SUMMARY.

Investigations on the Genetics of the homothallic Ascomycete Aspergillus nidulans. (Eidam) Winter. by L. M. Hemmons.

Aspergillus nidulans is a homothallic fungus with both a vegetative and a sexual cycle.

Genetical analysis of Aspergillus nidulans had previously been carried out using a technique of recombinant selection. This method gives no information about the position of the centromeres, the incidence and types of unusual asci, if any, or certain modalities of crossing-over.

The first object of the work reported in this thesis was to investigate some of these problems. To this end a technique of ascus dissection suitable for A.nidulans was developed. Among 136 asci of crossed origin isolated from crosses of various strains of A.nidulans, eleven abnormal asci were found. The three main probable causes of abnormality were:

a) mutation during the first meiotic division (3 asci);

b) supernumerary divisions in the ascus (2 asci);

c) inclusion of two nuclei, instead of one, in the ascospore (6 asci).

The incidence of inclusion of two nuclei in one ascospore was found to be about 2%.

That A.nidulans is homothallic, was rigorously established in four original strains by means of dissection of all the

spores of one ascus through two successive complete sexual cycles.

To locate some of the centromeres in A.nidulans, a cross in which four loci were segregating (w/W, lys/LYS, y/Y and bi/BI) was studied. The choice of strains proved unfortunate as low viability of the ascospores from "crossed"asci occurred. Backcrosses to the wild type were carried out, but little improvement was shown after two backcrosses.

From these and other results of ascus analysis, it appeared that genetic and/or cytoplasmic differentiation had taken place between the original white strain (and its derivatives) and the wild type strain (and its derivatives). The former had been isolated in 1936 from wild type and kept genetically separated since.

Some of the details of karyogamy in heterokaryons were investigated, and the interesting fact was discovered that the asci of one perithecium tend to be all of one type (either all selfed or all crossed). This made it possible to develop a new method of genetical analysis, applicable to A.nidulans and presumably also to other homothallic species, which did not necessitate the selection of particular classes of recombinants. Perithecia containing only (or almost only) "crossed" asci were picked, and classification as to all genes segregating was carried out,without selection of ascospores, from those perithecia which showed correct genetic ratios

for certain "markers".

This technique ("Perithecium analysis") is used to most advantage in crosses where the proportion of perithecia containing "crossed" asci is high and the loci, between which recombination has to be estimated, are not too closely linked.

UNIVERSITY OF GLASGOW.

INVESTIGATIONS ON THE GENETICS OF THE HOMOTHALLIC ASCOMYCETE A S P E R G I L L U S N I D U L A N S. (EIDAM) WINTER.

by

Lois M. Hemmons, B.Sc.

Thesis presented for the Degree of Ph.D.
November, 1952.

INVESTIGATIONS ON THE GENETICS OF THE HOMOTHALLIC ASCOMYCETE ASPERGILLUS NIDULANS (EIDAM) WINTER.

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GENERAL INTRODUCTION.

An ever-increasing use is being made of micro-organisms in genetical studies, due to the introduction, in recent years, of suitable techniques making possible genetical analysis in those micro-organisms which show a sexual cycle and in which deliberate mating can be controlled, as Saccharomyces, (Winge and Laustsen 1937 and Lindegren 1949) Neurospora (Wilcox 1928, Lindegren 1932 a and b, and Dodge 1927) and Paramecium (Sonneborn 1950) and in those which show a sexual cycle but in which mating cannot be controlled, as Aspergillus nidulans (Pontecorvo 1952a).

Suitable techniques have also been developed for organisms in which sexual reproduction is not known to occur, but in which, nevertheless, recombination and segregation of hereditary factors can be demonstrated. The latter group include bacteria, such as E.coli studied by Lederberg 1947, bacteriophages (Hershey 1946, Delbrück and Bailey 1946, Burnet 1951) and the Imperfect Fungi (Roper 1952, Pontecorvo and Roper 1952).

Thus the range of organisms suitable for genetical work has been greatly increased. It extends from the multicellular diploid organisms of classical genetics, such as man, Drosophila, maize and the mouse, to include fungi - homothallic (Pontecorvo 1952) as well as heterothallic (Kniep 1929, Lindegren 1932, 1949, Dodge 1927, Winge 1935) - and single-celled organisms, as Paramecium (Sonneborn 1947, 1950). The study of cell

heredity in tissues is even possible now (Medawar 1947).

There are enormous possibilities of widespread application of the genetics of micro-organisms in applied fields such as industrial fermentations, also in human, plant and animal pathology. New approaches have been made to the study of the relationship between genes and other cell constituents as in Saccharomyces (Ephrussi et alii 1949) and in Paramecium (Sonneborn 1947, Beale 1952).

Some of the advantages of using micro-organisms are that a single cell or the offspring from a single cell may be studied, morphological mutants of organisms can be detected directly by inspection and the effect of environment on single cells may be studied directly and conditions changed as required. The generation cycle is shorter in micro-organisms than in higher organisms.

Genetical analysis in micro-organisms was extended to include homothallic moulds by the development of a suitable technique (Pontecorvo 1949). In 1934 Henrard had attempted to work with the homothallic Ascomycete Aspidulans, but was completely at a loss when faced with the difficulty of distinguishing between asci of selfed and crossed origin. In 1946, Pontecorvo showed that genetical analysis of a homothallic fungus was not only possible but, in certain respects, easier than in a heterothallic organism.

PROBLEMS.

Genetical analysis of Aspergillus nidulans had previously been carried out using a technique of recombinant selection (Pontecorvo 1949); by using "markers" to distinguish between the products of selfed and crossed karyogamy, the latter could be selected and classified as to genetic characters other than those used as "markers".

This method gives no information about the position of the centromeres, certain modalities of crossing-over, or the incidence and types of unusual asci, if any.

The object of the work reported in the present thesis was to investigate some of these problems. To this end a technique of ascus dissection suitable for A.nidulans was developed.

From ascus dissection the occurrence of abnormal asci was detected and homothallism was rigorously established. As by-products: (1) some of the details of karyogamy in a heterokaryon were discovered, among which the fact that the asci of one perithecium tend to be all of the same genetic origin
and (2) a technique (Perithecium analysis) was developed for genetical analysis in A.nidulans, and presumably for other homothallic species, which did not necessitate the selection of recombinants.

DESCRIPTION OF ASPERGILLUS NIDULANS.

Aspergillus nidulans (Eidam) Wint., a homothallic Ascomycete, (Order Plectascineae, Family Aspergillaceae) was used in the work described. It exhibits both a vegetative and sexual cycle (see Plate 1).

Haploid uninucleate deep-green vegetative spores, the conidia, are formed and mutants for spore colour have been produced by other workers and are useful as "markers". The hyphae are multi-nucleate and may anastomose, with subsequent migration of nuclei, thus making studies on heterokaryosis possible.

A.nidulans will grow on a simple medium containing a source of organic carbon, nitrate and organic salts.

The fruiting bodies or perithecia - more accurately called cleistothecia - are spherical, from 100 to 200 μ in diameter and enclose from 10 to 100,000 asci.

The asci which are 10 - 15μ in diameter, contain eight brown-red ascospores which are the products of a single cell which has undergone meiosis. The ascospores are bi-nucleate (Adam, unpublished) and discoidal (diameter 3.5 x 5μ) with two characteristic ridges and can be handled easily using a micro-manipulator. Ripe asci liberate ascospores quite easily but the perithecia do not burst unless crushed, when asci and free ascospores are liberated. The ascospores are not arranged in a definite linear order

in the asceus, as in Neurospora and therefore the centromeres cannot be located one by one, but only three at a time (Whitehouse 1950).

As A.nidulans is homothallic, when two strains are grown in mixed culture, products of both selfed and crossed karyogamy are formed. For genetical analysis one must distinguish between the two and this can be done either by the technique of recombinant selection or (this thesis) by dissecting asci and using only those of crossed origin. As products of selfed and crossed karyogamy are indistinguishable until dissected and germinated, there is an enormous waste of labour in dissecting asci.

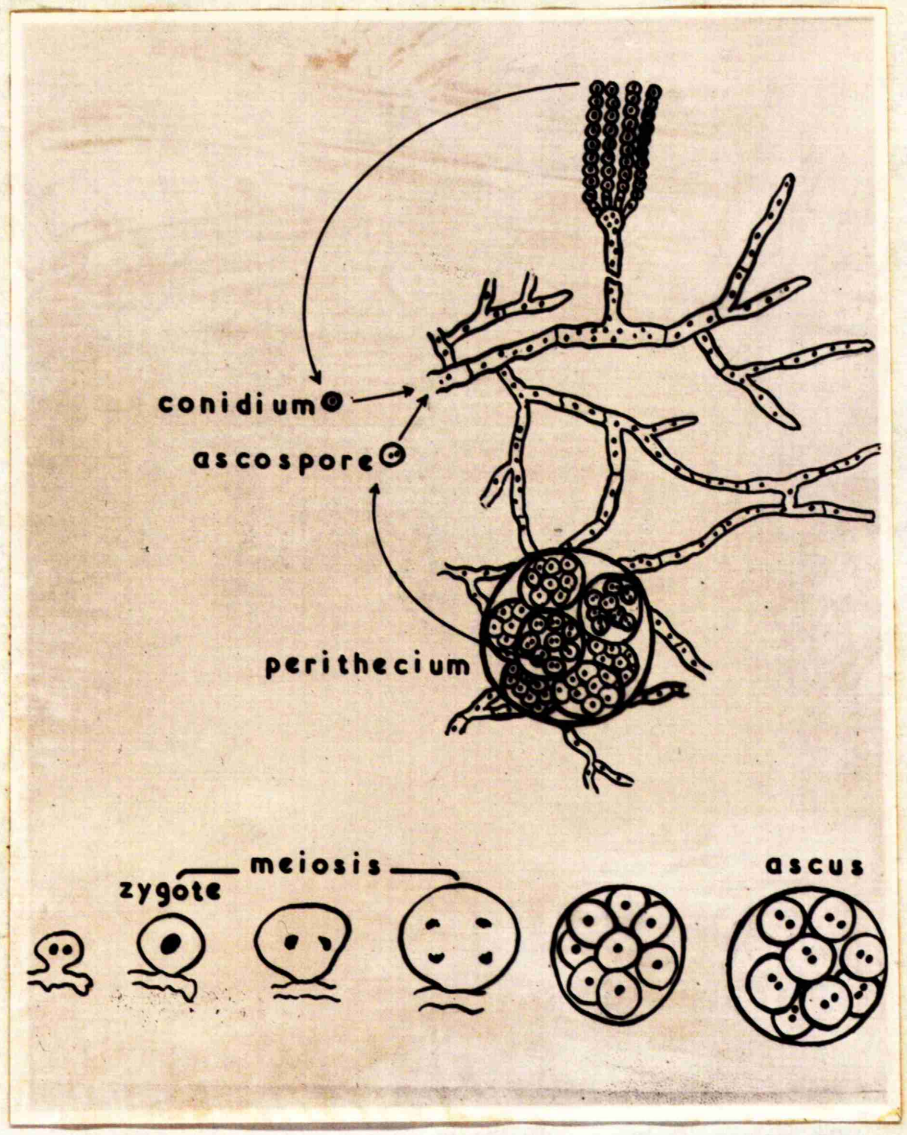


Plate 1. The life cycle of Aspergillus nidulans. Multinucleate hyphae originate from haploid uninucleate vegetative spores - the conidia - or from haploid binucleate ascospores. The sexual cycle takes place in the fruiting bodies (perithecia) and leads to the formation of asci each with eight ascospores.

Below: Formation from the zygote of an 8-spored ascus containing binucleate ascospores.

Crosses of A.nidulans are made in two ways (Pontecorvo 1952a).

1. "Heterokaryon cross". A "balanced" heterokaryon is formed from two strains and among the asci produced there are a proportion which originate from karyogamy between two haploid nuclei, one of each kind.

2. "Mixed-inoculum cross". A very thick suspension of conidia, of the two kinds to be crossed, is inoculated in agar medium and a proportion of asci produced is of crossed origin.

In order to study the formal genetics of A.nidulans, a supply of mutants was needed and they were produced mainly by irradiation, by other workers in this laboratory. Some of the loci (18 in all) have been determined with a greater or lesser precision and seven, and perhaps eight, are in one linkage group (Pontecorvo 1952a).

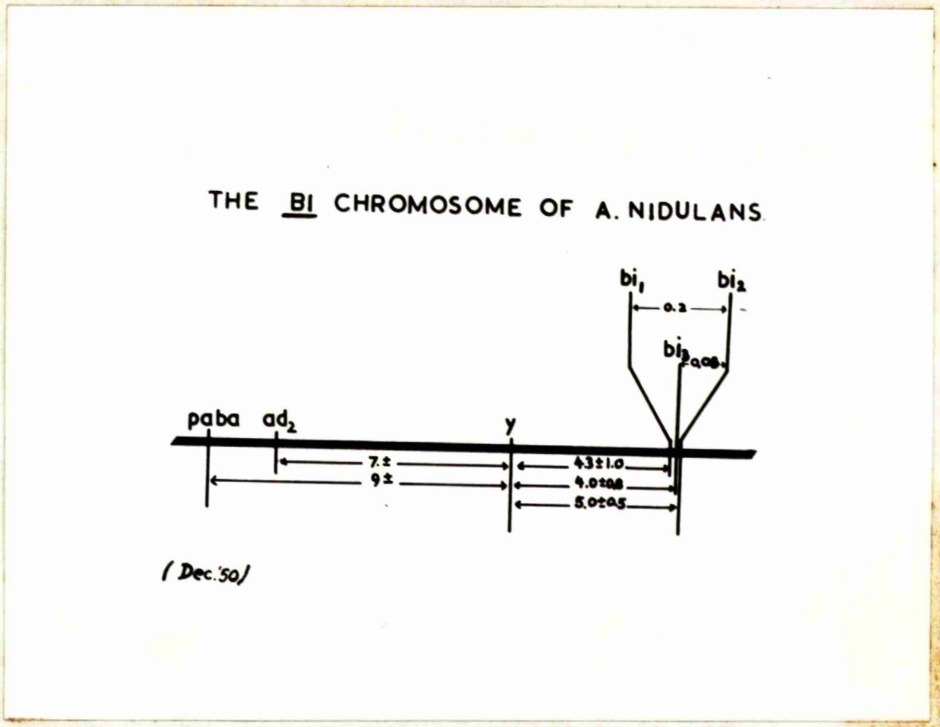


Plate 2. Map of chromosome region of Aspergillus nidulans including the three bi loci, y, ad_2 and paba. w is situated on the opposite side of paba to ad_2. (For details see Pontecorvo 1952a, Roper 1950).

STRAINS AND MEDIA USED.

STRAINS.

W AD_1 Y BI LYS S_d: wild type	Green conidia, prototroph. Referred to as "+" in the text. Supplied by J.L.Yuill. Referred to as A.69 by E. Yuill (1939).
W_a: white alba	A spontaneous mutant, isolated by E. Yuill (1939) from A.69. Referred to as "w" in the text.
y: yellow	X-ray mutant, with yellow conidia, from A.69.1947.
bi: biotin	Biotin-requiring mutant strain produced by X-ray of wild type.1947.
y bi: yellow biotin	Yellow conidia, biotin-requiring. Recombinant obtained from cross y ad_1 x bi. 1948.
y ad_1: yellow adenine-1	Mutant of yellow produced by X-rays. 1946.
W_a lys: white alba lysine	Mutant strain, white conidia, lysine-requiring, produced by X-ray from white alba. 1948. Referred to in text as "w lys".
s_d: s delta	Sulphite/thiosulphate-requiring strain produced by N-mustard from wild type. Received from Dr. Hockenhull 1948.

Note that green conidia (as in the wild type) are produced by genotype W Y, yellow conidia by genotype W y and white by genotype w Y. The latter, however, is epistatic and therefore genotype w y also gives white conidia. Every one of the other differences from wild type has been shown to be monogenic (Pontecorvo 1952a).

Requirements of strains for growth in Minimal Medium.

Strain.	Growth factor.	Concentration mg/ml. for optimal growth.
Biotin-requiring	Biotin or desthiobiotin	0.000002
Lysine-requiring	L-Lysine monohydrochloride	0.2
Adenine-requiring	Adenine hydrochloride	0.08

MEDIA USED.

Minimal medium (liquid)

	%
$NaNO_3$	0.60
KH_2PO_4	0.15
$MgSO_4$	0.05
KCl	0.05
Dextrose	1.00

Traces $FeSO_4$ and $ZnSO_4$.

For solid medium add 1.5% agar.

Complete medium. (Details Pontecorvo 1952a).

As minimal medium, with the addition of 0.2 gms. peptone, 0.1 gms. Yeastrel, 0.3 mls. hydrolysed yeast nucleic acid and 0.5 mls. casein hydrolysate per 100 mls.

Vitamins added as required after sterilisation.

Suspending fluid.

$\frac{1}{10,000}$ calsolene oil in sterile distilled water or saline.

PART I. INVESTIGATION OF INDIVIDUAL ASCI.

Introduction.

Tetrad analysis is the direct isolation and classification of all four haploid products of a single meiosis. Using this technique it is possible to:

1. Locate the centromeres.
2. Detect abnormal segregation of alleles.
3. Investigate certain modalities of crossing-over.

To locate some of the centromeres in A. nidulans, a cross (Table II, Cross VII) in which four loci were segregating (w/W, lys/LYS, y/Y and bi/BI, the last two closely linked) was studied. The choice of strains proved unfortunate, as differential viability occurred and insufficient numbers of asci of crossed origin with five or more spores germinating, were produced. Backcrosses to the wild type were carried out to improve the strains and make it worthwhile to repeat the analysis. Unfortunately the improvement in the backcrosses was negligible and accordingly, attention was turned to the other two problems.

As a start, it was necessary to evolve a technique of ascus dissection suitable for A. nidulans (See Section I).

a) Comparison of tetrad analysis and random sampling of gametes.

Using tetrad analysis, all four products of a single meiosis are obtained together and their relationship can be studied, but this is not possible in a random sampling of gametes as the products of many meioses are mixed up. By both methods, the location of genes in respect to each other is carried out by estimating recombination fractions, but tetrad analysis offers the further possibilities mentioned before.

Papazian (1952) points out that when estimating linkage from recombination frequencies, random sampling is more efficient than spores in tetrads. Two random products of meiosis give about as much information as all four of a tetrad. To offset this, when working with tetrads, if the genotype of three products is known, that of the fourth can be deduced and with two or more loci segregating, if only two products are known, but they have the same allele for all but one of the loci, then the other two products can be deduced. In A. nidulans, an ascus contains four pairs of sister spores, so the same amount of information can be obtained from four as from eight spores, if one of each pair is picked.

In most higher organisms genetical analysis is perforce restricted to a random sample of the products of a large

number of meioses, but recovery of all four products of meiosis is possible in Ascomycetes (Lindegren 1949) and Basidiomycetes (Perkins 1949) in mosses and probably in algae. Tetrad analysis is possible for Chlamydomonas (Moewus 1941) but not for Paramecium, as in the latter only one of the four products of meiosis remain in the one organism (Sonneborn 1947).

The spores in a tetrad may be arranged in a definite linear order - ordered tetrads - as in Neurospora (Dodge 1927, Wilcox 1928) or they may be unordered, (Lindegren 1929, Perkins 1929). In ordered tetrads, in the case of organisms where the spindles are orientated parallel to the sides of the ascus, as in N.sitophilia (Wilcox 1928), products of the first meiotic division go to opposite poles and remain there without overlapping. The two spindles of the second division are well apart, so the two centre nuclei do not usually exchange positions. By observing the arrangement of the spores it is possible to find whether segregation of any one pair of alleles occurred at the first or second meiotic division, (first or second division segregation), and from the order of separation, information is obtained as to map distance between any locus and its centromere (Lindegren 1932b, Whitehouse 1942).

In Saccharomycodes Ludwigii (Winge and Laustsen1939) and N.tetrasperma (Dodge 1927), the second division spindles are placed close together and overlap, so this has to be taken

into account when examining asci.

Classification by inspection of ascospores in the ascus is possible in Bombardia lunata, in crosses between strains with dark and light ascospores (Catcheside 1944) and hence immediate deduction from the spore arrangements as to whether first or second division segregation has occurred.

In unordered tetrads, where there is no definite arrangement of spores, other methods are necessary (Lindegren 1949, Perkins 1949).

Using tetrads, information on chromatid interference can be estimated using only two loci. When more than two loci are used, it is possible to distinguish between chromatid and chiasma interference (Lindegren and Lindegren 1942, Whitehouse 1942). From a random sample of spores, chromatid interference can only be detected in a few exceptional cases where recombination values are in excess of 50%.

Abnormal asci are detectable by tetrad analysis using adequate "markers" but from a random sampling of gametes, only in special cases can irregular segregations be detected, as in non-disjunction in Drosophila.

b) Ascus analysis of ordered and unordered tetrads.

i. Ascus analysis of ordered tetrads.

Ascospores arising from tetrads in which the products of meiosis are arranged in linear order, as in Neurospora, form one of six ascus patterns, depending on whether segregation occurred at the first or second meiotic division.

With a pair of allelomorphs A/a, two alternative arrangements result from first division segregation:

A		a
A	or	a
a		A
a		A

These two differ only in relation to proximal and distal ends of the ascus, as a mirror image, as do the first two arrangements from second division segregation, where one of the following four arrangements arises:

A		a		A		a
a	or	A	or	a	or	A
A		a		a		A
a		A		A		a

The number of spores representing each product of meiosis will depend on the organism studied. In Bombardia and Neurospora sitophila a further mitotic division follows meiosis, before the formation of the ascospores, resulting in the duplication of each product of meiosis: in Chromocrea, two such divisions follow meiosis so that each product is represented by four spores.

The frequency of crossing-over depends on the distance

of a locus from the centromere, thus by assessing the relative frequency of first and second division segregation at a locus, the position of the centromere may be plotted on a crossover map of the chromosome. Half the percentage of second division segregation for a gene is the uncorrected map distance of the gene from the centromere.

ii. Ascus analysis of unordered tetrads.

The situation is quite different with unordered tetrads. The frequencies of first and second division segregation cannot be determined using one locus; but only by testing two by two can the segregation of three loci be studied (Whitehouse 1950).

In a cross A B x a b, when two unlinked loci segregate, three segregation types of asci are distinguishable, according to the segregation of the alleles into the four products of meiosis:

Type I	2 A B, 2 a b.	= all parental combinations.
Type II	2 A b, 2 a B.	= all recombinant combinations.
Type III	A B, a b, A b, a B.	= parental and recombinant combinations.

These types of asci may be formed in the following ways, considering single and double crossovers only:

Table 1. Segregation types of unordered asci resulting from single and double crossingeover with two unlinked loci segregating in a cross.

