclei, homozygous for sex.

(13) That the third division in the ascus is mitotic.

(14) That the diploid nucleus delimited in the ascospore is reduced in the next two divisions, which constitute a meiosis.

(15) That this reduction may result in a maximum of four kinds of haploid nuclei being produced in the thallus originating from a single ascospore.

The first four considerations have been discussed and evidence for them has been presented. The fifth consideration, that is, that the fusion nucleus from the perithecium initial divides mitotically until attaining the mononucleate crozier initial, involves no difficult assumptions.

Evidence for the fact that the crozier divisions are reductional follows: Table XV shows that perithecium

233 produced five different kinds of asci. This is easily explained provided the perithecium originated from a single tetraploid fusion and second-division reduction took place frequently with the random fusion of the non-sister nuclei which were produced by the second division. Only 35 percent first-division segregation of cultural characters took place in the ascus as a result of this mating, or 65 percent second-division segregation of cultural characters took place. If the factors were segregated with the same frequency in both the crozier and the ascus divisions, that is, if a factor that is segregated most of the time in the first division in the ascus, is also segregated most of the time in the first division of the crozier nucleus, it is obvious that over 30 percent of exceptional asci would be expected, if 65 percent second-division reduction took place in the crozier. The fact that the cultures at this stage of the investigation had not been inbred, makes calculations impossible, but a large variety of asci are shown in Table XV, as resulting from the mating 114-1x7 (w x t).

In the mating, 338-1x4, which has been discussed at length, tan and wild-type factors were segregated in the first division of the ascus in 85 percent

of the asci. If first-division segregation in the crozier took place in the same percentage of the cases, the recombinations should reproduce the original fusion nucleus in a large number of the cases, and most of the asci from a perithecium should be alike. Only two mixed perithecia in 19 were encountered. We may conclude that if a high percentage of second-division segregation in the ascus occurs, a variety of asci from a single perithecium can be expected, but if a high percentage of first-division segregation occurs, most perithecia will produce asci which are all alike.

The only difficulty with this assumption lies in the fact that although sex is segregated in the first division in 85 percent of the cases, no asci have ever been encountered from which all of the ascospores were of one sex. This necessitates the assumption that homozygosis in the tetraploid ascus initial is a zygote lethal. The fact that less than two-thirds of the asci initiated ever mature, makes this assumption easy, for only about 8 percent of the asci would be expected to be homozygous for sex.

The eighth consideration, namely, that the reduced nucleus in the crozier is diploid, follows on the basis of the assumption that only a single meiosis takes place in the crozier, although either pre- or post-reduction may occur.

The other considerations have all been considered in detail.

The variations encountered in this study are all explained as arising in an orderly way through reduction and fusion and the assumption of mutations is unnecessary. So far in this study there is no evidence to show that any point mutations have been found.

V. SUMMARY.

A microdissection apparatus was devised to remove single spores in order from the ascus in a study of Neurospora.

The ascospores of Neurospora required heat-treatment to initiate germination. A hybrid between N. sitophila and N. crassa, and a race of N. sitophila studied, were both shown to produce two kinds of ascospores in regard to response to heat-treatment. It was shown that these differences were genetic. In the case of the hybrid, it was shown that the factors determining sex and absence or presence of conidia were linked to the factors determining response to heat-treatment.

Sex was shown to be segregated in the first division in N. crassa in 85 percent of the asci and in the second division in 15 percent of the asci. It was shown that genic factors do not influence this ratio.

A cytological study shows that four nuclei are delimited in the initial of the perithecium of N. crassa.

Cross-breeding of pure lines shows that nuclear interchange between thalli of opposite sex occurs.