Type of crossover	Crossing-over between:	Combinations formed	Type of ascus.
1. None		All parental	I
		All recombinant	II
2. Single	One locus & the centromeres	(2 parental (2 recombinant	III
	Both loci & centromeres	All parental	I
		All recombinant	II
		(2 parental (2 recombinant	III
3. Double a) 2-strand	One locus & the centromeres	All parental	I
		All recombinant	II
b) 3-strand	" " " "	(2 parental (2 recombinant	III
c) 4-strand	" " " "	All recombinant	II
		All parental	I
4. Double a) 2-strand	Both loci & the centromeres	All parental	I
		All recombinant	II
b) 3-strand	" " " "	All parental	I
		All recombinant	II
		(2 parental (2 recombinant	III
c) 4-strand	" " " "	All recombinant	II

Table 1 shows that the types of asci formed when no crossing-over takes place are similar and indistinguishable from those formed from the following types of crossing-over;

a) single crossing-over between both loci and their respective centromeres,

b) 2 and 4-strand double crossing-over between one locus and the centromere,

c) 2-strand double crossing-over between both loci and their respective centromeres.

Double crossovers between both loci and their respective centromeres occur only infrequently.

Types I and II may be called di-type tetrads and Type III a tetra-type tetrad (Whitehouse 1949). Equal numbers of Type I and II show there is no linkage between the two loci or that they are more than 50 units apart. When Type I is more frequent than Type II, linkage is indicated. If each of two (unlinked) loci is 50 or more units from its centromere, the ratio of Types I:II:III is 1:1:4.

As the proportion of di- to tetra-type tetrads depends on the frequency of crossing-over between the loci and their respective centromeres, Whitehouse (1950) shows the following relationship:

If p = proportion of 4-type tetrads

x and y = " " second division segregation at A and B loci,

then $p = x + y - \frac{3xy}{2}$

The precise frequencies of first and second division segregation cannot be determined from this expression, but by introducing a third independent locus C, showing no linkage with either A or B loci, the required proportions of first and second division segregation can be determined for all three loci.

If q and r are the proportions of tetra-type tetrads from loci B and C and loci A and C respectively and z is the proportion of second division segregation at the C locus then:

$$q = y + z - \frac{2yz}{2} \quad \text{and} \quad r = x + z - \frac{2xz}{2}$$

In the case of two linked genes, either located on the same chromosome arm or on opposite arms, the formulae may be modified, though on account of double crossovers, the results are less reliable and reasonably accurate results can only be expected when the two loci are both comparatively near the centromere.

In order to map the chromosomes with accuracy it is essential to have 3 <u>independent</u> loci.

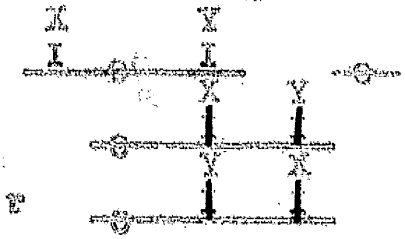
The following formulae (Whitehouse. Private communication.) show the mathematical treatment in the case of two linked genes:

Unordered tetrads with 2 linked genes.

There are three possible positions for the genes:

One gene on each arm.

2 genes on one arm.



and hence 3 alternative sets of formulae, all of which in general must be tried, to find which gives a real solution.

Let A, B and C be 3 loci. A and B linked and C independent.

" x, y & z be their respective second division segregation frequencies.

" p, q & r be the frequencies of 4-type tetrads of A and B, B & C and C & A respectively.

" P be the recombination frequency of A and B,

Then $q = y + z - \frac{3yz}{2}$ - - - - - - - - - (1)

$r = x + z - \frac{3xz}{2}$ - - - - - - - - - (2)

and $2P = x + y$ or $x - y$ or $y - x$, - - (3)

Solving for x, y and z gives:

$2P =$	$x + y$	$x - y$	$y - x$
$x =$	$\frac{2P - q + r - 3Pr}{4 - 3q - 3r}$	$\frac{2}{3}\ \frac{3Pr - q + r - 2P}{r-q}$	$\frac{2}{3}\ \frac{3Pr + q - r - 2P}{q-r}$
$y =$	$\frac{2P + q - r - 3Pq}{4 - 3q - 3r}$	$\frac{2}{3}\ \frac{3Pq - q + r - 2P}{r-q}$	$\frac{2}{3}\ \frac{3Pq + q - r - 2P}{q-r}$
$z =$	$\frac{q + r - 2P}{2 - 3P}$	$\frac{q - r + 2P}{3P}$	$\frac{2P - q + r}{3P}$

NOTE: Owing to double crossovers equation (3) is only true if A and B are near the centromere. Thus, 2 genes in the same chromosome arm may be 20 or 30 units apart and yet have almost

identical second division segregation frequencies, if they are both some distance from the centromere. Hence, if x or y are greater than 0.5 approx., the results are unreliable.

Section I. Technique of Ascus dissection.

In order to investigate the constitution of individual asci of Aspergillus nidulans, a technique of ascus dissection was developed.

With ascus analysis of yeast, one coverslip is used for the isolation and germination of ascospores from a number of asci (Winge and Laustsen 1937, Lindegren 1949). In Aspergillus, growth of hyphae over the coverslip renders this technique impracticable, so a modification was devised whereby the eight individual ascospores of a single ascus were transferred to separate coverslips for germination.

A De Fonbrune micro-manipulator was used in conjunction with a binocular microscope (eyepiece x 12, objective x 20, magnification x 360) and micro-instruments were made on a De Fonbrune microforge. Using soda glass tubing $\frac{1}{8}$" internal diameter, drawn out twice, a micro-loop of 15 - 20μ internal diameter and 3μ in thickness was made. The shaft of the loop was bent up at an angle of 40 degrees to enable the loop to enter the suspension easily. The loop was the most satisfactory instrument to employ, as it could be used for all manipulations required:

1) to separate individual asci from squashed-perithecium preparations.

2) to transfer asci and ascospores from one coverslip to another.

3) to break unripe asci by means of the shaft.

The rough covering of the ascospores aided their manipulation. The loop was cleared of adhering ascospores or agar by inserting it momentarily in a droplet of sterile agar medium. If adhering matter was not removed by this method the loop was left in chromic acid over-night and washed very thoroughly in sterile water before use. Alcohol and ether were employed for sterilisation of the loop.

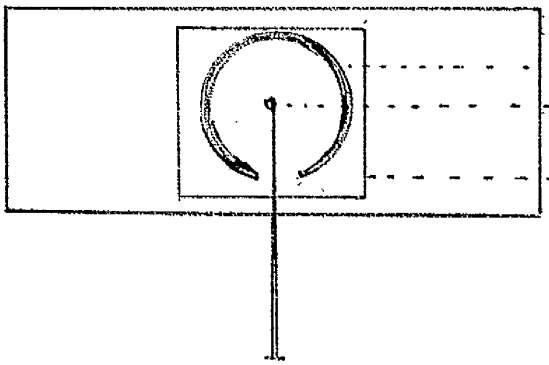
The moist isolating chamber.

From $\frac{5}{8}$" internal diameter glass tubing, a $\frac{1}{4}$" length was taken. A slot, $\frac{1}{4}$" wide was then cut down the entire length of the tubing, thus producing a horseshoe-shaped form. One end of this opened tubing was then firmly attached to a 3" x 1" glass slide, with the slot facing across the width of the slide. Enough water was introduced to cover the bottom of the chamber.

Preparation of ascus suspension.

Using a dissecting needle, a suitable perithecium was picked from a "mixed-inoculum" cross (see p. 35) using a low power binocular microscope, and transferred to a droplet of sterile water on a sterile $\frac{7}{8}$" No. 2 coverslip. The perithecium was lightly punctured, to liberate its contents and the coverslip was then placed in an inverted position on the prepared moist chamber, with the droplet of suspension in a central position. The moist chamber was placed on the microscope stage so that the micro-loop could be introduced

from the front.



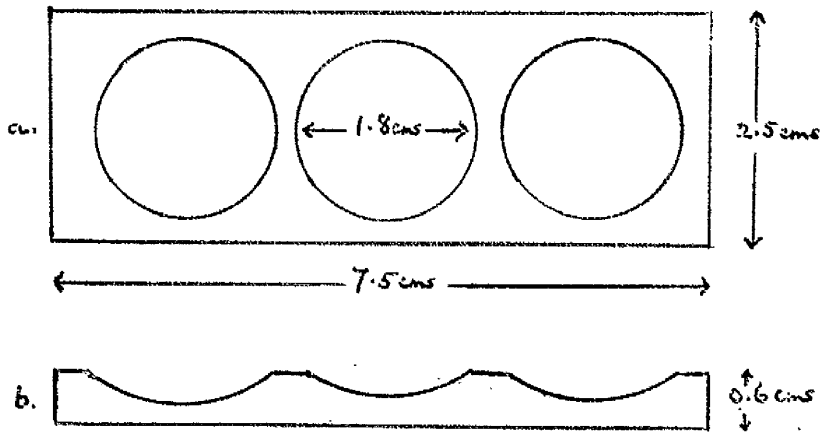
Moist chamber and loop as seen from above.

Ascus dissection.

Having focused on the preparation with the low power objective (x 10), the preparation was removed, to allow the micro-loop to be introduced safely into the moist chamber. The micro-loop was adjusted in a central position and at the same focus as the preparation, then lowered slightly whilst the cover-slip was returned to position. The x 20 objective was used for dissection. A deep-green and an orange filter were used together on the light source.

An ascus was removed from the suspension and freed of conidia and any adhering free ascospores. It was then removed in the loop from the coverslip, and transferred to an agar drop on the underside of a second coverslip, substituted for the first. The ascus was broken up, the number of ascospores checked and each separate ascospore placed on an individual agar-drop on a coverslip. The numbered agar-drop preparations were inverted on specially prepared perspex "depression" slides, as designed by Dr. Beale (private communication). The slides were 7.5 x 2.5 cms. and 0.6 cms. in depth. Three rounded

depressions 1.8 cms. in diameter and 0.3 cms. deep were spaced out along the slide (see diagrams) and were deep enough to hold a few drops of water. A little vaseline was applied to keep the cover-slips in position.



"Depression" slide

a) from above
b) transverse section

Micro-loop viewed from the side and from above. (Greatly magnified).

The slides were placed on a wire rack, over water, in a large covered dish and incubated at 37 degrees C. After 24 hrs. the preparations were examined for germination, under the low-power binocular, then returned to the incubator for another two days before classification for colour and nutritional requirements.

Only one ascus was isolated from each perithecium, for ascus analysis. Thus the number of asci dissected was equal to the number of perithecia from which ascospores were obtained, but not to the total number of perithecia sampled, as perithecial suspensions which did not contain whole asci were discarded.

Estimation of the proportion of asci of crossed origin from a random sample of ascospores.

In order to avoid crosses in which a very low proportion of asci of crossed origin are formed, the proportion of asci of crossed origin can be estimated on the basis of recombinants formed, in crosses involving two or more genes, from a preliminary plating of ascospores carried out before commencing ascus dissection (see Plate 3).

In asci from crosses in which two genes are segregating (as Y/y and W/y) ½ of the ascospores are recombinants, if the two loci are not linked.

From a random sample of ascospores from a cross y x w, the proportion of ascospores giving green colonies (recombinants) can be assessed and by multiplying this figure by four,

the proportion of ascospores arising from asci of crossed origin and hence the proportion of asci of crossed origin can be calculated.

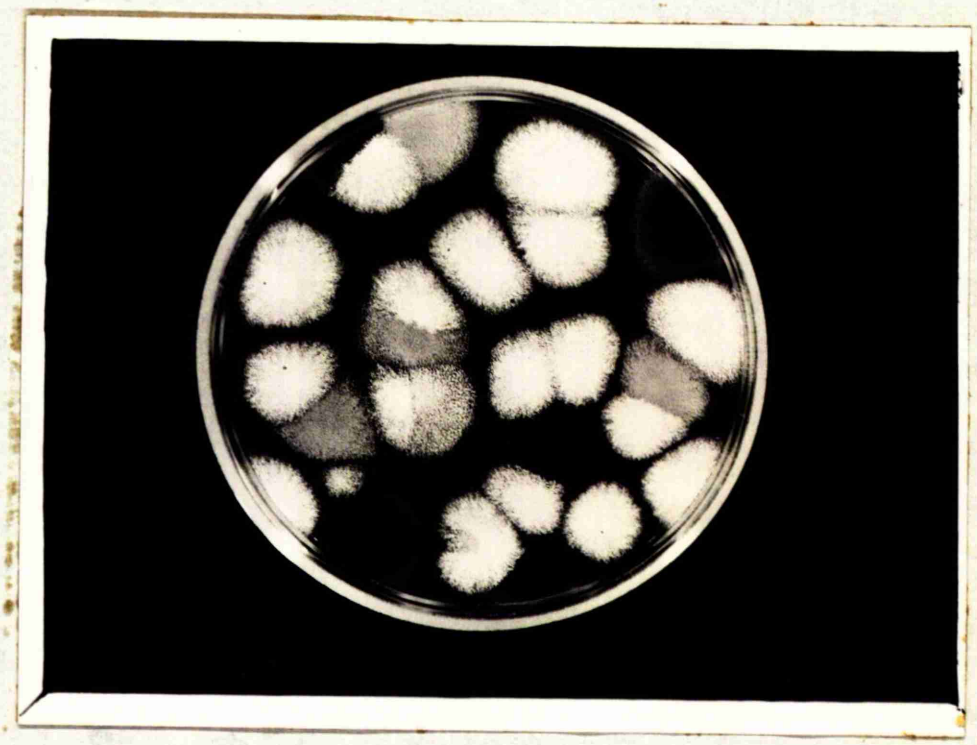


Plate 3. Plating of a random sample of ascospores from a cross w Y x W y (2 genes segregating) showing green recombinants, W Y (dark colonies) and parental types (light and medium colonies).

In Cross III y bi x w (Table C) from a random sample of ascospores the following results were obtained:

White colonies	=	552
Yellow colonies	=	51
Green colonies	=	27
Total colonies	=	630
Proportion of green recombinants	=	0.043 ± 0.009
Ascospores arising from asci of crossed origin	=	27 x 4
Proportion of asci of crossed origin	=	$\frac{27 \times 4}{630}$
	=	0.17

A similar procedure can be applied to crosses involving a nutritional character and a colour "marker" or two nutritional characters. It is obviously more laborious however, as classification for nutritional characteristics requires testing, whereas inspection is sufficient when colour "markers" are used.

The following results were obtained from a cross involving a colour "marker and a nutritional character. Cross y bi x + The y and bi loci are linked and show 0.051 ± 0.004 recombination. (Pontecorvo 1952a).

From this cross the following types of ascospores are expected: green prototrophs, green biotin-requirers, yellow prototrophs and yellow biotin-requirers. As the recombinants (yellow prototrophs and green biotin-requirers) cannot be

distinguished by inspection from the parental types, approximately a hundred green and a hundred yellow colonies were tested for biotin requirements, on minimal agar and minimal agar + biotin.

The results were as follows:

green prototrophs = 110
yellow biotin-requirers = 108
} Parental types.

yellow prototrophs = 2
green biotin-requirers = 0
} Recombinants.

Proportion of crossovers = $\frac{2}{220}$

= 0.009 ± 0.006

But 0.051 crossovers expected

Therefore, proportion of asci of crossed origin = $\frac{0.009}{0.05}$

= 0.18

Clearly, in this case, the estimate is affected by an enormous error due to the scarcity of crossovers.

Comparison of the proportion of asci of crossed origin from a random sample of ascospores and from ascus dissection.

Comparing the percentage of crossed asci obtained from ascus dissection with the expected figure calculated from recombinants, in crosses in which green recombinants occurred, it is shown in Table 2 that more than the expected number were obtained in all crosses but No. VII. However, as the percentage of crossed asci from ascus dissection was calculated on the number of classifiable asci, it is clear that a high proportion of unidentified asci, as in Crosses I, II, V and VI

would render the result inaccurate.

In crosses III and VII, whilst similar results are obtained from ascus dissection, a higher proportion of green recombinants from plating of ascospores are obtained in cross VII. Plating of ascospores was carried out in the latter cross approximately two months after preparation of the "mixed-inoculum" cross, whilst only a month elapsed before plating ascospores from Cross III. It was noticed that the older the "mixed-inoculum" cross, the fewer the conidia adhering to the perithecia. On counting the ascospores in the ascospore suspension, account was taken of conidia present - about 20% in the case of Cross III and a negligible number in Cross VII. If the viability of the conidia differed from that of the ascospores, whilst the number of green-recombinants obtained from colonial counts would not be affected, the proportion of green recombinants to total ascospores might be upset. It does not appear that differential ripening of green recombinants took place in Cross III and Cross VII as proportions of 22/437 and 18/417 respectively were found on ascus dissection (see Section III Table 10).

Table 2. Table showing the percentage crossed asci estimated by ascus dissection and from green recombinants in a random plating of ascospores.

Cross	Plating of ascospores		Asci from ascus dissection.				
	Green/Total	% crossed asci	Classifiable	Non-classifiable	Total	Crossed Ec.	% classifiable
I y x w	1/96	4	19.	34	53	5	26
II y x w	8/190	23	49	15	64	16	33
III y bi x w	27/630	17	60	25	85	17	28
V y bi x w	9/630	6	9	9	18	3	33
VI y bi x w	28/668	20	4	7	11	1	25
VII y bi x w lys	67/709	37	64	18	82	18	27

(Green ascospores represent recombinants ½ of the ascospores from crossed asci).

Section II. Improvements in the technique of Ascus analysis

Only the products of crossed karyogamy are of use for ascus analysis and in A.nidulans if karyogamy were at random between nuclei A and B in a heterocaryon, the upper limit of crossed A x B asci would be 50% when nuclei A and B were in equal proportions.

We know now however, though it was not known when the work was commenced, that certain combinations of strains may give more than 50%, up to 100% crossed asci, i.e. relative heterothallism (Hemmons, Pontecorvo and Bufton 1952), showing that karyogamy is not at random, at least in certain combinations. It was unfortunate that none of the combinationsused at the start showed relative heterothallism.

One problem therefore was that of finding conditions which would favour the formation of as high a proportion as possible (then believed to be up to 50%) of crossed asci. This was tackled firstly by attempting to improve the technique of "mixed-inoculum" cross, with regard to :

i. optimal initial density of conidia;

ii. optimal proportions of conidia of the two strains, and

iii. pre-incubation of the conidial suspension of one strain before mixing; also

iv. by the development of a suitable sporulation medium.

The second problem of obtaining a high proportion of germinable ascospores was tackled genetically, by backcrossing

to the wild type. The third problem was that of extracting as much information as possible from incomplete asci in which, in spite of all efforts, not all the ascospores had germinated. In the treatment of this last problem Dr. L. L. Cavalli kindly gave his help.

1. Improvements in the technique of "mixed-inoculum cross".

The general method (Pontecorvo 1952a) of preparing "mixed-inoculum" crosses is as follows: A layer of complete medium is poured in a Petri dish and when set, 0.1 mls. of a mixed suspension of the conidia of the two strains to be crossed, is spread on this surface. A top layer is then poured.

This method makes use of the fact that hyphal anastomoses occur readily, soon after germination of the conidia and therefore the packing together, in equal proportions, of conidia of two strains in non-selective agar medium, ensures a high number of inter-strain anastomoses. If the inoculation is of such a high density that very little growth can take place before the formation of the perithecia, a high proportion of the heterokaryotic hyphae formed will remain heterokaryotic up to the time of the formation of the perithecia. A proportion of perithecia will thus start with nuclei of both kinds. (Pontecorvo 1952a).

Equal initial numbers of conidia in a suspension will not necessarily produce conditions favouring the production of the highest percentage of asci of crossed origin, due to causes such as differential viability and germination time and various factors affecting karyogamy in the mycelium. The optimal proportions of conidia of the two strains used must therefore be found for each cross.

Simultaneous germination of the conidia of the two

strains is important, to ensure a high number of inter-strain anastomoses and avoid overgrowth of one strain by the other. Thus it may be necessary to pre-incubate the conidial suspension of a strain that germinates slowly, before making the mixed conidial suspension.

In certain crosses between y or y bi and w, (Table B Cross II and Table C Cross III) a bias was found in favour of selfed white asci. Thus, in investigating optimal proportions of the two strains, equal numbers of yellow and white conidia or an excess of yellow conidia were used. An investigation of the optimal proportions of the two strains and the optimal density of conidia was carried out in the same experiment.

i & ii) Optimal density of conidia and optimal proportions of the two strains.

Suspension of conidia from 5 day old cultures of y bi and w strains were prepared in calsolene, by sucking up and down a drawn-out Pasteur pipette until all conidial chains were broken up. Counts using a haemocytometer cell, were made on the conidial suspensions and the numbers adjusted to approximately 6 million per ml.

Using varying proportions of the two suspensions, mixtures were made to obtain proportions of white/yellow of $\frac{1}{100}$, $\frac{1}{10}$, and $\frac{1}{1}$ and total numbers of 0.2, 1.0 and 6.0×10^6 conidia per ml. 1 ml. of each mixture was

added to 1 ml. of warm complete medium, giving a final concentration of 0.1, 0.5 and 5.0 x 10^6 conidia per ml. Two 1 ml. portions of each mixture were run over the surface of complete medium slopes, in large test tubes (6" x 1"). The slopes had been prepared from approximately 7.5 ccs. of agar and warmed in the 37°C. incubator for a couple of hours before using, to facilitate the even spreading of the conidial layer.

Small aliquots of the mixed conidial suspension were plated on complete medium, to determine the actual ratios of viable white and yellow conidia (Column 2. Table 3).

After incubating the slopes for a period of 34-47 days at 37°C., ascospore counts were made on samples of ascospores from large numbers of perithecia. The perithecia were first freed of conidia, by rolling on the surface of well-dried agar, then transferred to a tube containing a few mls. of calsolene or saline, and squashed. The ascospore suspension, suitably diluted, was plated on complete medium and the colonies counted and classified for colour. The percentage of asci of crossed origin was calculated from the proportion of green recombinants (W Y).

Table 3 shows results obtained for a cross w x y bi.

It appears that a high proportion of yellow conidia favours a higher proportion of crossed asci when the total yellow conidia are less than 49.5 x 10^4 per slope. With equal proportions of yellow and white conidia, an increase of density

Table 3 Cross w x y bi. Showing the effect of the initial conidial density and the proportion of white and yellow conidia in a "mixed-inoculum cross" on the % asci of crossed origin formed.

Initial ratio of white and yellow conidia		Age of cross in days	Conidia plated per slope x 10^4			Type and number of colonies from ascospores.				% Green	% asci of crossed origin
Aimed at:	Viable counts obtained		White	Yellow	Total	Yellow	White	Green	Total		
1:99	Plates over-crowded	40	0.1 ?	9.9 ?	10	91	176	34	301	11 ± .018	44.0
		41	0.5 ?	49.5 ?	50	1,363	420	34	1,817	1.9 ± .003	7.5
		47	3 ?	297.0 ?	300	271	152	1	424	0.2 ± .002	1.0
1:9	1:4	37	2	8.0	10	40	202	17	259	6.6 ± .015	26.2
	(179:1039)	38	10	40.0	50	62	115	24	201	12.0 ± .026	48.0
		39	60	240.0	300	856	1,339	30	2,225	1.3 ± .002	5.2
1:1	1:0.8	34	5	5.0	10	23	1,979	20	2,022	1.0 ± .002	4.0
	(261:352)	35	25	25.0	50	87	1,771	86	1,944	4.4 ± .004	17.6

Figures in brackets show actual number of white and yellow colonies counted.

In all ratios, the figure for white is printed first (1 white : 99 yellow etc.)

? = Exact figure not known, as no viable count obtained.

Green ascospores represent recombinants and represent $\frac{1}{4}$ of the ascospores from crossed asci.

increases the proportion of crossed asci.

Plotting the total white and yellow conidia per slope against the percentage of asci of crossed origin obtained, (see Graph I, p.40) an increase in the percentage of crossed asci is shown as the total number of white conidia is decreased (and total yellow increased) to 10 x 10^4 per slope. The higher the proportion of yellow conidia, the fewer the total number required to obtain a certain number of crossed asci.

iii) Effect of pre-incubation of the conidial suspension of one strain before mixing.

In certain crosses, an excess of selfed asci of one type was found. In Crosses II y x w (Table B) and III y bi x w (Table C) an excess of yellow to white selfed asci occurred and in Crosses V (Table F) and VI (Table G) both y bi x w, the few selfed asci that occurred were all selfed white. Cross VII y bi x w lys (Table H) shows the opposite result, of an excess of selfed yellow, but when the following experiment was planned, ascus dissection of Cross VII had not been carried out.

When conidia of two strains are mixed, if conidia of one strain germinate even slightly faster than those of the other, they will overgrow the second strain. Consequently a preponderance of selfed asci of one type will be found and a deficit of asci of crossed origin. To investigate whether the excess of selfed white asci in crosses involving y or y bi and w, was due to faster germination of the white strain, the yellow strain was "pre-germinated" for 2 hrs. at room temperature before the mixed conidial suspension was prepared.

The experiment was carried out as follows: "Mixed-inoculum" crosses were prepared using conidial mixtures of y bi and w strains, with ratios of white/yellow as 1:1, 1:4 and 1:9 and a total of 5 million conidia per plate. In one

series of experiments the conidial suspensions were prepared at the same time and mixed, in the other series, the yellow conidial suspension was prepared first. The latter was diluted with saline to the required concentration and allowed to stand for 2 hrs. at room temperature, to start germinating, before mixing with a freshly prepared conidial suspension of the w strain. Conidial counts on the two conidial mixtures were made.

Ascospore counts, as carried out in the previous experiment (p. 37) were made on the plates after 26 days. Table 4 shows that except with an initial ratio of white to yellow of 1:4, there was no difference between the percentage of asci formed: a) when the mixed conidial suspension was prepared from two freshly prepared suspensions of the two strains or b) using a pre-incubated suspension of the y bi strain. Also changing the ratio of white/yellow from 1:9 to 1:1 did not affect the results. It might well be that pre-incubation of the yellow strain for a different length of time, or at 37°C., would prove advantageous. However, under the conditions of the experiment carried out, pre-incubation of the yellow strain made no difference to the results obtained, with an initial white/yellow ratio of 1:9 and 1:1. A reciprocal test of pre-incubation of the w strain was not carried out.

With what we know now about relative-heterothallism (p.33) it is not suprising that experiments based, as this

one was, on a strictly kinetic approach to karyogamy should give often inconclusive or unexpected results.

Table 4 Cross y bi x w.

The effect of "pre-incubation" of the y bi strain before preparing the mixed conidial suspension with the w strain in a "mixed-inoculum" cross.

Immediate mixing					Yellow "pre-incubated" for 2 hrs.				
Initial ratio of white to yellow conidia.		Viable counts from ascospores		% asci of crossed origin	Initial ratio of white to yellow conidia.		Viable counts from ascospores		% asci of crossed origin
Aimed at:	Viable count obtained	Green/total No.	Green/total %		Aimed at:	Viable count obtained	Green/total No.	Green/total %	
1:9	overcrowded	24/605	4.0 ± .008	16	1:9	1 : 9.4 (39:366)	9/259	3.6 ± .011	14
1:4	1 : 4.4 (79 : 352)	9/517	1.7 ± .006	7	1:4	1 : 3.0 (92:275)	30/629	4.7 ± .013	19
1:1	1 : 1.1 (119 : 137)	6/195	3.1 ± .012	12	1:1	1 : 0.8 (179:140)	2/73	2.7 ± .019	11

Figures in brackets show actual numbers of white and yellow colonies counted.

Green ascospores represent recombinants and represent ½ of the ascospores from crossed asci.

iv) Development of a suitable sporulation medium.

Examination of the literature relating to nutritional factors and sporulation, revealed both the diversity of approach of the investigators and the variation in requirements of different organisms. It was clear that many factors influence sporulation and each organism requires individual treatment. Optimal nutritional factors for sporulation are often quite different from those for vegetative growth. (Thom and Raper 1945, Hawker 1950, Lilly and Barnett 1951).

Association with other organisms or their products was shown to stimulate sporulation in Melanospora destruens (Asthana and Hawker 1936) and in Zygosaccharomyces (Nickerson and Thimann 1941). Hawker (1938) identified aneurin as a necessary growth factor concerned with the fruiting of Melanospora destruens and Nickerson and Thimann (1943) found riboflavin and glutamic acid to be necessary in Zygosaccharomyces.

The carbon source and the proportion used is important (Hawker 1939), and Westergaard and Mitchell (1947) demonstrated the importance of the carbon/nitrate ratio in Neurospora, showing that a lower concentration of nitrate than that used in ordinary laboratory media was beneficial to perithecium production.

Some attention has been paid to the significance of

Calcium in increasing sporulation in Chaetomium species (Basu 1951). Gypsum slopes are widely used for yeast sporulation (Stelling-Dekker 1931).

A.nidulans can be grown successfully on a simple medium containing a Carbon and Nitrogen source and inorganic salts. As the work of Westergaard and Mitchell (1947) was carried out on an Ascomycete, it was decided to investigate the importance of the carbon/nitrate ratio in Aspergillus, by varying the amounts of sodium nitrate and dextrose used in solid minimal medium.

Three strains of A.nidulans were used: +, y bi, and w. As the y bi strain required biotin for growth in minimal medium, 0.02 γ /ml. of desthiobiotin was added to the medium used. 0.05%, 0.1% and 0.5% concentrations of $NaNO_3$ were used in combination with 1.5%, 2.0% and 2.5% of dextrose. Three 10 ml. amounts of the various combinations were prepared in boiling tubes (1" x 6") and tests set up in triplicate. 1 ml. amounts of conidial suspension, containing 5 million conidia per ml. were added to the warm molten medium before sloping. After eleven days at 37°C., the tubes were examined for perithecium formation. Controls were set up using complete medium.

Table 5. Formation of perithecia by strains + and y bi, using varying concentrations of $NaNO_3$ and dextrose in solid minimal medium.

% $NaNO_3$	% dextrose 1.5	2.0	2.5
0.5	+++	+++	+++
0.1	+++	+++	+++
0.05	+++	+++	+++

+++ Good perithecium formation.

With all combinations a good "crop" of perithecia was obtained from strains y bi and +. Perithecia were largest for the y bi strain with the combination of 1.5% dextrose and 0.05% $NaNO_3$ and for the + strain with 2.5% dextrose and 0.5% $NaNO_3$.

Table 6. Formation of perithecia by strain w, using varying concentrations of $NaNO_3$ and dextrose in solid minimal medium.

% $NaNO_3$	% dextrose 1.5	2.0	2.5
0.5	---	---	+
0.1	++	++	++
0.05	---	---	+

--- No perithecia formed

\+ A few perithecia formed

++ Moderate number of perithecia formed.

It is evident that perithecium production by strain w is affected by the % $NaNO_3$. To find whether other concentrations of dextrose were more suitable, 0.1%, 2.5%, 5% and 10% concentrations were tried in conjunction with 0.1% $NaNO_3$. Perithecia were not formed with 5% and 10% dextrose. The addition of 0.1% peptone, 0.1% yeast extract or 0.3γ/ml. aneurin to minimal medium containing 2% dextrose and 0.1% $NaNO_3$ caused no increase in perithecium formation.

For a sporulation medium, a modified minimal medium containing 0.1% $NaNO_3$ and 2% dextrose was adopted.

2. Backcrossing to the wild type.

When working with strain w, or derivative strains, in certain crosses, Cross II y x w (Table B), Cross III y bi x w (Table C) and Cross VII y bi x w lys (Table H), difficulty was experienced in obtaining ascospores of high germinability. Strain w was isolated by Yuill 1936, as a naturally occurring mutant and since then it has been genetically isolated from the present strains. By backcrossing to wild type it was hoped to eliminate as many gene and cytoplasmic differences as possible.

Lindegren, Beanfield and Barber (1939) found mutant strains of N. crassa often extremely infertile, but managed to increase the fertility from 5.9 to 59.0% in the fourth generation by selection and inbreeding. Fincham (1950) found that after the third backcross, an interspecific cross of N.sitophila and N.crassa which was originally highly sterile, became fully fertile. As in Cross VII w lys x y bi (Table H), marked differential germination was shown between selfed and crossed asci. By repeated backcrossing of the two strains to wild type and re-isolation from asci containing eight spores if possible, it was hoped to increase the germinability of the spores from crossed asci. The strains to be backcrossed were both isolated from the same crossed ascus, No. 158, which contained six viable ascospores of the following phenotypes:

Ascus 158.	Spore 1.	White BI lys
	2.	Green BI LYS
	3.	White bi lys
	4.	White BI lys
	5.	Yellow bi LYS
	6.	Green BI LYS

Spores 1 and 4 were sister spores, but on account of epistasis their genotype might be w Y BI lys or w y BI lys. As no yellow recombinants among 1,860 ascospore colonies examined were found on backcrossing Spore 1 to wild type, its genotype was presumed to be w Y BI lys. As the percentage germination of ascospores from this cross was low (see Cross IX Table J), to save unnecessary labour in ascus dissection whole asci isolated from the first backcross were simply crushed on cellophane squares dipped in nutrient agar. Asci showing green and white sectors were presumed to be of crossed origin and white sectors in these asci were tested for lysine requirement. Of 50 asci examined, only one showed a white sector which proved to be lysine-requiring. From a plating of ascospores from a repeat of the backcross, perithecia bordering the white colonies were picked and asci isolated and squashed as before. (13 asci were dissected Cross VIII Table I). Six crossed asci from a total of 42 asci isolated showed green and white sectors. From a white sector of one of these asci, Number 6, a white lysine-requiring strain was isolated and backcrossed again

to wild type. 30 asci were isolated from this second backcross and four showed green and white sectors. A white lysine-requiring strain was isolated from an ascus No.24, which showed sectors: white lysine-requiring, white lysine-non-requiring and green lysine-requiring, as shown in the diagram.

Little selection was possible using this method of picking a sector and further backcrossing was necessary.

Table 7. Backcrossing of the w lys strain isolated from Cross VII - w lys x y bi.

w lys x y bi

Ascus 158 (Spore 1) w Y BI lys x + (1st backcross)

Ascus 6

White lysine-requiring strain isolated from a squashed-ascus preparation showing white and green sectors.

White lysine-requiring strain x + (2nd backcross)

Ascus 24

White lysine-requiring strain isolated from a squashed-ascus preparation showing white and green sectors.

The re-isolation of the y bi strain after back-crossing proved to be much easier than that of the w lys strain. From the first backcross, the ninth ascus isolated by ascus dissection was an eight-spored ascus of the following composition:

Ascus 9. 2 W y bi
2 W Y bi
2 W y BI
2 W Y BI

From the second backcross (Cross X Table K), Ascus 9 proved to be of crossed origin, with seven viable ascospores. A "mixed-inoculum" slant was used. Spore 2 of Ascus 9 was cultured and a single-ascospore strain established.

Table 8. Backcrossing of the y bi strains isolated from Cross VII w lys x y bi.

w lys x y bi

Ascus 158 (Spore 5) W y bi lys x + (1st backcross)

Ascus 9

W y bi W y bi W Y bi W Y bi W y BI W y BI W Y BI W Y BI

x + (Cross X) (2nd backcross)

Ascus 9

W y bi W y bi W y bi W Y BI W Y BI W Y BI W Y Bi

Single ascospore strain.

Conclusion.

No noticable improvement resulted after two backcrosses of the w lys strain. This was not unexpected as little selection was possible when re-isolating from a colony

showing green and white sectors.

Re-isolation of the y bi strain proved much easier and backcrossing proved more successful. A single ascospore strain was isolated from an eight and a seven-spored crossed ascus in the first and second backcrosses respectively.

3. Information from incomplete asci.

The tables of dissected asci in the appendix show that various numbers of ascospores germinated in the dissected asci. Asci with less than eight germinated ascospores were called "incomplete" asci. Their classification depended on the number and type of ascospores present. Information was required as to whether an ascus was selfed or crossed and if crossed, of which type.

1. Identification of asci of selfed and crossed origin. Asci of crossed origin will be referred to as "crossed".

a) One gene segregation. In crosses with one gene segregating, as Cross XII (y x +), asci with 5 or more viable ascospores are fully classifiable and those with 2, 3 or 4 ascospores are classifiable if at least one ascospore differs from the other or others, in the segregating allele. An ascus containing 2, 3 or 4 ascospores all of one type may either be of selfed or crossed origin. An ascus containing only one viable ascospore is not classifiable.

b) Two or more genes segregating. As with one gene segregating, asci with 5 or more viable ascospores are fully classifiable and with 1 to 4 germinated spores, the ascus can be identified as crossed if it contains at least one recombinant ascospore (Cross VII. Ascus 225) or if two phenotypes are represented (e.g., CrossII. Ascus 91. wh, wh, yell, and Cross VIII Ascus 1. wh LYS, gn lys, gn lys).

2. Identification of types of crossed asci.

This is necessary when locating the centromere in order to find the proportion of Type III (tetra-type) tetrads (see p 19).

Asci in which seven or eight ascospores germinate can be fully classified as to type, but if fewer germinate there may be difficulty. To overcome this, Cavalli (private communication 1950) worked out an analysis of incomplete tetrads, using those with five or six germinated spores. An insufficient number of crossed asci were obtained from Cross VII y bi x w lys to make application of the formula possible. It had been hoped to obtain sufficient information from this cross to locate three centromeres.

Cavalli expresses the loss of information due to the failure of some of the spores to germinate in terms of the additional number of asci required to compensate for it (Table 9).

If p represents the sum of the frequencies of crossed asci of types I and II, and thus the frequency of type III is 1-p, then for instance, at p = 0.5, 100 asci with 5 viable spores give the same information as 70 asci with full viability.

Table 9. Amount of information from 5 or 6-spored asci relative to that from 7 or 8-spored asci for various values of p.

The efficiency ratios are given in %.

p	ASCI	
	6-spored	5-spored
0.9	93	77
0.8	92	76
0.7	91	74
0.6	90	72
0.5	89.6	70

(p = the frequency of Type I and Type II asci.)

When four or less ascospores germinate it is possible to distinguish the types of asous when certain colour combinations are present.

Yellow and green ascospores only occur together in Type III asci, so 2, 3 and 4 germinated ascospores per ascus showing a combination of yellow and green are classifiable. If three phenotypes are represented, this also indicates a Type III ascus.

Section III. Reliability of the technique of Ascus analysis.

The reliability of the technique of ascus analysis was investigated from two angles:

1. efficiency of the technique for recovering all products of one meiosis.
2. efficiency of the technique in giving expected results of segregation.

An examination of the asci dissected from various crosses (Tables A to Q) showed that in certain crosses, as Cross IV y bi x + (Table D) and Cross XI y bi x + (Table L) in a high proportion of dissected asci, of both selfed and crossed origin, 7 to 8 spores would germinate. In other crosses, on the other hand, (Cross VII y bi x w lys Table H. and Cross II y x w Table B) a high proportion of ascospores germinated in asci of selfed origin but this was not the case for those of crossed origin.

In crosses where results of samples of ascospores from dissected asci and random samples of plated ascospores were not homogeneous, the effect was apparently due to the following causes:

a) the technique leads to selection of one type of ascus,
b) differential viability between selfed and crossed asci.

From various crosses, the total number of ascospores of different types obtained from ascus dissection, were compared in each case with a random sample of ascospores obtained from plating and prior to ascus dissection. (Table 10)

Table 10 Comparison of random sample of plated ascospores with samples of ascospores from dissected asci.

		Number and types of ascospores.				Test for homogeneity.		
		White	Yellow	Green	Total	χ^2	Degrees of freedom	p
Cross III y bi x w	Plating	552	51	27	630	6.27	2	0.04
	Ascus dissection (25)	380	55	22	457			
Cross IV y bi x +	Plating	-	176	204	380	14.26	1	< 0.001
	Ascus dissection (79)	-	344	242	586			
Cross V y bi x w	Plating	463	158	9	630	7.17	2	0.02 to 0.05
	Ascus dissection (18)	64	4	3	71			
Cross VI y bi x w	Plating	405	135	28	568	2.20	2	0.35
	Ascus dissection (11)	25	4	1	30			
Cross VII y bi x w lys	Plating	113	528	67	708	24.3	2	< 0.001
	Ascus dissection (23)	37	362	18	417			

In brackets, number of dissected asci from which the ascospores were obtained: only one ascus was dissected from each perithecium.

It appears that in Crosses IV, V and VII the results from a random sample of ascospores and from ascus dissection are not homogeneous. The results of Cross III are just on the borderline.

In Cross V, the data from dissected asci are too few to place any weight on them and homogeneity in Cross VI is based on very few dissected ascospores.

In Cross IV, there is a deficit of green ascospores on ascus dissection. Analysis of the results of ascus dissection in Cross IV, to find the number of selfed green, selfed yellow and crossed asci and the average number of ascospores per ascus gave the following results:

Table 11. Cross IV. y bi x + Numbers and types of dissected asci and mean germinable ascospores from each type.

	Selfed yellow	Selfed green	Crossed	Total
No. of asci.	32	20	27	79
Ascospores from these asci.	245	145	99 yellow) 97 green) 196	586
Mean germinable ascospores per ascus.	7.7	7.3	7.3	7.4

N.B. In this cross, classification as "selfed" or "crossed" is practically correct as 77 out of 79 asci gave more than four germinated ascospores. (Table D. Appendix)

It appears that there is no differential germination between the two types of selfed asci, or between selfed and crossed asci. Furthermore, clearly the yellow and green ascospores from crossed asci do not differ in germinability.

The proportions of yellow and green nuclei in Cross IV, as shown by the random sample of ascospores (Table 10), are 0.463 and 0.537 respectively. On the assumption of random mating between nuclei, the proportion of crossed asci should be 2 x 0.463 x 0.537 = 0.497, that of selfed yellow should be 0.463^2 = 0.2145, that of selfed green 0.537^2 = 0.2884.

The actual numbers of selfed yellow, selfed green and crossed asci, among the dissected asci were as follows:

Table 12

Cross IV. y bi x + Observed and expected numbers of selfed and crossed asci among dissected asci.

	Selfed Yellow	Selfed green	Crossed	Total
Found	32	20	27	79
Expected *	21	29	50	100

(* On the assumption of random karyogamy and estimating the proportion of yellow to green nuclei from the random sample of ascospores.)

This table shows that there is a serious bias in the sampling of asci for dissection, i.e. the deficit of green ascospores as shown in Table 12 can only be due to the picking of relatively more yellow asci for dissection. The technique (p. 24) consisted in picking individual perithecia, crushing gently and picking one whole ascus from each crushed perithecium. Perithecial suspensions which did not contain a single unbroken ascus were discarded. If green asci burst more easily than yellow, a bias would be produced in favour of the yellow in the sample of dissected asci.

One possibility accounting for this selection could have been that green asci mature earlier in the colony than yellow. This possibility is excluded by the following considerations: ascus dissection was carried out over a period of six weeks on a "mixed-inoculum" cross a month old at the start. The types of asci and ascospores obtained in the first and last fortnight of ascus dissection were as given in Table 15 and the distributions of asci in the two periods appeared homogeneous (p = 0.3 to 0.5).

Thus, the bias is apparently not accounted for by a different proportion of dissectable asci correlated with the age of the colony. The only other obvious alternative is that ripe yellow asci remain for a longer time in a state where, when opening a perithecium, they do not break up.

Table 13

Cross IV (y bi x +)

Asci dissected at the beginning and end of a period of six weeks, from a plate one month old at the start.

Types of Asci:	Dissected in the first fortnight				Dissected in the last fortnight			
	Selfed Yellow	Selfed Green	Crossed	Total	Selfed Yellow	Selfed Green	Crossed	Total
No. of asci	20	9	18	47	11	8	6	25
Ascospores from these asci	155	63	65 yell.) 66 gn..) 131	349	83	60	20 yell.) 21 gn.) 41	184
Mean germinable ascospores per ascus	7.8	7.0	7.3	7.4	7.5	7.5	6.8	7.4

Test for homogeneity of the distributions of the three types of dissected asci in the first and last fortnight. χ^2 = 2.12 for two degrees of freedom.

p = 0.3 to 0.5

Against this hypothesis are the results obtained by the ascus-squash method. (Table 27. Part IIa).

Cross VII. Data from Cross VII (y bi x w lys) Table H, showed an excess of yellow ascospores on ascus dissection and a deficit of white and green. The latter types were in the same proportion as obtained in the random sample of ascospores.

The results of ascus dissection, analysed to find the number of selfed white, selfed yellow and crossed asci and the average number of ascospores per ascus, are as follows:

Table 14.

Cross VII. y bi x w lys.

Numbers and types of dissected asci and mean germinable ascospores from each type.

	Selfed Yellow	Selfed Green	Crossed		Total
No. of asci	46	0	19		65
Ascospores from these asci	325	0	16 yell.) 32 white) 66 18 gn.)		391
Mean germinable ascospores per ascus	7.1		3.4		6.0

The mean number of germinating ascospores per ascus is much lower in crossed asci than in selfed yellow. In the crossed asci, the allele ratios are not significantly different from 1:1 as shown in the following table:

Table 15. Cross VII (y bi x w lys)

Segregation of w/W, y/Y, bi/BI and lys/LYS in crossed asci dissected.

W y				W y + w Y				W Y				
	16				32				18			
BI		bi		BI		bi		BI		bi		
1		15		13		19		16		2		
LYS	lys	LYS	lys	LYS	lys	LYS	lys	LYS	lys	LYS	lys	Total
1	0	10	5	10	3	6	13	10	6	2	0	66

Allele ratios.

w/W	:	32/36	bi/BI	:	36/30
y/Y	:	16/18	lys/LYS	:	27/39

Tests for linkage (results from Table 15).