The genetic results obtained are explained, without assuming the occurrence of any mutations, by means of the following nuclear mechanism:

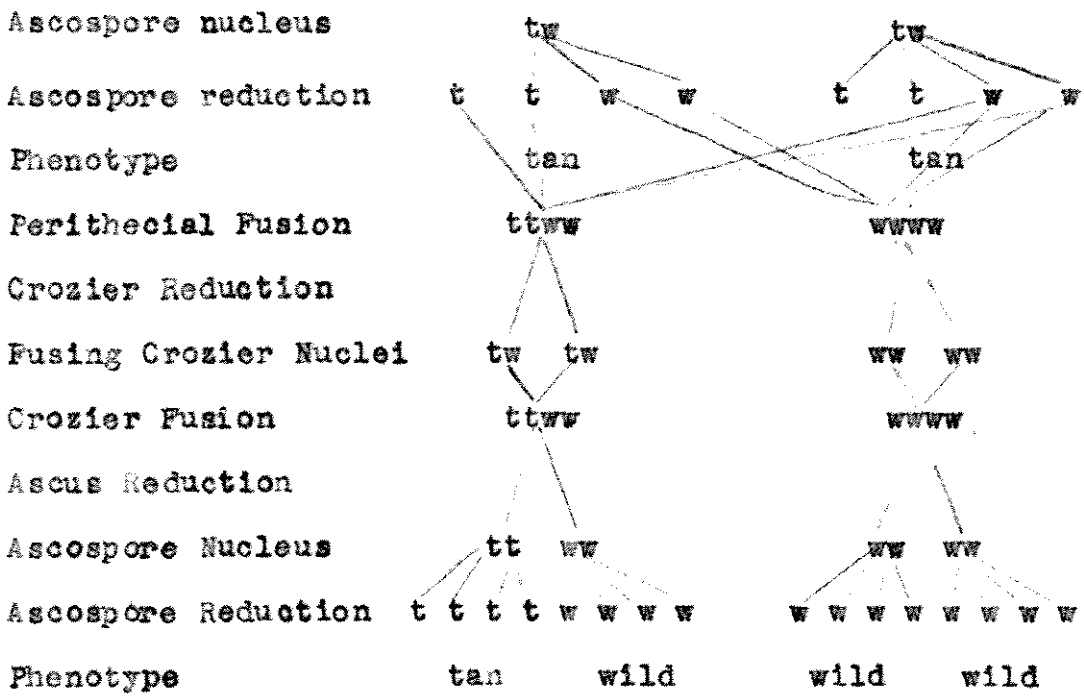
1. Tetraploid fusion of two pairs of nuclei (one from each parent) in the perithecium initial.
2. Mitotic division of this nucleus until the crozier is attained and a single meiosis there, with the same frequency of first and second division segregation as follows in the ascus.
3. Fusion of two of these reduced diploid nuclei to form a single tetraploid ascus zygote nucleus.
4. Reduction of this tetraploid nucleus to a diploid nucleus in the first two divisions in the ascus.
5. Mitotic division in the third division of the ascus.
6. Meiosis in the first two divisions of the ascospore nucleus with the possibility of producing as many as four different kinds of haploid nuclei in the thallus formed.

A scheme is presented,(Fig. 12) to show that this nuclear mechanism could account for the genetic results obtained.

Fig. 12. Scheme conforming to the assumptions made in the text showing how it might be possible to mate two tan mycelia and obtain half tan and half wild type offspring in one perithecium and all wild type in another perithecium.

Each letter indicates a gene and the number of letters indicates whether a nucleus is haploid, diploid, or tetraploid.

By replacing the w's by other letters, as a (albinistic) or e (even), it is possible to explain other genetic results obtained by this scheme.



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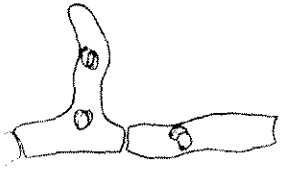

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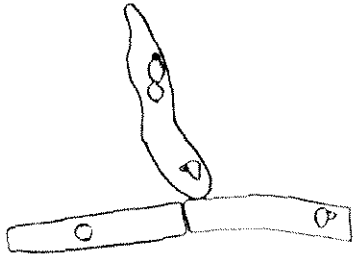