1. Between w/W and lys/LYS

	lys	LYS	Total
w	16	16	32
W	11	23	34
	27	39	66

Parental types = 39
Recombinant types = 27

2. Between bi/BI and lys/LYS.

	lys	LYS	Total
bi	18	18	36
BI	9	21	30
	27	39	66

Parental types = 27
Recombinant types = 39

3. Between y/Y and lys/LYS.

	lys	LYS	Total
y	5	11	16
Y	6	12	18
	11	23	34

Parental types = 17
Recombinant types = 17

4. Between y/Y andbi/BI.

	bi	BI	Total
y	15	1	16
Y	2	16	18
	17	17	34

Parental types = 31
Recombinant types = 3

Linkage is evident only between y/Y and bi/BI, where it is already known to occur (Pontecorvo 1952a). Even though the present data do not make the shortage of lys over LYS significant, the lys allele is known from other data (p.142) to reduce viability to 0.60.

For this reason, in crossed asci where half the spores are lysine-requiring, among these, a decrease in the number of germinated ascospores might be expected and the reduction estimated, as in Table 16, assuming no other complicating factors.

Table 16 Decrease in number of germinable ascospores per crossed ascus expected as a consequence of the 0.6 viability of the lys allele.

Spores per ascus.	Spores per ascus obtained due to 0.6 viability of the lys allele.
5	$5 - (0.4 \times \frac{1}{2}5) = 4.0$
6	$6 - (0.4 \times \frac{1}{2}6) = 4.8$
7	$7 - (0.4 \times \frac{1}{2}7) = 5.6$
8	$8 - (0.4 \times \frac{1}{2}8) = 6.4$

As selfed yellow asci are not affected by the viability effect of lys, crossed asci of the range 4.0 to 6.4 germinable spores correspond to selfed yellow asci of the range 5.0 to 8.0 germinable ascospores. It is clear that this does not fully account for the difference in distribution between the selfed yellow and crossed asci. (see summary of Cross VII y bi x w lys Table H)

Whilst y bi x + (Cross IV Table D) gives a high proportion of both selfed and crossed asci with 7 and 8 spores germinating, in Crosses VIII (Table I) and IX (Table J) both of w lys x + , poor germination was shown. As w lys was derived from white alba, crosses involving white alba were also examined to find whether there was any evidence of differential germination due to this gene or to other causes.

A comparison of types and numbers of ascospores per ascus was made between five crosses involving white alba or its derivatives, as shown in Table 17.

When examining the table for evidence of differential germination, the unidentified asci must be taken into account, as they may be either of selfed or crossed origin.

In Cross I, 34/53 asci were unidentified and with such a high proportion, no conclusion can be drawn. Similarly in Crosses V and VI, data were too meagre to place any weight on them.

However, in Cross II, there is evidence in favour of differential germination. Even if the unidentified asci were all of selfed origin, the majority of white selfed asci would still show 5 or more spores germinating, in contrast to the crossed asci, in which in all but one ascus, less than 5 spores germinated. Similarly in Cross III the majority of selfed white asci would show five or more spores germinated, even if all unclassified white asci were considered as of selfed origin.

It would thus appear that in Cross VII (y bi x w lys), allowing for the viability effect of lys, differential germination operates between selfed yellow and crossed asci.

Table 17

Types of asci and numbers of spores germinating from crosses involving the strain white alba or derivatives, as one parent, and wild type or derivatives as the other parent.

Asci	Identified			Un-identified			Total
	Selfed Yellow	Selfed White	Crossed	Yellow	White	No germination.	
Ascospores germinating .No.	5 6 7 8	5 6 7 8	1 2 3 4 5 6 7 8	1 2 3 4	1 2 3 4	0	
Cross							
I y x w	2 4 5 3	- - - -	1 - 2 - 1 1 - -	5 3 5 8	2 2 - -	9	53
II y x w	- 2 1 -	2 10 11 6	- 1 9 5 1 - - -	2 1 - -	2 6 2 2	1	64
III y bi x w	2 1 - -	10 8 15 7	- - 3 2 4 3 3 2	2 2 1 2	1 3 5 8	1	85
V y bi x w	- - - -	1 1 3 1	1 - - 1 - 1 - -	- 1 - -	1 2 3 1	1	18
VI y bi x w	- - - -	- 1 2 -	- 1 - - - - - -	1 - 1 -	2 1 - -	2	11

Discussion.

In crosses involving white alba or its derivatives, the crossed asci contain fewer germinable ascospores than the selfed asci (including the selfed white). However, the allele ratios within the crossed asci are not significantly different to 1 : 1.

Hence, the differential viability is not due to any of the alleles known to segregate. It could be due:
a) either to a chromosome rearrangement or to complementary genes, unlinked to any of the "markers", for which white alba has become differentiated since its isolation and/or
b) to cytoplasmic differences which reduce the viability of the ascospores in crossed asci.

Chromosome rearrangement e.g. a translocation involving chromosomes or chromosome parts, other than the ones where the "markers" are situated, would automatically cause a proportion of spores from crossed asci to be unbalanced, due to duplications or deficiencies, and therefore they would fail to germinate. The result would be similar if genetic differentiation had taken place, as complementary systems in the white alba strain and its derivatives, would be different from those of the wild type strain and its derivatives.

Cytoplasmic differentiation might also account for the results, provided a neucleus- cytoplasmic balance was involved which was different in the two groups of strains. Further work will be necessary to decide between the alternatives.

Section IV. Abnormal Asci

Introduction.

From genetical work with micro-organisms, using ascus analysis, it has been shown that in certain cases abnormal asci occur, as in yeast (Lindegren1949) and Bombardia (Zickler 1934). Some abnormal asci may have a normal complement of spores which produce colonies of phenotypes not according with Mendelian segregation and which cannot be readily accounted for by suppressors, modifying factors or polyploidy; other abnormal asci may not contain the expected number of spores. (Winge and Roberts 1950a).

It is important to distinguish between actual irregular segregations and survival of the products of meiosis in abnormal proportions due to secondary causes. Actual irregular segregations have been shown in Drosophila in the case of preferential segregation in Chromosome IV trisomics (Sturtevant 1936).

Considering firstly asci in which abnormal numbers of spores were found, Winge and Roberts (1950a) report yeast asci containing 5 and 6 ascospores. Ratios for maltose fermentation (M:m) of 2:3 and 3:1 were found respectively. The abnormal asci were explained by assuming that an additional mitotic division took place in the ascus, with subsequent degeneration of certain spores. That 8-spored asci occur normally in certain yeasts is shown by 8-spored Schizosaccharomyces octosporus. (Winge and Roberts 1950b). The isolation of

5 and 7-spored asci has been reported by Powell (1951) in S.cerevisiae.var.ellipsoideus. In A.nidulans, 16-spored asci have been isolated from heterozygous diploids (Roper 1952).

The expected number of spores may not be obtained in an ascus if anomalies in spore formation occur and fewer or more nuclei than normal are enclosed in a single ascospore. In these cases however, the overall allele ratios are as expected (Shear & Dodge 1927, Dowding 1931).

Zickler (1934) working with the ascomycete Bombardia lunata, heterozygous for rubiginosa (r^+/r), reported asci in which segregations of r^+ (wild type) and r occurred as 4:0, 3:1 and 1:3. Miss Mathieson (unpublished) working also on Bombardia lunata confirmed these results. Segregations were mainly of the 3:1 type and abnormal asci of these types were found with relative frequencies of $\frac{37}{8204}$ and $\frac{11}{9320}$ by Zickler and Miss Mathieson respectively. From further work, Miss Mathieson showed that two of the eight spores in each abnormal ascus represented a mutation from one allele to the other. The 3:1 and 1:3 segregation ratios obtained by Miss Mathieson indicate that mutation always occurred at the same stage of meiosis, i.e., during the first division; a mutation during the second meiotic division or the final mitotic division would give a 3:5 ratio.

Other cases of 3:1 and 4:0 ratios have been reported in Ustilago levis by Dickinson (1928 and 1931) and in work on maltose fermentation in yeast (Winge and Roberts 1948, 1950b).

Wülker (1935) found 2:2, 0:4 and 1:3 segregation ratios in N.sitophila, but as he used only one pair of markers, could not decide in favour of any specific theory. Dickinson was in favour of "conventional mendelian explanations" for his findings. Winge and Roberts explained their results by a system of three polymeric genes and in 1950 Winge demonstrated a fourth.

Unlike Winge, Lindegren (1949) sought to explain allele ratios, within one ascus, which differed from the normal by new suppositions, as cytoplasmic transfer and gene conversion. Conclusive evidence of the latter theories is difficult to obtain however and it is safer to explore all the possibilities before discarding interpretations based on present genetical knowledge.

Supposedly abnormal asci may be found as a result of faulty technique, as overlapping of generations (Winge and Roberts 1950b), or in mating mixtures containing residual haploid or self-diploidized cells (Fowell 1951). The use of a sufficient number of "markers" is necessary to guard against the latter.

Among 136 crossed asci which we have isolated from crosses of various strains of A.nidulans, 11 abnormal were found. The three main causes of abnormality were apparently due to:

1) mutation during the first meiotic division ($\frac{5}{136}$),

2) supernumerary divisions in the ascus ($\frac{2}{136}$), (plus a possible three more),

3) inclusion of 2 nuclei, representing two different products of meiosis, in the same ascospore ($\frac{0}{138}$).

Detection of Abnormal Asci.

On culturing, ascospores from a cross between two strains differing in colour "markers", give rise to colonies of different colours. For short, we shall call "yellow" an ascospore giving rise to a yellow colony, "green" an ascospore giving rise to a green colony and "white" an ascospore giving rise to a white colony.

Classifying by colour, any crossed ascus is abnormal which has:

a) more than 4 spores of any one colour;

b) more than 2 yellow or 2 green spores in a tricolour ascus.

Classifying by the nutritional characters used in the present work, any crossed ascus is abnormal in which each nutritional "marker" is represented in more, or less, than 4 ascospores.

Abnormal asci isolated.

In the course of work on ascus analysis in A.nidulans, of a total of 539 asci dissected, 401 were classifiable and of these, 136 were crossed asci. Of these crossed asci, 55 were fully classifiable as to type because they gave 7 or 8 germinable spores.

Among the 136 crossed asci, abnormal distribution of the segregating characters among the ascospores was found in various crosses (Table 18).

Table 18 Abnormal crossed asci isolated from A.nidulans.

Details of cross	Ascus No.	Total germinating ascospores per ascus	Segregation in the ascus
No.II y x w	70	4	3 yell:1 gn
" III y bi x w	83	5	4 gn BI : 1 wh BI
" IV y bi x +	37	7	5 yell bi 1 gn bi 1 (gn BI / yel bi) two types of mycelium
" "	4 and 30	7	3 yell bi 3 gn BI 1 (gn BI / yell bi) "
" "	44	7	2 yell bi 1 yell BI 2 gn bi 1 gn BI 1 (gn BI / yell BI) "
" VII y bi x w lys	164	2	1 wh BI LYS 1 (wh BI LYS / yell BI LYS) "
" XII y (light) x + (light)	48e	8	2 yell: 6 gn
" XIII y bi (medium) x + (light)	1h	6	1 yell bi light 2 yell bi medium 1 (yell bi light / gn BI light) " 1 gn BI light 1 gn BI dark
" "	20c	7	2 yell bi medium 2 yell BI light 2 gn BI light 1 gn BI medium
1st backcross of y bi x +	9	8	2 yell BI light 2 yell bi medium 2 gn bi dark 1 gn BI light 1 gn BI dark

Notation as used in tables of Crosses, see Appendix Part 1.

Light, medium and dark refer to background colour of colony from germinated ascospores. See Background colour variation.

Cross II. Ascus 70. In Cross II (2 genes segregating y/y and w/w), from the combination of yellow and green ascospores in Ascus 70 it appeared that the ascus was type III. A normal type III ascus should have 2 green, 2 yellow and 4 white ascospores and a type II ascus should have 4 white and 4 green ascospores. As no normal type of ascus in which there is a green ascospore should carry more than 2 yellow, we may assume that this is a type III ascus.

Cross IV. Ascus 37 and Cross XII. Ascus 48e. In crosses IV and XII/as only one colour gene (y/y) is segregating, crossed asci would be expected to show a 1:1 ratio for yellow/green. In both asci a 3:1 ratio was found, though in Ascus 37 it was for yellow/green and in Ascus 48e for green/yellow.

Cross III. Ascus 83. Five spores only germinated in Ascus 83 (Cross III) but they were all BI (biotin non-requiring) whereas at least one biotin-requiring spore was expected. As only five ascospores were present, it was impossible to tell whether the segregation of green and white was normal or not. As four green ascospores were present, it appeared that the ascus was type II.

Cross XIII. Ascus 20c. An excess of biotin-non-requiring ascospores was also found in ascus 20c. Ascospores are found in pairs in the ascus, so the non-germinated spore was presumably BI, making the ratio 6 BI ; 2 bi.

The underside of colonies from four spores of Ascus 20c

showed light background colour (see p 86.) and three (Nos 1, 2 and 7) showed medium, whereas in no other ascus had more than two spores with medium background colour been found. Spores 1 and 2, 3 and 4, and 5 and 6 were sister spores, but the corresponding sister spore to Number 7 was missing. This was unfortunate as it would have been a check. Misclassification of spore 7 as showing medium instead of dark background colour would explain the anomaly.

Cross IV. Asci 37, 4, 30, 44.
Cross VII. Ascus 164.
Cross XIII. Ascus 1h.

These six asci all exhibited an interesting phenomenon of one ascospore producing mycelium of two types; yellow and green. Seven spores germinated in all asci from Cross IV, whilst only six germinated in Ascus 1h.Cross XIII and two in Ascus 164. Cross VII.

1st backcross of y bi x +. Ascus 9. Details of this ascus are given in the section on background variation (p86.).

Possible causes of abnormal asci.

A segregation ratio of 3:1 was found in ascus 37 (Cross IV) for yellow:green, in ascus 48c (Cross XII) for green:yellow and in ascus 20c (Cross XIII) for BI:bi, on the assumption that in the latter case the missing ascospore of the fourth pair was biotin non-requiring. These ratios are similar to the 3:1 ratios obtained by Miss Mathieson (unpublished) on Bombardia, indicating the occurrence of mutation during the first meiotic

division. The mutation of Y to y is known to occur and probably that of y to Y takes place, but the back mutation of bi to BI has never been obtained, from extensive work by Roper (unpublished) nor by any other worker in the laboratory. It thus seems unlikely that back mutation is the cause of abnormal ascus 20c.

Cross III. Ascus 83. As only five out of the eight spores of this ascus were viable, a full analysis for biotin requirement was not possible. In crossed asci from this cross (y bi x w), green and white non-biotin requiring spores would only occur together in type III asci of the following constitution:

Cross y bi x w.

Type III ascus.	2 w Y BI	white - parental type	) Types
	2 W Y BI	green - recombinant	) isolated.
	2 W y bi	yellow- parental type	
	2 w y bi	white - recombinant	

Normally, only two green recombinants occur in type III asci but if an extra mitotic division occurred, four green recombinants would be formed. Subsequent degeneration of all but the five spores isolated may have taken place, similar to the situation described in yeast by Winge and Roberts (1950a).

Cross II Ascus 70. A similar situation may have arisen in Ascus 70, thus explaining the combination of more than two yellow ascospores with a green one, but the possibility of mutation Y to y cannot be excluded. With only four viable ascospore it is difficult to draw any definite conclusion.

Cross IV, VII and XIII. From the occurrence of ascospores

producing mycelium of two types (for short, bi-colour ascospores) it would appear that an irregularity in the distribution of nuclei took place; two of the eight, which resulted from a meiotic followed by a mitotic division of the diploid nucleus in the ascus primordium, being included in one ascospore, and none in another. The fact that only 7 ascospores germinated in the abnormal asci of this kind (Nos. 4, 30, 37 and 44 in Cross IV) supports this theory.

Ascospores cut out in such a way as to carry two out of the eight nuclei could only be detected in cases where the two nuclei were of unlike type, i.e. in a certain proportion of crossed asci but never in selfed asci. In this work, for obvious technical reasons, it was only possible to detect those cases in which two nuclei, unlike in colour "markers" were included in one spore. Their incidence must therefore probably be higher than is apparent from the number of "bi-colour" spores found, i.e. detectable di-karyotic spores.

Incidence of "di-karyotic" ascospores.

Di-karyotic spores are those which contain two kinds of nuclei.

The chance of nuclei of unlike type going together into one spore, and thus forming a colony containing two detectably different kinds of mycelium, varies according to the number of types of nuclei present in one ascus, e.g. in an ascus, segregating for Y/y, which contains four y and four Y products of the third division:

if n = the total number of nuclei produced at the third division

and r = the number of nuclei going together into one spore

then combinations of nCr = $\frac{n!}{(n-r)!r!}$

i.e. with 8 recovered nuclei,

$$nCr = \frac{8!}{6!\,2!}$$

$$= 28$$

Each y nucleus can form a detectable di-karyotic or "bi-colour" spore with any one of four Y nuclei.

∴ No. of possible yellow-green di-karyotic spores out of 28 combinations = 16

Incidence of " " " spores in an ascus where all 8 nuclei are recovered = $\frac{16}{28}$

$$= \frac{4}{7}$$

Similarly, in a tetra-type ascus, the proportion of detectable di-karyons $= \frac{6}{7}$

The probability of detecting the di-karyotic spores differs according to the number of recovered nuclei (ie. number of germinable ascospores + 1) in an ascus, as well as to the proportions of the two types of nuclei present, as shown in Table 19.

Table 19.

Combinations of Y and y nuclei in crossed bi-type asci with 6 to 8 recovered nuclei, showing proportion of detectable di-karyotic spores in each case.

No. of germinating ascospores.	No. of recovered nuclei.	Possible combinations of yellow and green nuclei.	Proportion of detectable di-karyotic spores.
7	8	4 yell + 4 gn	$\frac{4}{7}$
6	7	4 yell + 3 gn.	$\frac{4}{7}$
		3 yell + 4 gn.	$\frac{4}{7}$
5	6	3 yell + 3 gn.	$\frac{9}{15}$
		2 yell + 4 gn.	$\frac{8}{15}$
		4 yell + 2 gn.	$\frac{8}{15}$

Calculations on the assumption that only one di-karyotic spore per ascus occurs.

As varying total numbers of nuclei from 5, 6, 7 and 8 spored asci were found (see Table 19A) the mean proportion of

detectable di-karyotic spores was 56.6% and thus 43.4% of the di-karyotic spores were undetected.

From the results of ascus dissection of Crosses shown in Appendix I and considering only those crossed asci with 5, 6, 7 and 8 spores germinating, di-karyotic spores were found in Crosses IV (y bi x +) and XIII (y bi x +) see Table 19A. Among those crosses in which $y/_y$ was segregating, of a total of 207 green nuclei and 214 yellow, one green and one yellow went together in five cases, i.e. in five detectable cases. Assuming that spores containing two like products of the third division are formed in a similar fashion, a further 5 x .43 x 2 = 4.3 undetectable cases(yellow/yellow and green/green) would have arisen. The incidence of inclusion of two third division nuclei in one spore is thus $\frac{9.3}{421}$ x 100 = 2.2%

On the whole, this kind of abnormality is not frequent enough to cause trouble either in ascus analysis or in random sampling of ascospores, and this even without considering that four out of the nine abnormal asci obtained were found among the first twenty dissected in Cross IV: this suggests that some special conditions were operating in the early part of the work in Cross IV, which favoured the occurrence of this type of abnormality.

As regards the incidence of two di-karyotic spores in an ascus, it is obvious that :

1) they would only occur in asci with 6, 5, 4, 3 or 2 spores germinating,

2) only cases in which <u>two</u> yellow-green spores occurred would have been detected (if one yellow-green spore occurred the chances of another one occurring in the same ascus = $\frac{2}{15}$)

3) asci in which one yellow-green + one yellow-yellow or green-green spore occurred would not be distinguishable from asci in which a single yellow-green spore occurred.

As the incidence of <u>detectable</u> single di-karyotic spores was 2.2%, it is unlikely that any cases of two per ascus would have been found if the process were at random.

Table 19A.

Incidence of crossed asci, with 5 or more germinable ascospores, containing bi-colour ascospores, from various crosses.

Cross	Asci showing one bi-colour ascospore.	No. of crossed asci with spores germinating: 8	7	6	5	
I y x w	-	-	-	1^T	1^T	
II y x w	-	-	-	-	1	
III y bi x w	-	2	$3\binom{2D}{1T}$	$3\binom{2D}{1T}$	$4\binom{2D}{2T}$	
IV y bi x +	4(7)	13	5	2	1	
V y bi x w	-	-	-	1^T	-	
VI y bi x w	-	-	-	-	-	
VII y bi x w lys	-	-	-	2^T	1	
VIII + x w lys	-	-	-	-	-	
IX + x w lys	-	-	-	-	-	
X y bi x +	-	-	1	-	-	
XI y bi x +	-	-	-	-	2	
XII y (bt) x + (lt)	-	3	8	7	2	
XIII y bi (m) x + (lt)	1(6)	4	3	1	5	
XIV y bi x w lys	-	-	-	-	-	
Di-type asci	5	22^D	19^D	12^D	14^D	Total 72^D
Tetra-type asci	-	0	1^T	5^T	3^T	9
Total	5	22	20	17	17	81
Taking asci from crosses y x X	5	20	17	10	10	62
No. of yellow & green nuclei from these asci. Yellow	22	78	54	34	26	214
Green	17	82	53	26	24	207

Total nuclei = 421

(Details of crosses in Appendix Part I)

Numbers in brackets in second column indicate number of germinated ascospores.

T = tetra-type asci

D = di-type asci (All asci of this type unless otherwise marked)

Background colour variation.

When viewing an inverted plate, it was observed that colonies formed from platings of ascospores or conidia of certain crosses, differed in colour of the background. Colonies formed from dissected ascospores of crossed asci from certain crosses also showed "background colour" variation. Three types of background colour were distinguishable and they were referred to as "light", "medium" and "dark", e.g. Cross XIII Table N.
y bi (medium background colour) x + (light background colour).

From the first backcross of y bi x + , a crossed ascus was isolated in which the segregation of biotin requirements and conidial colour was as expected, (Plate 4), and four pairs of sister spores were apparent, but they were in 3:3:2 ratios for dark, light and medium background colour (Plate 5). In this ascus, spores 3 and 4, which were sister spores showed light and dark colour background, respectively.

The constitution of this abnormal ascus, Number 9, was as follows:-

Cross y bi (dark background) x + (light background)

	Spore Number.	Spore colour.	Biotin requirement.	Background colour.
Ascus 9.	(1	Green	bi	Dark
	(2	Green	bi	Dark
	(3	Green	BI	Light
	(4	Green	BI	Dark
	(5	Yellow	bi	Medium
	(6	Yellow	bi	Medium
	(7	Yellow	BI	Light
	(8	Yellow	BI	Light

(Brackets indicate sister spores).