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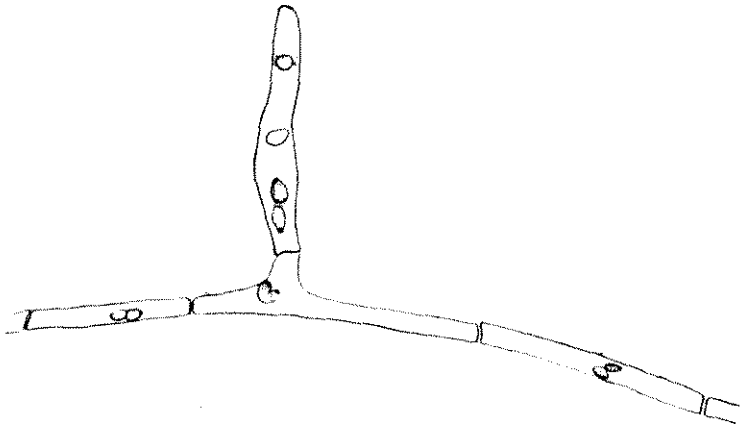

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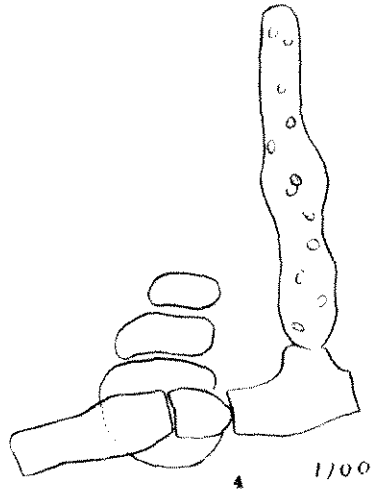

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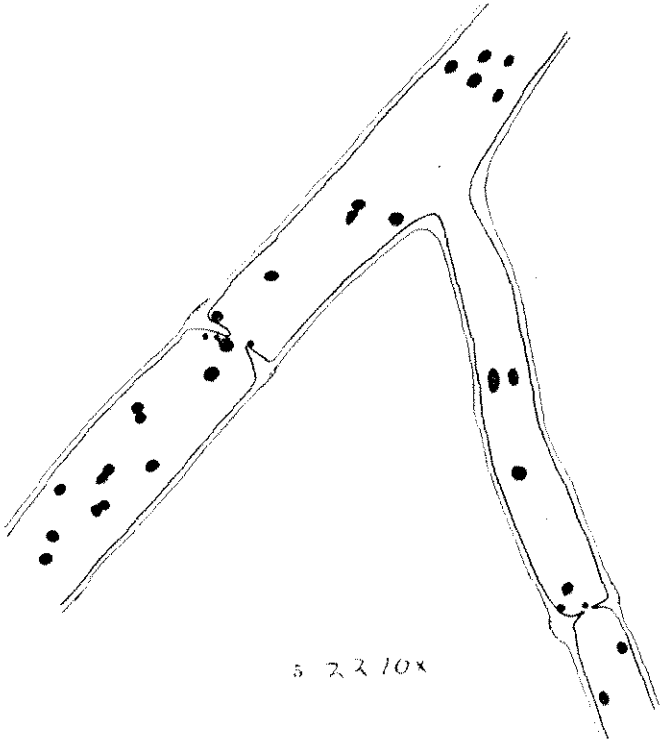

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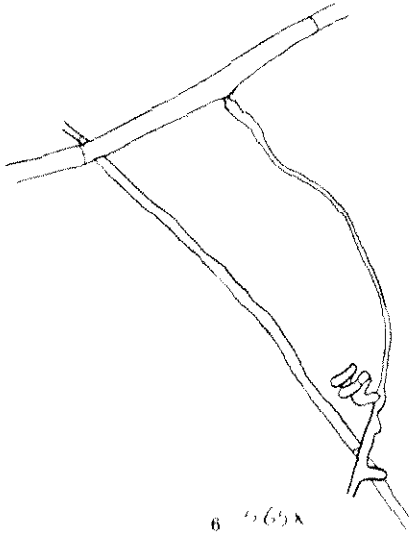

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