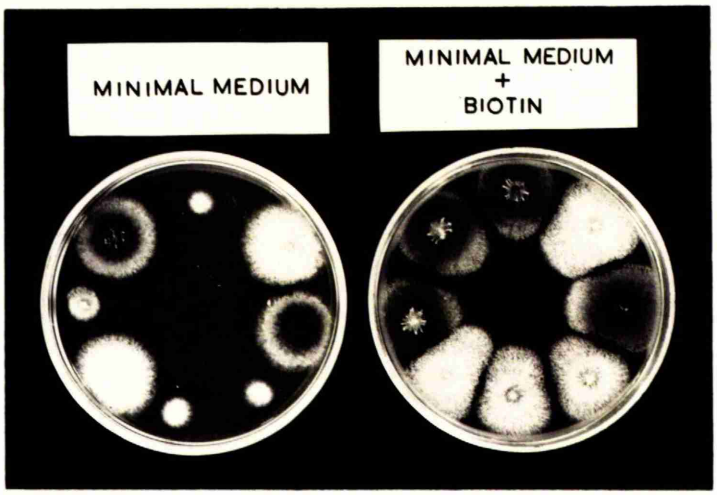


Plate 4. Segregation for $y/_{y}$ and $bi/_{BI}$ in Ascus 9, (a tetra-type ascus), isolated from the first backcross of y bi x + .

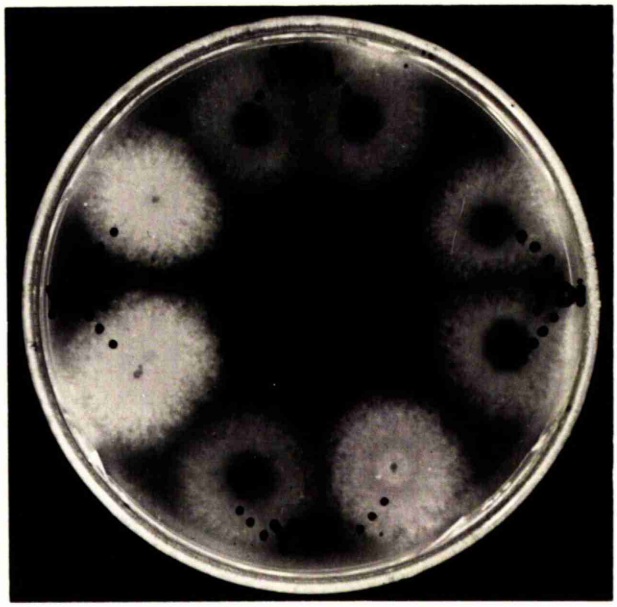


Plate 5. Pairs of monoascosporic cultures from Ascus 9, isolated from the first backcross of y bi x + : dark background from $\frac{5}{8}$ colonies, pair Y/BI shows one dark and one light.

As segregation of background colour was found in Ascus 9 and in other crossed asci showing background colour variation, experiments were made:

a) to investigate the genetic basis of background colour in Ascus 9 and various crosses made from its ascospores,

b) to discover whether background colour variation was easily classifiable and reliable and could be used as a "marker".

Henrard (1934) had reported a deep-red background colour produced where certain colonies of A.nidulans met, when plated on Raulin gelatine. A certain minimum depth of medium was necessary before the phenomenon was apparent and using Raulin agar the effect was less noticeable. A preliminary study was made on the effect and depth of medium on background colour variation.

Comparative effect of Complete medium and Minimal medium + biotin

Subcultures from the ascospore colonies of Ascus 9 were made onto plates containing complete medium and minimal medium with 0.005γ per ml. of biotin. Differentiation of background colour was increased using the minimal medium. It was noticed that the background colour of spores 5 and 6 was not as dark as that of spores 1, 2 and 4, and could be classified as medium dark or "medium" as it was more convenient to call it.

Effect of concentration of biotin and depth of medium.

10 and 20 ml. amounts of minimal medium, containing a total of 3γ and 30γ of biotin in each case, were poured into petri dishes and inoculated from the colonies of Ascus 9. The

background colour of the resultant colonies was examined after 2, 3 and 4 days at 37°C. Differentiation was not very clear after 2 days but was fully developed after 4 days.

Table 20. Cross W y bi (dark background) x + (light background)

Effect on background colour of media of different depths and containing different concentrations of biotin.

Total addition of biotin.	Thick medium (20 mls).	Conc. of biotin per ml.	Thin medium (10 mls).	Conc. of biotin per ml.
30 γ	4 dark.4 light (deep)	1.5 γ	2 dark.2 medium 4 light.	3.0 γ
3 γ	2 dark.2 medium. 4 light.	0.15γ	2 dark.2 medium. 4 light	0.3γ

These results show that with increase of biotin concentration or depth of agar, there is a shifting of background colour towards dark. With a total concentration of 1.5 γ biotin per ml. and thick medium, no colonies with medium background colour are differentiated. To obtain differentiation of the three background colours, concentrations of 0.15 to 0.3 γ/ml of biotin, or less, should be used in solid minimal medium.

As the optimal concentration of biotin, for growth of biotin-requiring strains, is 0.002 γ per ml. in minimal medium, it is unexpected that these concentrations well above the optimal should have affected the formation of background colour as they did.

Continuing the investigation on background colour, minimal medium containing 0.15 to 0.3 γ/ml. of biotin was used.

a) Genetic investigations on background colour variation.

Crosses were made between various ascospores of Ascus 9, to try and discover what genetic differences existed between them. Both ascus analysis and plating of a random sample of ascospores was carried out on two crosses but as the former method was lengthy and tedious, in subsequent crosses, only plating of ascospores was employed.

Table 21 shows that light x light gives only light whilst medium x light and dark x light gives light, medium and dark background colour. From Table N. Cross XIII (medium x light background colour) we see that a single ascus may contain ascospores showing:

a) dark and light background, in the ratio of 4:4 (Ascus 1d) or

b) dark:light:medium as 1:2:1 (Ascus 1e)

Not more than four light and/or four dark spores were found in any ascus showing dark or light spores, and except in Ascus 20c - which was probably abnormal (Table 18) - not more than two spores showed medium in any ascus in which this type appeared at all.

A Table, see Appendix. Table P, of the segregation of y/Y, $bi/_{BI}$ and background colour was made from the results of ascus analysis of Cross XIII. Table N.

The allele ratios of $bi/_{BI}$ and y/Y were not significantly different from 1:1 and recombination between the loci for y/Y and $bi/_{BI}$ was about 0.05. There was no evidence that

Table 21. Analysis of types of "background colour" of ascospore colonies obtained from various crosses of strains from Ascus 9. (p 86.)

Cross	Types of background colour					
	Ascus dissection			Plating of Ascospores		
	Light	Medium	Dark	Light	Medium	Dark
XII) y BI (light) (8) x Y BI (light) (3)	126 yell. 68 gn.	-	-	985 yellow 811 green	-	-
XIII) y bi (medium) (5) x Y BI (light) (3)	28 yell. 30 gn.	9 yell. 5 gn.	12 yell. 12 gn.	4 yellow 2 green	-	92 yellow 3 green
				REPEAT 42 yellow 1547 green	54 yellow - unclassifiable	38 yellow
y BI (light) (8) x Y bi (dark) (2)				9 yellow 27 green	14 yellow 26 green	6 yellow 10 green
Y BI (light) (3) x Y bi (dark) (2)				4 Classification very	588 difficult	6

N.B. In brackets, ascospore of Ascus 9 from which the strain was derived.

either type of background colour was linked to $y/_{Y}$ or $bi/_{BI}$.

Segregation ratios of 13:54:24 were obtained for medium light and dark background colour respectively. χ^2 for the hypothesis of a 1:2:1 ratio was 4.5 (p = 0.1 to 0.2).

Genetic constitution of Ascus 9.

Ascus 9 was obtained from a backcross of y bi (dark background) x (light background) and this y bi strain had originated from a crossed ascus from Cross VII y bi x w lys. Ascus 158 - spore 5. As the y bi stock strain showed light background colour, crossing with the w lys strain had evidently caused change in background colour.

As light, medium and dark background colour is found on crossing light x medium, it appears that this variation in background colour may be due to two genes, which can be called L_1 and L_2. As light x light background colour gives only light (Cross XII Table M) we can classify spore 3 (light) as L_1 L_2 and spores 5 and 6 (medium) as l_1 L_2. By deduction, the dark spores Nos. 1 and 2, will be l_1 l_2 and the remaining light spores, Nos 7 and 8, as L_1 l_2, with L_1 epistatic to l_2/L_2.

The genetic constitution of Ascus9from this reasoning would be therefore:

Cross: y bi (dark background) x + (light background

	Spore Number	Genotype	Phenotype
Ascus 9	(1	W Y bi l_1 l_2	dark green
	(2	W Y bi l_1 l_2	dark green
	(3	W Y BI L_1 ? L_2	light green
	(4	W Y BI	dark green
	(5	W y bi l_1 L_2	medium yellow
	(6	W y bi l_1 L_2	medium yellow
	(7	W y BI L_1 l_2	light yellow
	(8	W y BI L_1 l_2	light yellow

(Brackets indicate sister spores)

From results of ascus analysis, a ratio of 1:2:1 for medium: light: dark background colour has been found and the same ratio would hold for Ascus 9, except for Spore 4. As spores 3 and 4 were sister ascospores as regards colour and biotin requirements, it would appear that a mutation from light to dark had occurred in spore 4. If this were the case, a mutation at both loci would be needed to change L_1 L_2 to l_1 l_2. However, if spores 7 and 8 were of genotype L_1 L_2 and spores 3 and 4 of genotype l_1 l_2, a mutation, in spore 4, from L_1 to l_1 would give dark (l_1 l_2) instead of light background colour.

The following constitution of Ascus 9 therefore appears more probable:

Cross: y bi (dark background x + (light background)

Ascus 9.	Spore Number.	Genotype	Phenotype	
	(1	W Y bi l_1 l_2	dark	green
	(2	W Y bi l_1 l_2	dark	green
	(3	W Y BI L_1 l_2	light	green
	(4	W Y BI l_1 l_2(m)	dark	green
	(5	W y bi l_1 L_2	medium	yellow
	(6	W y bi l_1 L_2	medium	yellow
	(7	W y BI L_1 L_2	light	yellow
	(8	W y BI L_1 L_2	light	yellow

(Brackets indicate sister spores)
(m) = mutation from L_1 l_2

Conidia from colonies 3 and 4 of Ascus 9 were plated out to find whether there was any evidence of a high mutation rate of dark to light, or visa versa. No mutations were found when at least 4,000 conidia from each colony were tested.

The wild type strain (light background colour), from which Ascus 9 was derived, showed no mutants for dark background colour in 500 colonies and no change in background colour had previously been reported.

It is unlikely that spore 4 was a contaminant, as the green parent culture had a light background.

Background colour variation - Summary

From experiments carried out on background colour it appeared that variation (light, medium, dark) might be due to two genes, L_1 L_2, with L_1 epistatic to l_2/L_2. The differential effects of the various alleles and combinations of them on the phenotype, were very sensitive to environmental conditions (e.g., amount and type of medium) as well as to the residual genotype. There appeared to be no obvious genetic linkage or physiological correlation between background colour and y/y or bi/BI.

Light background colour could be detected easily but differentiation between dark and medium was often difficult. The typing of background colour of ascospores from a plating was often extremely difficult and in the Cross Y BI (light)x Ybi (dark), Table 21, classification was nearly impossible. For this reason, background colour was useless as a "visible" marker.

It appeared to be unprofitable to continue with further analysis of the products of crossing of various combinations of spores of exceptional Ascus 9, in which the two members of one pair of sister spores differed from one another in background colour.

Section V. Modality of crossing-over investigated by means of ascus analysis.

In Crosses with 2 or more genes segregating, ascospores from crossed asci can be analysed for parental and recombinant types. (see Table E Cross IV y bi x +). Recombinant types only are produced from 4-strand double crossovers. e.g., Cross IV Ascus 8.

Chromatid interference can be more readily detected using ascus analysis than from analysis of a random sample of spores.

Negative chiasma interference has been inferred from results of tetrad analysis on N.crassa (Lindegren and Lindegren 1937 and 1942, Whitehouse 1942). Using recombination frequencies, only in the house mouse is there some evidence for chromatid interference (Carter and Robinson 1952).

In A.nidulans the y and bi loci are linked and show 0.051 ± .004 recombination. In a cross involving these loci, approximately 10% of the crossed asci found are expected to give 4 out of 8 spores showing recombination between $y/_{y}$ and $bi/_{BI}$ and about 1% of crossed asci showing only recombinant types.

In Table D. Cross IV y bi x + , of a total of 27 crossed asci, 3 asci were found in which all the ascospores showed crossing-over between bi and y. These asci constitute 11% of the total. If they were genuine cases of 4-strand double crossovers they would be expected to constitute about 1%,

barring negative chromatid interference.

The abnormally high number of asci showing ascospores as those produced by 4-strand double crossovers may have been due:

a) to a rare event,

b) to a fault in technique,

c) to abnormalities in the development of these asci,

d) to an extremely high negative chromatid interference.

These asci were not distributed at random among the asci isolated but were found among the first 20 asci, of a total of 80. This favours supposition (b) rather than (a) or (d), and no further evidence of a high proportion of 4-strand double-crossovers was found again in a similar cross (Table O. Cross XIII y bi x +).

A third possible explanation is that these asci were abnormal asci in which crossover nuclei only had survived and undergone an extra mitotic division.

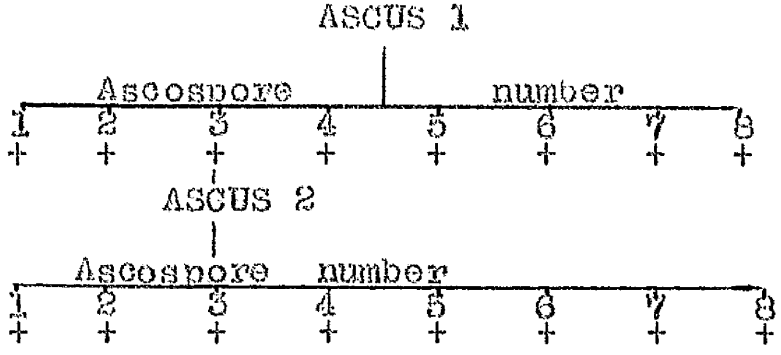
Section VI. Homothallism.

Homothallism may be defined as "the ability of a strain, whose nuclei are derived from a single haploid nucleus, to go through a complete sexual cycle".

Schwartz (1928) affirmed that four strains of A.nidulans which he studied were homothallic but it was not till 1934, that Henrard gave proof of what he termed "sexual homothallism", by growing single-spore isolates of the fifteen cultures of A.nidulans that he used and finding that they all produced perithecia.

The three original strains used in the Dept. of Genetics, Glasgow, from which all others were derived, (+, w, s_d) together with a yellow spore mutant (y) were tested for homothallism, by dissecting eight ascospores from a single ascus of each strain, (derived from a single-ascospore culture) examining the single-ascospore strains thus established for production of perithecia, and again dissecting eight ascospores from an ascus of one of these single-ascospore strains.

Method of ascus-dissection employed for each strain.



\+ = fertile strain established from single-ascospore culture.

In the case of the wild type strain, only 7 out of 8 ascospores germinated from the first isolation, but the other strains gave 8 out of 8 single-ascospore cultures from each ascus.

The majority of mutant and recombinant strains derived from the four strains tested were still self-fertile after single-ascospore or single-conidium isolation.

Homothallism was therefore established in the four strains tested, from which all the others in this laboratory have been derived, as single-ascospore cultures went through two successive complete sexual cycles and the majority of derivatives of the strains remained self-fertile, even after single-conidium or single-ascospore isolation.

SUMMARY.

From investigations of individual asci, using a special technique of dissection developed for Aspergillus nidulans, information was gathered that was not obtainable from examining a random sample of ascospores.

Firstly, it was possible to detect abnormal asci and the causes of abnormality were apparently due to:

(1) mutation during the first meiotic division,

(2) supernumerary divisions in the ascus,

(3) inclusion of two nuclei in one ascospore.

It was only possible to detect cases of the latter in ascospores in which two nuclei unlike in colour "markers" were included, as these gave rise to colonies with two detectably different kinds of mycelium.

Among those crosses in which y/Y was segregating and considering asci with 5, 6, 7 and 8 spores germinating, five asci showing one bi-colour ascospore were found.

The incidence of two third division nuclei in one ascospore was 2%. No cases of two detectable di-karyons per ascus were found.

In Cross IV (y bi x +), four of the 22 crossed asci showed inclusion of two genetically different nuclei in one ascospore and these four were among the first twenty asci dissected. This suggests some special conditions were operating in the early part of the work which favoured the occurrence of this type of abnormality.

An insufficient number of ascospores germinating and the use of too few "markers" rendered exact interpretation of results in (1) and (2) difficult.

Although no actual cases of asci containing more than eight ascospores were found during work on ascus dissection of A.nidulans, their occurrence has been reported by Roper (1952) in diploid strains.

From an investigation on background colour variation it was found that variation (light, medium, dark) was probably due to two genes, L_1, L_2, with L_1 epistatic to l_2/L_2. Unfortunately background colour was useless as a "visible" marker, due to difficulty of unequivocal classification.

From results of ascus dissection on four strains of A.nidulans, homothallism was firmly established: single-ascospore cultures went through two successive complete sexual cycles and the majority of the strains derived from these four remained self-fertile on culturing, even though derived from a single conidium or ascospore.

Data from ascus analysis showed that germinability was poor in all crosses of white alba (or a derivative) with wild type (or a derivative). As white alba had been isolated from wild type in 1936 and kept genetically isolated ever since, genetic and/or cytoplasmic differentiation could have occurred. The poor germinability of spores from crossed asci was not differential.

By varying the density of plating and the proportions of the conidia of two strains used, it was found that there was a relationship between the proportion of the two kinds of nuclei and the percentage of crossed asci formed. Within limits, departure from the theoretically optimal proportions of 50:50 could be compensated for by an increase in the density of a plating.

ABBREVIATIONS USED IN TABLES

self	=	selfed ascus.
cr.	=	crossed ascus.
?	=	type of ascus not identifiable. Discussed in text p. 54.
*	=	abnormal asci. Discussed in text. (Part I. Section IV).
yell	=	yellow
wh	=	white
gn	=	green

White strains may be of genotype y w or Y w.

Lt	=	light background colour of colony
M	=	medium " " " "
D	=	dark " " " "

Tables A - Q found in the appendix of Part I.

Tables R and S found in the appendix of Part II.

SUMMARY OF CROSSES

Table		Cross	Asci dissected				
			Total	Classifiable	Non-classifiable	of crossed origin No.	%
A	I.	y x w	53	19	34	5	26
B	II.	y x w	64	49	15	16	33
C	III.	y bi x w	85	60	25	17	28
D	IV.	y bi x +	79	79	0	27	34
F	V.	y bi x w	18	9	9	3	33
G	VI.	y bi x w	11	4	7	1	25
H	VII.	y bi x w lys	82	64	18	18	27
I	VIII.	w lys x +	13	4	9	1	25
J	IX.	w lys x +	8	3	5	0	0
K	X.	y bi x +	9	7	2	1	14
L	XI.	y bi x +	26	23	3	2	9
M	XII.	y (light) x + (light)	29	29	0	21	) Several) asci
N	XIII.	y bi (medium) x + (light)	32	23	9	15	) sampled) per
Q	XIV.	y bi x w lys	30	20	10	9	) perith-) ecium.
			539	393	146	136	

y and bi are linked, 5.1 units apart. (Pontecorvo 1952a)

Ascospores from crossed asci tested for all characters segregating: those from selfed asci scored only for $w/_{w}$ and $y/_{y}$, except in Cross VII.

Crosses with:

1 gene segregating	2 genes segregating	3 genes segregating	4 genes segregating
XII. y/y	I. $y/_{y}$, $w/_{w}$	III. $y/_{y}$, $w/_{w}$, $bi/_{BI}$	VII. $y/_{y}$, $w/_{w}$, $bi/_{BI}$, $lys/_{LYS}$
	II. $y/_{y}$, $w/_{w}$	V. $y/_{y}$, $w/_{w}$, $bi/_{BI}$	XIV. $y/_{y}$, $w/_{w}$, $bi/_{BI}$, $lys/_{LYS}$
	IV. $y/_{y}$, $bi/_{BI}$	VI. $y/_{y}$, $w/_{w}$, $bi/_{BI}$	
	VIII. $w/_{w}$, $lys/_{LYS}$		
	IX. $w/_{w}$, $lys/_{LYS}$		
	X. $y/_{y}$, $bi/_{BI}$		
	XI. $y/_{y}$, $bi/_{BI}$		
	XIII. $y/_{y}$, $bi/_{BI}$		

Abnormal Asci.

Number of Cross	Number of abnormal ascus.
II	70
III	83
IV	4, 30, 37, 44
VII	164
XII	48e
XIII	1h, 20c

1st backcross of y bi x + 9

Cross I y × w (y/y and w/W segregating)

"Mixed-inoculum" cross prepared 10/10/49, ascus-dissection commenced 31/10/49, finished 10/11/49.

Asci all taken from one dish.

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
1	Self yell	yell	yell	yell	yell	yell	yell	yell	—
2	Self yell	yell	yell	yell	yell	yell	yell	—	—
3	crossed	wh	wh	yell	gn	gn	—	—	—
4	Self yell	yell	yell	yell	yell	yell	—	—	—
5	Self yell	yell	yell	yell	yell	yell	yell	—	—
6	?	yell	—	—	—	—	—	—	—
7	Self yell	yell	yell	yell	yell	yell	—	—	—
8	?	yell	yell	yell	yell	—	—	—	—
9	?	yell	yell	yell	yell	—	—	—	—
10	?	wh	wh	—	—	—	—	—	—
11	?	yell	yell	yell	—	—	—	—	—
12	?	yell	yell	yell	yell	—	—	—	—
13	?	—	—	—	—	—	—	—	—
14	?	—	—	—	—	—	—	—	—
15	?	—	—	—	—	—	—	—	—
16	?	yell	yell	yell	—	—	—	—	—
17	?	yell	—	—	—	—	—	—	—
18	?	yell	—	—	—	—	—	—	—
19	?	yell	yell	yell	yell	—	—	—	—
20	?	—	—	—	—	—	—	—	—
21	?	—	—	—	—	—	—	—	—
22	?	wh	—	—	—	—	—	—	—
23	Self yell	yell	yell	yell	yell	yell	yell	—	—
24	?	yell	yell	—	—	—	—	—	—
25	?	yell	yell	—	—	—	—	—	—
26	Self yell	yell	yell	yell	yell	yell	yell	yell	—
27	?	—	—	—	—	—	—	—	—
28	?	—	—	—	—	—	—	—	—
29	?	yell	yell	yell	yell	—	—	—	—
30	?	yell	—	—	—	—	—	—	—

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
31	self yell	yell	yell	yell	yell	yell	yell	yell	yell
32	?	yell	yell	–	–	–	–	–	–
33	?	yell	–	–	–	–	–	–	–
34	crossed	wh	wh	wh	yell	yell	gn	–	–
35	?	yell	yell	yell	yell	–	–	–	–
36	?	yell	yell	yell	yell	–	–	–	–
37	self yell	yell	yell	yell	yell	yell	yell	yell	–
38	self yell	yell	yell	yell	yell	yell	yell	yell	–
39	crossed	gn	–	–	–	–	–	–	–
40	self yell	yell	yell	yell	yell	yell	yell	yell	–
41	?	–	–	–	–	–	–	–	–
42	?	wh	wh	–	–	–	–	–	–
43	self yell	yell	yell	yell	yell	yell	yell	yell	yell
44	crossed	wh	wh	yell	–	–	–	–	–
45	?	yell	yell	yell	yell	–	–	–	–
46	Crossed	wh	yell	yell	–	–	–	–	–
47	?	yell	yell	yell	–	–	–	–	–
48	?	yell	yell	yell	–	–	–	–	–
49	?	wh	–	–	–	–	–	–	–
50	self yell	yell	yell	yell	yell	yell	yell	–	–
51	self yell	yell	yell	yell	yell	yell	yell	yell	
52	?	yell	yell	yell	–	–	–	–	–
53	?	–	–	–	–	–	–	–	–

SUMMARY

Types of asci:		Total	Asci with spores germinating.								Total
			0	1	2	3	4	5	6	7	8	
classifiable:	selfed yellow	19		–	–	–	–	2	4	5	3	14
	selfed white			–	–	–	–	–	–	–	–	–
	crossed			1	–	2	–	1	1	–	–	5
non-classifiable:	yellow	34		5	3	5	8	–	–	–	–	21
	white			2	2	–	–	–	–	–	–	4
	no germination		9									9
Total tested		53										

Cross II y × w (y/y and w/w segregating.)

"Mixed-inoculum" cross prepared 27/10/49, ascus-dissection commenced 11/11/49, finished 30/11/49.

Asci all taken from one dish.

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
54	self wh	wh	wh	wh	wh	wh	wh	—	—
55	self wh	wh	wh	wh	wh	wh	wh	wh	—
56	self wh	wh	wh	wh	wh	wh	wh	—	—
57	crossed	wh	wh	gn	gn	—	—	—	—
58	?	wh	wh	—	—	—	—	—	—
59	self wh	wh	wh	wh	wh	wh	wh	—	—
60	crossed	wh	gn	gn	—	—	—	—	—
61	self wh	wh	wh	wh	wh	wh	wh	wh	—
62	self wh	wh	wh	wh	wh	wh	wh	—	—
63	self wh	wh	wh	wh	wh	wh	wh	—	—
64	?	wh	wh	wh	wh	—	—	—	—
65	self wh	wh	wh	wh	wh	wh	wh	wh	wh
66	?	wh	wh	—	—	—	—	—	—
67	?	wh	wh	—	—	—	—	—	—
68	crossed	wh	gn	—	—	—	—	—	—
69	self wh	wh	wh	wh	wh	wh	wh	wh	wh
70	crossed *	yell	yell	yell	gn	—	—	—	—
71	crossed	wh	yell	gn	—	—	—	—	—
72	self wh	wh	wh	wh	wh	wh	wh	wh	wh
73	?	wh	—	—	—	—	—	—	—
74	?	yell	—	—	—	—	—	—	—
75	self wh	wh	wh	wh	wh	wh	wh	wh	—
76	crossed	wh	wh	gn	gn	—	—	—	—
77	crossed	wh	wh	yell	—	—	—	—	—
78	self wh	wh	wh	wh	wh	wh	wh	—	—
79	self wh	wh	wh	wh	wh	wh	wh	wh	—
80	self wh	wh	wh	wh	wh	wh	wh	wh	—
81	self wh	wh	wh	wh	wh	wh	wh	wh	—
82	self wh	wh	wh	wh	wh	wh	wh	—	—
83	self wh	wh	wh	wh	wh	wh	wh	wh	wh

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
84	?	wh	–	–	–	–	–	–	–
85	?	wh	wh	wh	–	–	–	–	–
86	crossed	yell	yell	gn	–	–	–	–	–
87	?	yell	yell	–	–	–	–	–	–
88	self wh	wh	wh	wh	wh	wh	–	–	–
89	?	yell	–	–	–	–	–	–	–
90	crossed	yell	gn	gn	–	–	–	–	–
91	crossed	wh	wh	yell	–	–	–	–	–
92	self wh	wh	wh	wh	wh	wh	wh	wh	–
93	crossed	wh	yell	yell	yell	yell	–	–	–
94	crossed	wh	yell	yell	gn	–	–	–	–
95	?	–	–	–	–	–	–	–	–
96	crossed	wh	gn	gn	–	–	–	–	–
97	self wh	wh	wh	wh	wh	wh	wh	wh	wh
98	crossed	wh	gn	gn	–	–	–	–	–
99	self wh	wh	wh	wh	wh	wh	wh	–	–
100	self wh	wh	wh	wh	wh	wh	wh	wh	–
101	self wh	wh	wh	wh	wh	wh	wh	wh	wh
102	?	wh	wh	wh	–	–	–	–	–
103	self wh	wh	wh	wh	wh	wh	wh	wh	–
104	?	wh	wh	–	–	–	–	–	–
105	self wh	wh	wh	wh	wh	wh	wh	–	–
106	self yell	yell	yell	yell	yell	yell	yell	–	–
107	crossed	wh	wh	gn	–	–	–	–	–
108	self yell	yell	yell	yell	yell	yell	yell	–	–
109	?	wh	wh	–	–	–	–	–	–
110	crossed	wh	wh	wh	gn	–	–	–	–
111	?	wh	wh	wh	wh	–	–	–	–
112	self wh	wh	wh	wh	wh	wh	wh	wh	–
113	self wh	wh	wh	wh	wh	wh	wh	wh	–
114	self yell	yell	yell	yell	yell	yell	yell	yell	–
115	self wh	wh	wh	wh	wh	wh	wh	–	–
116	self wh	wh	wh	wh	wh	wh	–	–	–
117	?	wh	wh	–	–	–	–	–	–

Cross II (contd.)

SUMMARY

Types of asci:		Total	Asci with ascospores germinating.								
			0	1	2	3	4	5	6	7	8	Total
classifiable:	selfed yellow	48		–	–	–	–	–	2	1	–	3
	selfed white			–	–	–	–	2	10	11	6	29
	crossed			–	1	9	5	1	–	–	–	16
non-classifiable:	yellow	16		2	1	–	–	–	–	–	–	3
	white			2	6	2	2	–	–	–	–	12
	no germination		1									1
Total tested		64										

Cross III y bi × w (y/y, w/w and bi/Bi segregating.)

"Mixed-inoculum" cross prepared 15/12/49, ascus-dissection commenced 9/1/50, finished 2/3/50.

Asci all taken from one dish

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
1	?	yell	—	—	—	—	—	—	—
2	self wh	wh	wh	wh	wh	wh	wh	wh	—
3	self wh	wh	wh	wh	wh	wh	wh	wh	—
4	?	wh	wh	wh	wh	—	—	—	—
5	?	wh	wh	wh	—	—	—	—	—
6	crossed	wh Bi	wh Bi	wh Bi	wh Bi	yell bi	yell bi	yell bi	yell bi
7	crossed	wh (?)	wh (?)	wh (?)	wh (?)	yell (?)	gn (?)	gn (?)	—
8	self wh	wh	wh	wh	wh	wh	wh	wh	wh
9	self wh	wh	wh	wh	wh	wh	wh	—	—
10	?	—	—	—	—	—	—	—	—
11	self wh	wh	wh	wh	wh	wh	wh	wh	—
12	self wh	wh	wh	wh	wh	wh	wh	wh	wh
13	crossed	wh (?)	yell (?)	yell (?)	—	—	—	—	—
14	self yell	yell	yell	yell	yell	yell	—	—	—
15	?	yell	yell	yell	yell	—	—	—	—
16	self wh	wh	wh	wh	wh	wh	wh	wh	—
17	self wh	wh	wh	wh	wh	wh	wh	—	—
18	self wh	wh	wh	wh	wh	wh	wh	wh	—
19	self wh	wh	wh	wh	wh	wh	wh	—	—
20	self yell	yell	yell	yell	yell	yell	—	—	—
21	self wh	wh	wh	wh	wh	wh	wh	wh	wh
22	self wh	wh	wh	wh	wh	wh	—	—	—
23	self wh	wh	wh	wh	wh	wh	—	—	—
24	?	wh	wh	wh	wh	—	—	—	—
25	crossed	wh bi	wh bi	wh bi	wh bi	gn Bi	gn Bi	gn Bi	gn Bi
26	self wh	wh	wh	wh	wh	wh	wh	—	—
27	?	wh	—	—	—	—	—	—	—
28	self wh	wh	wh	wh	wh	wh	wh	wh	—
29	self wh	wh	wh	wh	wh	wh	wh	wh	—
30	?	wh	wh	—	—	—	—	—	—

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
31	crossed	wh Bi	wh Bi	wh Bi	wh Bi	yell bi	yell bi	—	—
32	self wh	wh	wh	wh	wh	wh	—	—	—
33	self wh	wh	wh	wh	wh	wh	wh	wh	—
34	self wh	wh	wh	wh	wh	wh	—	—	—
35	?	wh	wh	wh	wh	—	—	—	—
36	crossed	wh Bi	wh Bi	wh bi	yell bi	yell bi	qn Bi	—	—
37	self yell	yell	yell	yell	yell	yell	yell	—	—
38	crossed	wh bi	wh bi	wh bi	qn Bi	qn Bi	qn Bi	qn Bi	—
39	self wh	wh	wh	wh	wh	wh	wh	wh	—
40	crossed	wh bi	wh bi	wh bi	wh bi	qn Bi	—	—	—
41	self wh	wh	wh	wh	wh	wh	wh	wh	wh
42	self wh	wh	wh	wh	wh	wh	wh	wh	wh
43	?	wh	wh	wh	wh	—	—	—	—
44	self wh	wh	wh	wh	wh	wh	—	—	—
45	self wh	wh	wh	wh	wh	wh	wh	—	—
46	?	yell	yell	yell	yell	—	—	—	—
47	crossed	wh Bi	wh Bi	wh Bi	wh Bi	yell bi	yell bi	yell bi	—
48	crossed	wh Bi	wh Bi	yell bi	yell bi	—	—	—	—
49	self wh	wh	wh	wh	wh	wh	wh	—	—
50	self wh	wh	wh	wh	wh	wh	wh	wh	—
51	?	yell	—	—	—	—	—	—	—
52	crossed	wh Bi	yell bi	yell bi	—	—	—	—	—
53	crossed	wh Bi	wh Bi	qn bi	—	—	—	—	—
54	?	wh	wh	wh	—	—	—	—	—
55	?	wh	wh	—	—	—	—	—	—
56	?	wh	wh	wh	wh	—	—	—	—
57	self wh	wh	wh	wh	wh	wh	—	—	—
58	crossed	wh bi	yell bi	qn Bi	qn Bi	—	—	—	—
59	?	wh	wh	wh	—	—	—	—	—
60	?	wh	wh	wh	wh	—	—	—	—
61	self wh	wh	wh	wh	wh	wh	wh	wh	—
62	self wh	wh	wh	wh	wh	wh	wh	wh	—
63	self wh	wh	wh	wh	wh	wh	wh	wh	—
64	crossed	wh bi	wh bi	wh bi	qn Bi	qn Bi	—	—	—

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
65	self wh	wh	wh	wh	wh	wh	wh	wh	—
66	?	wh	wh	wh	—	—	—	—	—
67	self wh	wh	wh	wh	wh	wh	wh	—	—
68	self wh	wh	wh	wh	wh	wh	—	—	—
69	self wh	wh	wh	wh	wh	wh	wh	wh	wh
70	self wh	wh	wh	wh	wh	wh	wh	wh	—
71	?	yell	yell	—	—	—	—	—	—
72	?	yell	yell	yell	—	—	—	—	—
73	self wh	wh	wh	wh	wh	wh	—	—	—
74	?	yell	yell	—	—	—	—	—	—
75	?	wh	wh	wh	wh	—	—	—	—
76	?	wh	wh	wh	—	—	—	—	—
77	?	wh	wh	—	—	—	—	—	—
78	?	wh	wh	wh	wh	—	—	—	—
79	self wh	wh	wh	wh	wh	wh	—	—	—
80	crossed	wh Bl	wh Bl	yell bi	gn Bl	gn Bl	—	—	—
81	self wh	wh	wh	wh	wh	wh	wh	—	—
82	crossed	wh Bl	wh Bl	wh Bl	wh Bl	yell bi	yell bi	—	—
83	crossed *	wh Bl	gn Bl	gn Bl	gn Bl	gn Bl	—	—	—
84	self wh	wh	wh	wh	wh	wh	—	—	—
85	self wh	wh	wh	wh	wh	wh	wh	wh	wh

SUMMARY

Types of asci		Total	Asci with spores germinating.								Total
			0	1	2	3	4	5	6	7	8	
classifiable	selfed yellow	60		—	—	—	—	2	1	—	—	3
	selfed white			—	—	—	—	10	8	15	7	40
	crossed			—	—	3	2	4	3	3	2	17
non-classifiable	yellow	25		2	2	1	2	—	—	—	—	7
	white			1	3	5	8	—	—	—	—	17
	no germination		1									1
Total tested		85										

Cross IV y bi x + (y/y and bi/Bi segregating)

"Mixed - inoculum" cross prepared 8/2/50, ascus - dissection commenced 7/3/50, finished 26/4/50.

Asci all taken from one dish

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No	Type	1	2	3	4	5	6	7	8
1	self yell	yell	yell	yell	yell	yell	yell	yell	yell
2	crossed	yell Bl	yell Bl	yell Bl	yell Bl	gn bi	gn bi	gn bi	gn bi
3	self gn	gn	gn	gn	gn	gn	gn	—	—
4	crossed *	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	{gn Bl / yell bi}	—
5	self yell	yell	yell	yell	yell	yell	yell	yell	yell
6	self gn	gn	gn	gn	gn	gn	gn	gn	gn
7	self yell	yell	yell	yell	yell	yell	yell	yell	yell
8	crossed	yell Bl	yell Bl	yell Bl	yell Bl	gn bi	gn bi	gn bi	gn bi
9	self yell	yell	yell	yell	yell	yell	yell	yell	yell
10	self yell	yell	yell	yell	yell	yell	yell	yell	—
11	self yell	yell	yell	yell	yell	yell	yell	yell	yell
12	self gn	gn	gn	gn	gn	gn	gn	—	—
13	self yell	yell	yell	yell	yell	yell	yell	yell	—
14	crossed	yell bi	gn Bl	gn Bl	—	—	—	—	—
15	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
16	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
17	self yell	yell	yell	yell	yell	yell	yell	yell	yell
18	crossed	yell bi	yell bi	yell Bl	gn bi	gn Bl	gn Bl	—	—
19	self gn	gn	gn	gn	gn	gn	gn	gn	gn
20	crossed	yell Bl	yell Bl	yell Bl	gn bi	gn bi	gn bi	gn bi	—
21	self gn	gn	gn	gn	gn	gn	gn	gn	—
22	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
23	self yell	yell	yell	yell	yell	yell	yell	yell	yell
24	self gn	gn	gn	gn	gn	gn	gn	gn	gn
25	self yell	yell	yell	yell	yell	yell	yell	yell	yell
26	self gn	gn	gn	gn	gn	gn	gn	gn	—
27	self yell	yell	yell	yell	yell	yell	yell	yell	yell
28	self yell	yell	yell	yell	yell	yell	yell	yell	yell
29	self yell	yell	yell	yell	yell	yell	yell	yell	yell
30	crossed *	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	{gn Bl / yell bi}	—

ASCUS NUMBER and TYPE of ASCOSPORES.

No.	Type	1	2	3	4	5	6	7	8
31	self gn	gn	gn	gn	gn	gn	gn	gn	gn
32	self gn	gn	gn	gn	gn	gn	—	—	—
33	self yell	yell	yell	yell	yell	yell	yell	yell	yell
34	crossed	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl	—
35	self yell	yell	yell	yell	yell	yell	yell	yell	—
36	self yell	yell	yell	yell	yell	yell	yell	—	—
37	crossed *	yell bi	yell bi	yell bi	yell bi	yell bi	gn Bl	{gn Bl / yell bi}	—
38	self yell	yell	yell	yell	yell	yell	yell	yell	yell
39	crossed	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	—	—
40	discarded								
41	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
42	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	—
43	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	—
44	crossed *	yell bi	yell bi	yell Bl	gn bi	gn bi	gn Bl	{gn Bl / yell Bl}	—
45	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
46	self yell	yell	yell	yell	yell	yell	yell	yell	yell
47	self yell	yell	yell	yell	yell	yell	yell	yell	yell
48	self yell	yell	yell	yell	yell	yell	yell	yell	yell
49	self gn	gn	gn	gn	gn	gn	gn	gn	gn
50	self yell	yell	yell	yell	yell	yell	yell	yell	—
51	self gn	gn	gn	gn	gn	gn	gn	—	—
52	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
53	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
54	self gn	gn	gn	gn	gn	gn	gn	gn	gn
55	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
56	self gn	gn	gn	gn	gn	gn	gn	gn	gn
57	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
58	self gn	gn	gn	gn	gn	gn	gn	gn	—
59	crossed	yell bi	yell bi	gn Bl	gn Bl	—	—	—	—
60	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
61	crossed	yell bi	yell bi	yell bi	gn Bl	gn Bl	—	—	—
62	self yell	yell	yell	yell	yell	yell	yell	yell	—
63	self yell	yell	yell	yell	yell	yell	yell	—	—
64	self yell	yell	yell	yell	yell	yell	yell	yell	yell

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
65	self yell	yell	yell	yell	yell	yell	yell	yell	—
66	self yell	yell	yell	yell	yell	yell	yell	yell	yell
67	crossed	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl	—
68	self gn	gn	gn	gn	gn	gn	gn	gn	—
69	self yell	yell	yell	yell	yell	yell	yell	yell	yell
70	self gn	gn	gn	gn	gn	gn	gn	gn	gn
71	self yell	yell	yell	yell	yell	yell	yell	yell	yell
72	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	gn Bl	gn Bl	gn Bl
73	self gn	gn	gn	gn	gn	gn	gn	gn	—
74	self gn	gn	gn	gn	gn	gn	gn	gn	gn
75	self yell	yell	yell	yell	yell	yell	yell	yell	yell
76	self gn	gn	gn	gn	gn	gn	gn	gn	gn
77	self yell	yell	yell	yell	yell	yell	yell	yell	—
78	self gn	gn	gn	gn	gn	gn	gn	gn	—
79	self yell	yell	yell	yell	yell	yell	yell	yell	yell
80	self yell	yell	yell	yell	yell	yell	yell	yell	yell

SUMMARY

Types of asci:		Total	Asci with ascospores germinating.								
			0	1	2	3	4	5	6	7	8	Total
classifiable:	selfed yellows			—	—	—	—	—	2	7	23	32
	selfed green	79		—	—	—	—	1	3	6	10	20
	crossed			—	—	1	1	1	2	9	13	27
non-classifiable:	yellows			—	—	—	—	—	—	—	—	—
	green	—		—	—	—	—	—	—	—	—	—
	no germination		—									—
Total tested		79										

Table E. Cross IV. y bi x + ($y/_{y}$ and $bi/_{BI}$ segregating)

Analysis of ascospores from crossed asci.

D = ascus showing a 4-strand double cross-over

S = ascus showing a single cross-over.

Ascus No.	Types of ascospores.			
	Parental		Recombinant	
	y bi	Y BI	y BI	Y bi
2. D	0	0	4	4
4	4	4	0	0
8 D	0	0	4	4
14	1	2	0	0
15	4	4	0	0
16	4	4	0	0
18 S	2	2	1	1
20. D	0	0	3	4
22	4	4	0	0
30	4	4	0	0
34	3	4	0	0
37	6	2	0	0
39	3	3	0	0
41	4	4	0	0
42	4	3	0	0
43	4	3	0	0
44 S	2	2	2	2
45	4	4	0	0
52	4	4	0	0
53	4	4	0	0
55	4	4	0	0
57	4	4	0	0
59	2	2	0	0
60	4	4	0	0
61	3	3	0	0
67	3	4	0	0
72	4	4	0	0
Total	85	82	14	15

Total ascospores of parental types = 167 % recombinants = $\frac{29}{196} \times 100 = 14.8$

Total ascospores of recombinant " = 29

TABLE F.

Cross V y bi × w (y/y, w/w and bi/Bl segregating.)

"Mixed-inoculum" cross prepared 5/4/50, ascus-dissection commenced 3/5/50; finished 16/5/50.

Asci all taken from one dish.

(Initial ratio of ybi : w as 1:3)

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
1	?	wh	wh	–	–	–	–	–	–
2	crossed	gn –	–	–	–	–	–	–	–
3	?	wh	wh	wh	–	–	–	–	–
4	self wh	wh	wh	wh	wh	wh	wh	wh	–
5	self wh	wh	wh	wh	wh	wh	wh	wh	–
6	self wh	wh	wh	wh	wh	wh	wh	wh	wh
7	self wh	wh	wh	wh	wh	wh	wh	wh	–
8	?	–	–	–	–	–	–	–	–
9	crossed	wh bi	wh Bl	wh Bl	yell bi	yell bi	gn Bl	–	–
10	?	wh	wh	wh	–	–	–	–	–
11	crossed	wh bi	wh bi	wh Bl	gn Bl	–	–	–	–
12	?	wh	wh	wh	wh	–	–	–	–
13	self wh	wh	wh	wh	wh	wh	wh	–	–
14	?	wh	wh	wh	–	–	–	–	–
15	self wh	wh	wh	wh	wh	wh	–	–	–
16	?	wh	–	–	–	–	–	–	–
17	?	wh	wh	–	–	–	–	–	–
18	?	yell	yell	–	–	–	–	–	–

SUMMARY

Types of asci:		Total	Asci with ascospores germinating.								
			0	1	2	3	4	5	6	7	8	Total
classifiable:	selfed yellow	9		–	–	–	–	–	–	–	–	–
	selfed white			–	–	–	–	1	1	3	1	6
	crossed			1	–	–	1	–	1	–	–	3
non-classifiable:	yellow	9		–	1	–	–	–	–	–	–	1
	white			1	2	3	1	–	–	–	–	7
	no germination		1									1
Total tested		18										

Cross VI y bi × w (y/y, w/w and bi/Bi segregating.)

"Mixed-inoculum" cross prepared 5/4/50, ascus-dissection commenced 16/5/50, finished 16/5/50.

Asci all taken from one dish.

(Conidial suspensions as used in Cross I, but ratio of y bi : w as 1·0 : 0·8)

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No	Type	1	2	3	4	5	6	7	8
19	?	yell	—	—	—	—	—	—	—
20	?	—	—	—	—	—	—	—	—
21	self wh	wh	wh	wh	wh	wh	wh	—	—
22	self wh	wh	wh	wh	wh	wh	wh	wh	—
23	self wh	wh	wh	wh	wh	wh	wh	wh	—
24	?	wh	—	—	—	—	—	—	—
25	?	wh	wh	—	—	—	—	—	—
26	?	yell	yell	yell	—	—	—	—	—
27	?	wh	—	—	—	—	—	—	—
28	crossed	wh (?)	gn (?)	—	—	—	—	—	—
29	?	—	—	—	—	—	—	—	—

SUMMARY

Types of asci:		Total	Asci with ascospores germinating.								
			0	1	2	3	4	5	6	7	8	Total
classifiable	selfed yellow	4		—	—	—	—	—	—	—	—	—
	selfed white			—	—	—	—	—	1	2	—	3
	crossed			—	1	—	—	—	—	—	—	1
non-classifiable	yellow	7		1	—	1	—	—	—	—	—	2
	white			2	1	—	—	—	—	—	—	3
	no germination		2									2
Total tested		11										

Cross VII y bi × w lys (y/y, w/w, bi/Bi and lys/LYS segregating.)

"Mixed-inoculum" cross prepared 22/6/50, ascus-dissection commenced 22/8/50, finished 12/9/50.

Asci all taken from one slant.

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
150	self yell	yell	yell	yell	yell	yell	yell	yell	yell
151	self yell	yell	yell	yell	yell	yell	yell	yell	yell
152	self yell	yell	yell	yell	yell	yell	yell	yell	yell
153	?	yell bi LYS	yell bi LYS	yell bi LYS	—	—	—	—	—
154	?	—	—	—	—	—	—	—	—
155	crossed	yell bi LYS	yell bi lys	yell bi lys	—	—	—	—	—
156	self yell	yell	yell	yell	yell	yell	yell	yell	—
157	crossed	wh bi LYS	wh bi LYS	gn Bl lys	gn Bl lys	—	—	—	—
158	crossed	wh Bl lys	wh Bl lys	wh bi lys	yell bi LYS	gn Bl LYS	gn Bl LYS	—	—
159	?	wh Bl lys	—	—	—	—	—	—	—
160	crossed	wh Bl LYS	wh Bl LYS	yell bi LYS	yell bi LYS	gn Bl lys	gn Bl lys	—	—
161	crossed	wh Bl LYS	wh Bl LYS	—	—	—	—	—	—
162	self yell	yell	yell	yell	yell	yell	yell	yell	—
163	self yell	yell	yell	yell	yell	yell	yell	—	—
164	crossed *	{wh Bl LYS / yell Bl LYS}	wh Bl LYS	—	—	—	—	—	—
165	self yell	yell	yell	yell	yell	yell	yell	—	—
166	self yell	yell	yell	yell	yell	yell	yell	yell	yell
167	crossed	yell bi LYS	yell bi LYS	gn Bl lys	—	—	—	—	—
168	?	—	—	—	—	—	—	—	—
169	self yell	yell	yell	yell	yell	yell	yell	yell	yell
170	crossed	wh bi lys	wh bi lys	yell bi LYS	yell bi LYS	—	—	—	—
171	self yell	yell	yell	yell	yell	yell	yell	yell	—
172	self yell	yell	yell	yell	yell	yell	yell	yell	yell
173	?	yell bi LYS	—	—	—	—	—	—	—
174	self yell	yell	yell	yell	yell	yell	yell	yell	—
175	self yell	yell	yell	yell	yell	yell	yell	yell	yell
176	self yell	yell	yell	yell	yell	yell	yell	yell	—
177	self yell	yell	yell	yell	yell	yell	yell	yell	—
178	self yell	yell	yell	yell	yell	yell	yell	yell	—
179	?	wh Bl lys	wh Bl lys	wh Bl lys	—	—	—	—	—

ASCUS NUMBERS 2 K and TYPE of ASCOSPORES.

No.	Type	1	2	3	4	5	6	7	8
180	self yell	yell	yell	yell	yell	yell	yell	—	—
181	self yell	yell	yell	yell	yell	yell	yell	yell	—
182	crossed	wh bi LYS	wh bi LYS	wh bi lys	—	—	—	—	—
183	discarded								
184	self yell	yell	yell	yell	yell	yell	yell	—	—
185	?	yell bi LYS	—	—	—	—	—	—	—
186	?	yell bi LYS	yell bi LYS	yell bi LYS	yell bi LYS	—	—	—	—
187	?	yell bi LYS	yell bi LYS	yell bi LYS	yell bi LYS	—	—	—	—
188	self yell	yell	yell	yell	yell	yell	—	—	—
189	?	yell bi LYS	yell bi LYS	yell bi LYS	yell bi LYS	—	—	—	—
190	?	yell bi LYS	yell bi LYS	—	—	—	—	—	—
191	?	yell bi LYS	yell bi LYS	yell bi LYS	yell bi LYS	—	—	—	—
192	self yell	yell	yell	yell	yell	yell	yell	yell	yell
193	self yell	yell	yell	yell	yell	yell	—	—	—
194	self yell	yell	yell	yell	yell	yell	yell	yell	yell
195	self yell	yell	yell	yell	yell	yell	yell	—	—
196	self yell	yell	yell	yell	yell	yell	yell	—	—
197	self yell	yell	yell	yell	yell	yell	yell	yell	yell
198	self yell	yell	yell	yell	yell	yell	yell	yell	yell
199	crossed	yell bi LYS	yell bi LYS	yell bi lys	yell bi lys	—	—	—	—
200	?	—	—	—	—	—	—	—	—
201	self yell	yell	yell	yell	yell	yell	yell	yell	yell
202	self yell	yell	yell	yell	yell	yell	yell	yell	yell
203	crossed	wh bi lys	wh bi lys	gn Bi LYS	gn Bi LYS	—	—	—	—
204	?	—	—	—	—	—	—	—	—
205	crossed	wh bi LYS	wh bi LYS	gn Bi LYS	gn Bi lys	—	—	—	—
206	self yell	yell	yell	yell	yell	yell	yell	yell	—
207	self yell	yell	yell	yell	yell	yell	yell	yell	—
208	self yell	yell	yell	yell	yell	yell	yell	yell	—
209	self yell	yell	yell	yell	yell	yell	yell	yell	
210	crossed	wh Bi lys	gn bi LYS	gn bi LYS	—	—	—	—	—
211	crossed	wh Bi LYS	wh Bi LYS	yell bi lys	—	—	—	—	—
212	crossed	wh bi lys	wh bi lys	gn Bi LYS	gn Bi LYS	gn Bi LYS	—	—	—
213	?	—	—	—	—	—	—	—	—

ASCUS NUMBER and TYPE of ASCOSPORES.

No.	Type	1	2	3	4	5	6	7	8
214	self yell	yell	yell	yell	yell	yell	—	—	—
215	crossed	wh bi lys	wh bi lys	gn BI LYS	gn BI LYS	—	—	—	—
216	self yell	yell	yell	yell	yell	yell	yell	yell	—
217	self yell	yell	yell	yell	yell	yell	yell	yell	—
218	?	—	—	—	—	—	—	—	—
219	self yell	yell	yell	yell	yell	yell	yell	yell	yell
220	?	yell bi LYS	—	—	—	—	—	—	—
221	self yell	yell	yell	yell	yell	yell	yell	—	—
222	self yell	yell	yell	yell	yell	yell	yell	yell	—
223	self yell	yell	yell	yell	yell	yell	yell	—	—
224	self yell	yell	yell	yell	yell	yell	yell	yell	—
225	crossed	wh bi lys	—	—	—	—	—	—	—
226	self yell	yell	yell	yell	yell	yell	yell	yell	yell
227	self yell	yell	yell	yell	yell	yell	yell	yell	—
228	self yell	yell	yell	yell	yell	yell	yell	yell	yell
229	?		—	—	—	—	—	—	—
230	self yell	yell	yell	yell	yell	yell	yell	yell	yell
231	crossed	wh BI LYS	wh BI LYS	wh bi lys	wh bi lys	—	—	—	—
232	self yell	yell	yell	yell	yell	yell	yell	—	—

(Ascospores from selfed yellow asci were all of genotype yell bi LYS.)

SUMMARY.

Types of asci:		Total	Asci with ascospores germinating.								Total
			0	1	2	3	4	5	6	7	8	
classifiable:	selfed yellow	64		—	—	—	—	3	9	16	18	46
	selfed white			—	—	—	—	—	—	—	—	—
	crossed			1	2	5	7	1	2	—	—	18
non-classifiable:	yellow	18		3	1	1	4	—	—	—	—	9
	white			2	—	1	—	—	—	—	—	3
	no germination		6									6
Total tested.		82										

Cross VIII w lys x + ($^{w}/_{W}$ and $^{lys}/_{LYS}$ segregating)

Asci - dissection commenced 25/11/50, finished 27/11/50.

Plating of a random sample of ascospores from first backcross of w lys x +.

Asci taken from perithecia at edges of wh lys colonies.

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
1	crossed	wh LYS	gn lys	gn lys	—	—	—	—	—
2	?	—	—	—	—	—	—	—	—
3	?	—	—	—	—	—	—	—	—
4	?	wh	wh	wh	wh	—	—	—	—
5	?	wh	wh	wh	—	—	—	—	—
6	?	—	—	—	—	—	—	—	—
7	?	—	—	—	—	—	—	—	—
8	self wh	wh	wh	wh	wh	wh	—	—	—
9	?	—	—	—	—	—	—	—	—
10	self wh	wh	wh	wh	wh	wh	—	—	—
11	?	—	—	—	—	—	—	—	—
12	self gn	gn	gn	gn	gn	gn	gn	—	—
13	?	wh	wh	wh	—	—	—	—	—

SUMMARY

Types of asci:		Total	Asci with ascospores germinating.								
			0	1	2	3	4	5	6	7	8	Total
classifiable:	selfed white	4		—	—	—	—	2	—	—	—	2
	selfed green			—	—	—	—	—	1	—	—	1
	crossed			—	—	1	—	—	—	—	—	1
non-classifiable:	white	9		—	—	2	1	—	—	—	—	3
	green			—	—	—	—	—	—	—	—	—
	no germination		6									6
Total tested		13										

Cross IX w lys × + (w/W and lys/LYS segregating)

"Mixed-inoculum" cross prepared 15/12/50, ascus-dissection commenced 1/2/51, finished 1/2/51.

Asci all taken from one dish.

(the w lys strain isolated from the first backcross of w lys × +)

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
101	?	qn	–	–	–	–	–	–	–
102	self qn	qn	qn	qn	qn	qn	–	–	–
103	self qn	qn	qn	qn	qn	qn	qn	qn	–
104	?	–	–	–	–	–	–	–	–
105	?	qn	qn	–	–	–	–	–	–
106	?	wh	–	–	–	–	–	–	–
107	?	wh	–	–	–	–	–	–	–
108	self qn	qn	qn	qn	qn	qn	qn	qn	qn

Types of asci:		Total	Asci with ascospores germinating.								Total
			0	1	2	3	4	5	6	7	8	
classifiable:	selfed white	3		–	–	–	–	–	–	–	–	–
	selfed green			–	–	–	–	1	–	1	1	3
	crossed			–	–	–	–	–	–	–	–	–
non-classifiable:	white	5		2	–	–	–	–	–	–	–	2
	green			1	1	–	–	–	–	–	–	2
	no germination		1									1
Total tested.		8										

Cross X y bi x + (y/y and bi/Bi segregating.)

"Mixed-inoculum" slant prepared 7/11/50, ascus-dissection commenced 17/1/51, finished 17/1/51.

Asci all taken from one slant.

(the y bi strain isolated from the first backcross of y bi x +)

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
1	self yell	yell	yell	yell	yell	yell	yell	yell	yell
2	self yell	yell	yell	yell	yell	yell	yell	yell	–
3	self yell	yell	yell	yell	yell	yell	yell	–	–
4	?	yell	yell	yell	yell	–	–	–	–
5	self yell	yell	yell	yell	yell	yell	yell	yell	yell
6	?	yell	yell	yell	yell	–	–	–	–
7	self yell	yell	yell	yell	yell	yell	yell	yell	–
8	self yell	yell	yell	yell	yell	yell	yell	yell	yell
9	crossed	yell bi	yell bi	yell bi	gn Bi	gn Bi	gn Bi	gn Bi	–

(The cross was the second backcross of y bi x +.)

SUMMARY

Types of asci:		Total	Asci with ascospores germinating								
			0	1	2	3	4	5	6	7	8	Total
classifiable:	selfed yellow	7		–	–	–	–	–	1	2	3	6
	selfed green			–	–	–	–	–	–	–	–	–
	crossed			–	–	–	–	–	–	–	1	1
non-classifiable:	yellow	2		–	–	–	2	–	–	–	–	2
	green			–	–	–	–	–	–	–	–	–
	no germination		–									
Total tested		9										

Cross XI y bi × + (y/y and w/W segregating.)

"Mixed-inoculum" cross prepared 7/11/50, ascus-dissection commenced 30/11/50, finished 14/12/50

Asci all taken from one dish.

(The y bi strain was isolated from the first backcross of y bi × +) (see p.52)

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
1	self yell	yell	yell	yell	yell	yell	yell	yell	—
2	self yell	yell	yell	yell	yell	yell	yell	yell	—
3	self yell	yell	yell	yell	yell	yell	yell	yell	yell
4	self yell	yell	yell	yell	yell	yell	yell	yell	—
5	self yell	yell	yell	yell	yell	yell	yell	—	—
6	self yell	yell	yell	yell	yell	yell	yell	—	—
7	self yell	yell	yell	yell	yell	yell	yell	—	—
8	self yell	yell	yell	yell	yell	yell	yell	yell	—
9	crossed	yell bi	yell bi	yell bi	yell bi	yell Bl	—	—	—
10	self yell	yell	yell	yell	yell	yell	—	—	—
11	crossed	yell bi	yell bi	yell bi	yell bi	gn Bl	—	—	—
12	self yell	yell	yell	yell	yell	yell	yell	—	—
13	?	—	—	—	—	—	—	—	—
14	?	yell	yell	yell	—	—	—	—	—
15	self yell	yell	yell	yell	yell	yell	—	—	—
16	self yell	yell	yell	yell	yell	yell	yell	yell	—
17	self yell	yell	yell	yell	yell	yell	yell	yell	yell
18	self yell	yell	yell	yell	yell	yell	—	—	—
19	self yell	yell	yell	yell	yell	yell	yell	yell	—
20	self yell	yell	yell	yell	yell	yell	yell	—	—
21	self yell	yell	yell	yell	yell	yell	yell	yell	yell
22	self yell	yell	yell	yell	yell	yell	yell	yell	—
23	self yell	yell	yell	yell	yell	yell	yell	yell	yell
24	self yell	yell	yell	yell	yell	yell	yell	—	—
25	self yell	yell	yell	yell	yell	yell	yell	yell	—
26	?	yell	yell	yell	—	—	—	—	—

(Summary overleaf.)

SUMMARY

Types of Asci		Total	Asci with spores germinating.								
			0	1	2	3	4	5	6	7	8	Total
classifiable:	selfed yellow	23		—	—	—	—	3	6	8	4	21
	selfed green			—	—	—	—	—	—	—	—	—
	crossed			—	—	—	—	2	—	—	—	2
non-classifiable:	yellow	3		—	—	2	—	—	—	—	—	2
	green			—	—	—	—	—	—	—	—	—
	no germination		1									1
Total tested		26										

Cross XII y (light background) x + (dark background)

"Mixed-inoculum" cross prepared 28/11/50, ascus-dissection commenced 8/2/51, finished 16/2/51.

Asci all taken from one dish.

(The y and + strains both isolated from Ascus 9 (1st first backcross of ybi x +. see p.52)

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
39a	crossed	yell	yell	yell	gn	gn	gn	—	—
39b	crossed	yell	yell	yell	gn	gn	gn	gn	—
39c	crossed	yell	yell	yell	yell	gn	gn	gn	—
39d	crossed	yell	yell	yell	gn	gn	gn	gn	—
39e	crossed	yell	yell	yell	gn	gn	gn	—	—
48a	crossed	yell	yell	yell	gn	gn	gn	gn	—
48b	crossed	yell	yell	yell	yell	gn	gn	gn	—
48c	crossed	yell	yell	yell	gn	gn	gn	—	—
48d	crossed	yell	yell	yell	gn	gn	gn	gn	—
48e	crossed *	yell	yell	gn	gn	gn	gn	gn	gn
48f	crossed	yell	yell	yell	yell	gn	gn	gn	gn
48g	crossed	yell	yell	yell	yell	gn	gn	—	—
51a	self yell	yell	yell	yell	yell	yell	yell	yell	—
51b	self yell	yell	yell	yell	yell	yell	yell	yell	yell
51c	self yell	yell	yell	yell	yell	yell	yell	yell	yell
51d	self yell	yell	yell	yell	yell	yell	yell	yell	—
51e	self yell	yell	yell	yell	yell	yell	yell	yell	—
51f	crossed	yell	yell	yell	gn	gn	gn	—	—
51g	self yell	yell	yell	yell	yell	yell	yell	yell	yell
51h	self yell	yell	yell	yell	yell	yell	yell	yell	—
51l	self yell	yell	yell	yell	yell	yell	yell	yell	—
53a	crossed	yell	gn	gn	—	—	—	—	—
53b	crossed	yell	yell	yell	yell	gn	gn	—	—
53c	crossed	yell	yell	yell	gn	gn	gn	gn	—
62a	crossed	yell	yell	yell	gn	gn	gn	gn	—
62b	crossed	yell	yell	gn	gn	gn	—	—	—
62c	crossed	yell	yell	yell	yell	gn	gn	—	—
62d	crossed	yell	yell	yell	yell	gn	—	—	—
62e	crossed	yell	yell	yell	yell	gn	gn	gn	gn

Cross XII (contd)

SUMMARY

Types of asci:		Total	Asci with ascospores germinating.								
			0	1	2	3	4	5	6	7	8	Total
Classifiable :	selfed yellow	29		—	—	—	—	—	—	5	3	8
	selfed green			—	—	—	—	—	—	—	—	—
	crossed			—	—	1	—	2	7	8	3	21
non-classifiable	yellow	-		—	—	—	—	—	—	—	—	—
	green			—	—	—	—	—	—	—	—	—
	no germination		—									—
Total tested		29										

Cross XIII — y bi (medium background colour) × + (light background colour)
(y/y, bi/Bi and background colour segregating)

"Mixed-inoculum" cross prepared 23/11/50, ascus-dissection commenced 10/3/51, finished 14/4/51.
Asci all taken from one dish.
(y and + strains both derived from Ascus 9, first backcross of y bi × + . p 86, ascospores Nos 5 and 3. respectively.)

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
II	crossed	yell bi D	yell bi D	yell Bi M	gn Bi Lt	–	–	–	–
1a	crossed	yell bi Lt	yell bi Lt	yell bi D	yell bi D	gn Bi Lt	gn Bi M	gn Bi M	–
1b	crossed	yell bi Lt	yell bi D	gn Bi Lt	gn Bi Lt	gn Bi D	–	–	–
1c	crossed	gn Bi Lt	gn Bi Lt	–	–	–	–	–	–
1d	crossed	yell bi Lt	yell bi Lt	yell bi Lt	yell bi Lt	gn Bi D	gn Bi D	gn Bi D	gn Bi D
1e	crossed	yell bi Lt	yell bi Lt	yell bi M	yell bi M	gn Bi Lt	gn Bi Lt	gn Bi D	gn Bi D
1f	crossed	gn Bi D	–	–	–	–	–	–	–
1g	crossed	yell bi M	yell bi Lt	–	–	–	–	–	–
1h	crossed *	yell bi Lt	yell bi M	yell bi M	{yell bi Lt / gn Bi Lt}	gn Bi Lt	gn Bi D	–	–
4a	self yell	yell	yell	yell	yell	yell	yell	–	–
4b	?	yell	yell	yell	–	–	–	–	–
4c	self yell	yell	yell	yell	yell	yell	–	–	–
4d	?	–	–	–	–	–	–	–	–
4e	self yell	yell	yell	yell	yell	yell	yell	yell	–
4f	self yell	yell	yell	yell	yell	yell	–	–	–
20a	crossed	yell bi Lt	yell Bi Lt	yell Bi M	gn Bi Lt	gn Bi Lt	–	–	–
20b	?	–	–	–	–	–	–	–	–
20c	crossed *	yell bi M	yell bi M	yell Bi Lt	yell Bi Lt	gn Bi Lt	gn Bi Lt	gn Bi M	–
20d	discarded								
20e	crossed	yell bi Lt	yell bi Lt	yell bi D	yell bi D	gn Bi Lt	gn Bi Lt	gn Bi M	gn Bi M
20f	crossed	yell bi Lt	gn Bi Lt	gn Bi Lt	gn Bi D	gn Bi D	–	–	–
21a	?	yell D	–	–	–	–	–	–	–
21b	self yell	yell	yell	yell	yell	yell	–	–	–
21c	self yell	yell	yell	yell	yell	yell	yell	–	–
21d	?	–	–	–	–	–	–	–	–
21e	self yell	yell	yell	yell	yell	yell	yell		–
21f	self yell	yell	yell	yell	yell	yell	yell	yell	–
72a	crossed	yell bi (?)	gn Bi Lt	gn Bi Lt	gn Bi Lt	gn Bi Lt	–	–	–

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No.	Type	1	2	3	4	5	6	7	8
72b	crossed	yell bi Lt	yell bi Lt	yell bi (?)	yell bi (?)	gn Bi Lt	gn Bi Lt	gn Bi (?)	gn Bi (?)
72c	crossed	yell bi Lt	yell bi (?)	yell bi (?)	gn Bi Lt	gn Bi Lt	gn Bi (?)	gn Bi (?)	–
72d	?	–	–	–	–	–	–	–	–
Ia	crossed	yell bi Lt	yell bi D	gn Bi Lt	gn Bi Lt	gn Bi D	–	–	–
Ib	crossed	yell bi D	yell bi D	yell bi D	yell bi D	gn Bi Lt	gn Bi Lt	–	–

SUMMARY

Types of asci:		Total	Asci with ascospores germinating.								Total
			0	1	2	3	4	5	6	7	8	
classifiable:	selfed yellow	23		–	–	–	–	3	3	2	–	8
	selfed green			–	–	–	–	–	–	–	–	–
	crossed			–	–	–	1	5	2	3	4	15
non-classifiable:	yellow	9		1	1	1	–	–	–	–	–	3
	green			1	1	–	–	–	–	–	–	2
	no germination		4									4
Total tested		32										

Table O. Cross XIII $\frac{y\ bi}{y\ y}$ (medium background) x $^{+}$ (light background)
$\frac{Y}{y}$, BI/bi and background colour (light, medium and dark) segregating.

Analysis of segregation types of ascospores from crossed asci:

results obtained by ascus analysis (Table N)

Ascus No.	Parental types						Recombinant types					
	y bi			Y BI			y BI			Y bi		
	Light	Medium	Dark	Light	Medium	Dark	Light	Medium	Dark	Light	Medium	Dark
II	-	-	2	1	-	-	-	1	-	-	-	-
1a	2	-	2	1	2	-	-	-	-	-	-	-
1b	1	-	1	2	-	1	-	-	-	-	-	-
1c	-	-	-	2	-	-	-	-	-	-	-	-
1d	4	-	-	-	-	4	-	-	-	-	-	-
1e	2	2	-	2	-	2	-	-	-	-	-	-
1f	-	-	-	-	-	1	-	-	-	-	-	-
1g	1	1	-	-	-	-	-	-	-	-	-	-
1h ≠	2	2	-	2	-	1	-	-	-	-	-	-
20a	1	-	-	2	-	-	1	1	-	-	-	-
20c ≠	-	2	-	3	-	-	2	-	-	-	-	-
20e	2	-	2	2	2	-	-	-	-	-	-	-
20f	1	-	-	2	-	2	-	-	-	-	-	-
72a	-	-	-	4	-	-	-	-	-	-	-	-
72b	2	-	-	2	-	-	-	-	-	-	-	-
72c	1	-	-	2	-	-	-	-	-	-	-	-
Ia	1	-	1	2	-	1	-	-	-	-	-	-
Ib	-	-	4	2	-	-	-	-	-	-	-	-
Total	20	7	12	31	4	12	3	2	0	0	0	0

N.B. Spore 1 from ascus 72(a), spores 3,4,7 & 8 from ascus 72(b) and spores 2,3,6 & 7 from ascus 72(c) omitted from this summary because they were not completely classified.

Table P. Cross XIII y bi (medium background) x + (light background)

Segregation of y/Y, bi/BI and background colour from results of Table C.

		Background colour			
		Light	Medium	Dark	Total
y	bi	20	7	12	39
	BI	3	2	0	5
Y	bi	0	0	0	0
	BI	31	4	12	47
		54	13	24	91

Allele ratios. $\frac{y}{Y} = \frac{44}{47}$ $\frac{bi}{BI} = \frac{39}{52}$

Background colour. Medium:light:dark = 13:54:24

(Recombination fraction y-bi = 0.055 $\pm$ 0.023)

Cross XIV y bi × w lys (y/y, w/w, bi/Bi and lys/LYS segregating)

"Mixed-inoculum" cross prepared 29/6/51, dissection commenced 22/8/51, finished 31/8/51.

Asci all taken from one dish. (The y bi strain isolated from Ascus 9, ascospore 1. see Cross X)

ASCUS		NUMBER and TYPE of ASCOSPORES.							
No	Type	1	2	3	4	5	6	7	8
1	self yell	yell	yell	yell	yell	yell	yell	–	–
2	self yell	yell	yell	yell	yell	yell	yell	yell	yell
3	self yell	yell	yell	yell	yell	yell	yell	yell	–
4	self yell	yell	yell	yell	yell	yell	–	–	–
5	self yell	yell	yell	yell	yell	yell	yell	yell	–
6	self yell	yell	yell	yell	yell	yell	yell	yell	yell
7	self yell	yell	yell	yell	yell	yell	–	–	–
8	self yell	yell	yell	yell	yell	yell	yell	yell	–
12a	Crossed	wh Bi LYS	–	–	–	–	–	–	–
12b	?	yell bi LYS	yell bi LYS	–	–	–	–	–	–
12c	?	–	–	–	–	–	–	–	–
12d	?	yell bi LYS	–	–	–	–	–	–	–
12e	?	–	–	–	–	–	–	–	–
17a	Crossed	wh bi LYS	yell bi LYS	yell bi LYS	–	–	–	–	–
17b	?	–	–	–	–	–	–	–	–
17c	Crossed	wh Bi LYS	wh Bi LYS	wh Bi LYS	–	–	–	–	–
17d	Crossed	wh Bi LYS	–	–	–	–	–	–	–
17e	?	wh (?)	wh (?)	–	–	–	–	–	–
17f	Crossed	wh Bi LYS	wh Bi LYS	yell bi LYS	yell bi LYS	–	–	–	–
18a	Crossed	wh Bi LYS	wh bi LYS	wh bi LYS	–	–	–	–	–
22a	Crossed	wh Bi LYS	wh Bi LYS	–	–	–	–	–	–
22b	?	–	–	–	–	–	–	–	–
18b	self yell	yell	yell	yell	yell	yell	yell	yell	–
23a	self yell	yell	yell	yell	yell	yell	yell	yell	yell
23b	self yell	yell	yell	yell	yell	yell	yell	yell	yell
24a	Crossed	wh Bi LYS	wh Bi LYS	wh Bi LYS	–	–	–	–	–
24b	Crossed	wh bi LYS	wh bi LYS	–	–	–	–	–	–
24c	?	–	–	–	–	–	–	–	–
25c	?	wh (?)	wh (?)	–	–	–	–	–	–
25d	?	yell (?)	–	–	–	–	–	–	–

Cross XIV (contd.)

(Asci 12 a-e inclusive and nos. 24c, 25c and 25d kept in the refrigerator for 2 days before dissection. Asci 17 a-f inclusive and nos 18a, 18b, 22a and 22b kept for one day in the refrigerator and one day at room temperature before dissection.)

SUMMARY.

Types of asci:		Total	Asci with ascospores germinating.									
			0	1	2	3	4	5	6	7	8	Total
classifiable:	selfed yellow	20		-	-	-	-	2	1	4	4	11
	selfed white			-	-	-	-	-	-	-	-	-
	crossed			2	2	4	1	-	-	-	-	9
non-classifiable:	yellow	10		2	1	-	-	-	-	-	-	3
	white			1	2	-	-	-	-	-	-	3
	no germination		4									4
Total tested		30										

PART IIA. ORIGIN OF THE NUCLEI OF A PERITHECIUM.

PART IIA. ORIGIN OF THE NUCLEI OF THE PERITHECIUM OF A. NIDULANS.

Introduction.

The perithecia of A. nidulans contain in the order of 10,000 8-spored asci (p. 127). In the ascus, two meiotic divisions take place, followed by a mitotic division, resulting in the formation of four pairs of haploid ascospores, as demonstrated by ascus dissection. (Tables A - Q).

The chief problems were:

(a) whether all the asci in one perithecium originate from two or more than two nuclei;

(b) how these nuclei multiply in the ascogenous hyphae;

(c) where the nuclei fuse.

The investigation of (c) was not pursued but the solution of the problem did not affect the issue; cytological work by Pontecorvo (1952a) suggests that this fusion occurs in the ascus primordium. Genetical methods were used to investigate the first two problems.

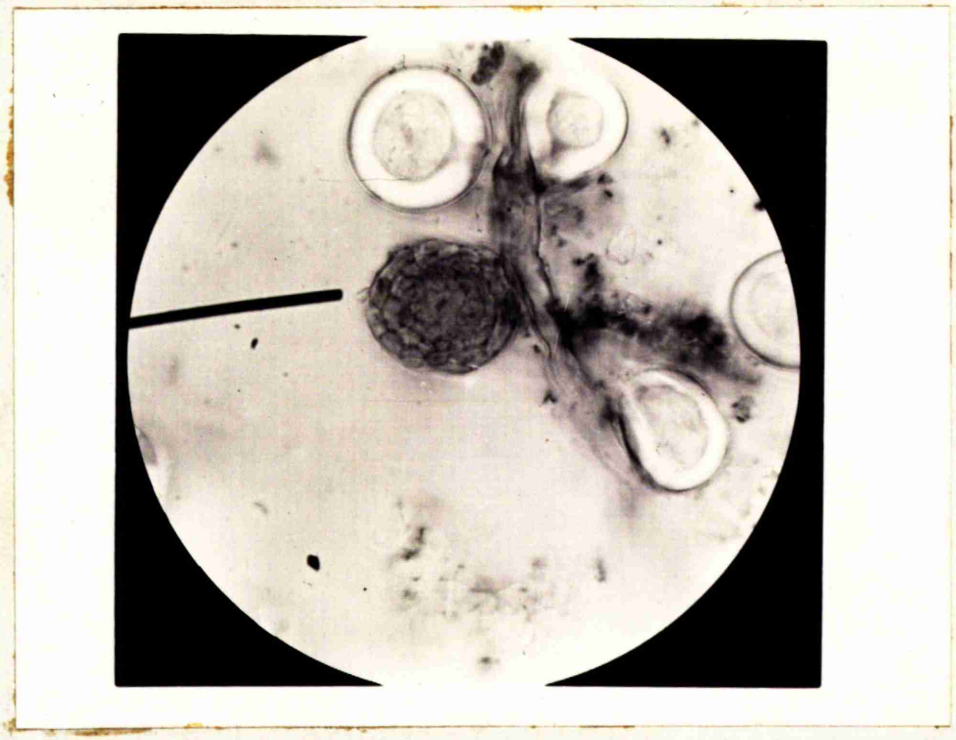


Plate 6. A young perithecium of Aspergillus nidulans.

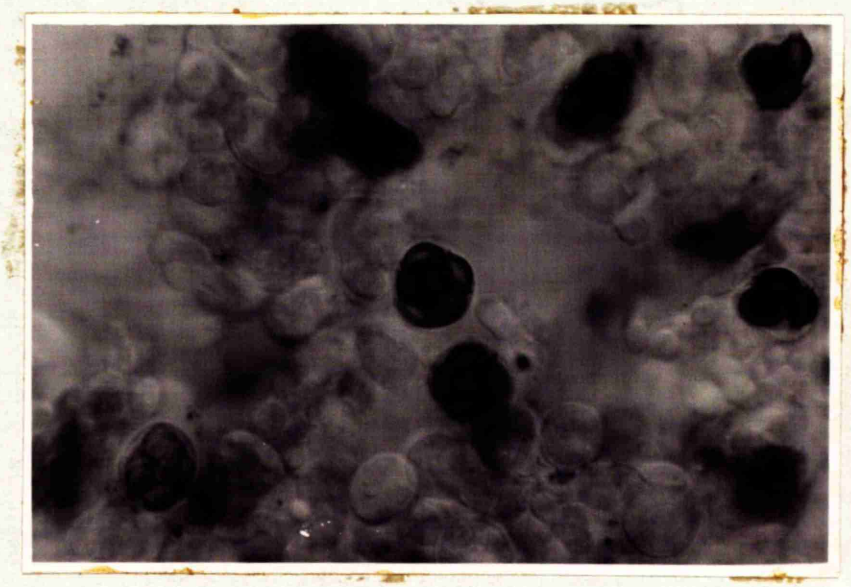


Plate 7. 8-spored asci from a crushed perithecium of Aspergillus nidulans.

The general morphology of A. nidulans was described by Eidam (1883) but the origin and development of the perithecium has never been fully investigated. There is no information either about the behaviour of nuclei of genetically different kinds, when present simultaneously in the hyphae of a homothallic organism. Clearly, this problem could not be investigated before the development of a technique for genetical analysis (Pontecorvo 1949).

Work has been carried out on N. crassa, a heterothallic Ascomycete, using heterocaryons to determine the origin of the ascogenous nuclei (Sansome 1947). In Neurospora, heterocaryons are produced from a cross of strains of the same mating type (Sansome 1946, Beadle and Coonradt 1944). Using two mutant strains A and B, the resultant heterocaryon can be crossed with the wild type strain of opposite mating type and perithecia produced examined for the type or types of asci they contain. If the ascogenous hyphae of one perithecium originated from more than one pair of nuclei, in certain perithecia at least, a mixture of crossed asci A x $^{+}$ and B x $^{+}$ would be recovered. If, on the other hand, they originated from only one pair of nuclei, <u>each</u> perithecium should carry asci either all of type A x $^{+}$ or all of type B x $^{+}$.

Sansome (1947) found that 4 out of 8 analysed perithecia contained asci of both types and she concluded that probably more than two nuclei were involved. However some or all of

the peritheeia giving two types of nuclei may have been "twins", i.e. one peritheeium enclosed or fused with a second one.

Lindegren (1934) working with crosses involving normal (n) and the mutable gene tan (T), found only 2/9 peritheeia containing asci of more than one type. He assumed that these were exceptions and concluded that the ascogenous hyphae normally originate from one pair of nuclei. However the proportion of "mixed" peritheeia is, obviously, dependent on the proportions of nuclei of the two kinds in the heterokaryon.

Whereas in Neurospora, all the products of karyogamy are of crossed origin, in A.nidulans, from a mycelium containing a mixture of two types of nuclei, products of both selfed and crossed karyogamy are formed.

Anastomosis and migration of nuclei occurs between hyphae of two different types, when two strains are grown in close proximity, as in the balanced-heterokaryon or in the "mixed-inoculum" cross (see p. 7&35), enabling a mixing of nuclei to take place. Its occurrence has been demonstrated by the finding of heads bearing conidial chains of two colours, in a cross using colour "markers" to differentiate between the two strains (Pontecorvo 1947 and 1952a).

In A.nidulans, in a cross y x Y, the products of karyogamy will be selfed y x y, selfed Y x Y and crossed y x Y. The type or types of ascus found in a peritheeium will therefore, in the first place, vary according to the origin

of the nuclei, i.e. according to the combination or combinations of nuclei. In the second place it will vary according to where the nuclei fuse.

The various possible origins of the nuclei of a perithecium will now be considered.

Possible origins of the nuclei of a perithecium.

All the nuclei in a perithecium may originate from one, two or more than two nuclei, though clearly perithecia cannot originate mainly from one nucleus, else perithecia containing crossed asci would not be found as shown in Table 26. Whatever the initial number of nuclei, it is obvious that further multiplication takes place as the perithecium starts as a small body (Figure 6) and ends up by containing more than 10,000 asci (p. 127). This multiplication may occur conceivably before or after fusion of two nuclei, and if before, it may or may not be followed by a system of conjugate divisions. Whilst from the experiments reported here it is not possible to distinguish between initial fusion, followed by multiplication in the diploid condition, and conjugate divisions followed by fusion in the ascus, some light is thrown on the problem of the number of nuclei which initiate a perithecium.

If a perithecium were formed from many original nuclei, the perithecia obtained from a cross, e.g. Y x y, would be mainly of a uniform kind and would contain three types of asci (Y/Y; y/y and Y/y). The proportion of these three types of asci would approach p^2: 2pq: q^2, where p and q represent the proportion of yellow and green nuclei in the mycelium. The same would hold whether or not fusions in the ascus were preceded by conjugate divisions or fusions took place at the start of the perithecium.

If perithecia were formed from few, but more than two original nuclei, again, perithecia would contain mostly three types of asci, but there would be a large variance in the proportions of the three kinds of nuclei between perithecia carrying crossed asci; most perithecia would contain nuclei only of the prevalent type, some would contain both types and a few, the rarer type only. Each of those containing both types of nuclei would have the three types of asci in binomial proportions, but p and q would be grossly different between perithecia. Similar results would be obtained at whatever stage fusion of nuclei took place.

Another possibility is that a perithecium could originate from only two nuclei which multiplied at random before fusion. In this case, from a cross y x Y, the two original nuclei would be either both y, or both Y, or one y and one Y. Perithecia originating from nuclei y + y or Y + Y would contain only selfed asci, but in the latter case (y + Y), selfed asci of both types and crossed asci would be formed. Each perithecium of this kind (y + Y) would contain three types of asci (Y/Y; y/y and Y/y) in proportions $p^2:q^2:2pq$ respectively, where the mean values of p and q = 0.50.

The two original nuclei might, alternatively, multiply by conjugate divisions before fusing two by two in the ascus or fuse immediately at the beginning of the perithecium and multiply subsequently as diploid. In either case, each

perithecium would contain only one type of ascus: the asci in each perithecium would be either all selfed of one type, or all crossed, or all selfed of the other type. Perithecia which contained crossed asci would thus contain only crossed asci. Perithecia of these three types should be in proportions of $p^2 + 2pq + q^2$ respectively, and if the mean number of asci per perithecium were the same in all three kinds, the asci from a random sample of perithecia should also be in $p^2 + 2pq + q^2$ proportions.

From the results it was shown that the majority of perithecia contained one type of ascus only and those perithecia which contained crossed asci at all, contained mostly crossed asci. In a few cases, two types of ascus were found in a perithecium but never more than two. Thus the case is favoured of a perithecium originating prevalently from two nuclei, which either multiply by conjugate divisions before fusing, or fuse immediately at the beginning of the perithecium. From an examination of the ascus content of a perithecium, these two cases are indistinguishable.

Section I. Typing of perithecia by ascus content.

As shown in the previous pages, the types and proportions of asci in perithecia will vary according to the original number of nuclei and how they multiply. By sampling and typing a suitable number of asci from a perithecium, information may be obtained as to its contents.

Only perithecia containing crossed asci are critical for the present investigation. As a preliminary sample two asci per perithecium were picked from a "mixed-inoculum" cross y x Y, (i.e. y x +) and if either or both these asci proved to be of crossed origin, further asci were picked and dissected from the original crushed perithecium preparation kept in the refrigerator. The first two asci picked were not dissected but crushed on cellophane squares dipped in nutrient medium (see Whole-ascus squash technique p. 116). After germination of the ascus, a colony with sectors of yellow and green indicated that an ascus was of crossed origin.

Two asci were picked from 18 perithecia and in some cases were of like type and in other cases different, as shown in the following table:

Table 22. Cross y x Y.

Combinations of asci found from a preliminary testing of perithecia by crushing.

Perithecia containing 2 asci of types:

Both yellow	Both green	Both crossed	1 crossed + 1 yellow	1 crossed + 1 green	1 yellow + 1 green	Total
0	3	3	2	2	0	18

Further, analysis of 5 perithecia which contained at least one crossed ascus, showed that from 3 perithecia, asci of only one type were isolated, but the other 2 contained a mixture of asci (selfed yellow and crossed). Table 23.

Table 23. Cross y x Y.

Types of asci obtained from 5 perithecia from a preliminary test by crushing and subsequent dissection.

	ASCI					
	Preliminary test		Dissected		Total	
Perithecium Number	selfed yellow	crossed	selfed yellow	crossed	selfed yellow	crossed
39	-	2	-	5	-	7
48	-	2	-	7*	-	9
51	1	1	8	1	9	2
53	1	1	-	4	1	5
62	-	2	-	5	-	7

* One ascus gave a ratio of 6 Gn. : 2 Yell. ascospores, See abnormal asci Table 18. Ascus 48e.

No selfed green asci were obtained from the above perithecia.

Pooling the results from ascus analysis, found in Table M, of numbers and types of ascospores from asci dissected from the 5 perithecia in Table 23, it was shown, Table 24, that no gross differential viability existed between yellow and green ascospores of crossed origin, or between crossed and selfed yellow. Although no selfed green asci were obtained in the sample there is evidence from similar crosses that a high proportion of selfed green ascospores per ascus do germinate. (See Table D. Cross IV y bi x +).

Table 24. Cross y x Y.

Number and types of ascospores of selfed and crossed origin germinating per ascus.

	Types of asci. Selfed yellow	Selfed green	Crossed	Total
No. asci sampled	8	0	21 (yell. 67, gn. 68)	29
Ascospores from these asci	59	-	135	194
Mean germinable ascospores per ascus	7.4		6.4	6.0

Continuing the investigation, to save unnecessary labour in ascus dissection, a method of isolation of whole undissected asci was used. This was possible, as when using colour "markers" in order to classify an ascus as selfed or crossed, it was unnecessary to isolate its ascospores. A crushed whole ascus would give origin to a colony of one colour only if it was of selfed origin, or a colony showing sectors of both colours, if it resulted from crossed karyogamy. Asci of crossed origin from a cross y (yellow) x Y (green-wild type) with one gene $^{y}/_{Y}$ segregating for colour, would show sectors of yellow and green. There is little error of misclassification due to non-germinability of ascospores. Even with 50% germinability, the chance of misclassifying an ascus of crossed origin is $0.5^4 = 0.065$, i.e. only 6% of the crossed

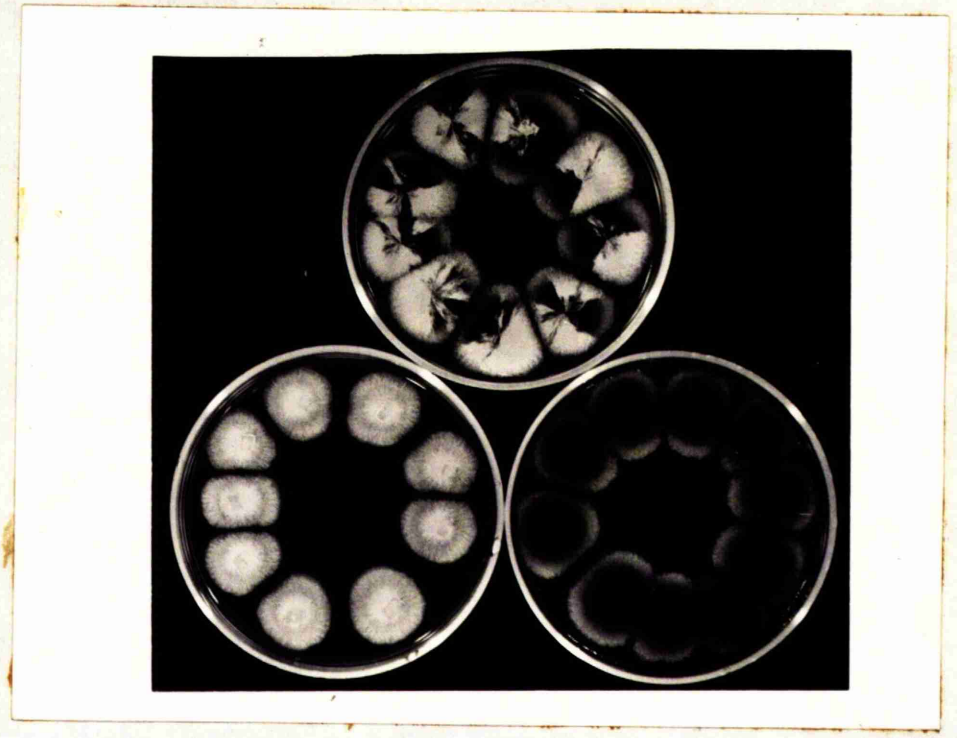


Plate 8. Cross y x Y (one gene segregating).

Whole ascus-squash preparations of asci. 1 plate per perithecium.

Top. Nine asci of crossed origin.

Bottom left. Nine selfed yellow asci.

Bottom right. Ten selfed green asci.

asci would be misclassified as selfed, assuming no differential viability. That germinability was well above 50% and that no appreciable differential viability existed was shown previously in Table 24.

To ensure a high proportion of mature asci, the perithecia must be fully ripe. Ripe perithecia do not burst unless broken by crushing, but in so doing, even as gently as possible, the majority of ripe asci liberate their ascospores. Hence, rarely can more than 10 unbroken mature asci be extracted from one perithecium. The method of isolation of whole un-dissected asci was as follows and was called:

The whole ascus-squash method.

Perithecia were picked from a "mixed-inoculum" cross, each one placed in a drop of sterile water and gently teased open with two needles. Seven or more whole asci were removed individually from each perithecium suspension, using the micro-manipulator and a micro-loop 15 - 20 μ in diameter.

Each ascus was placed on a cellophane square, coated with complete medium and adhering to the underside of a coverslip. The ascus was squashed, in order to check the presence of eight ascospores and ensure that no contaminating conidia or other ascospores were present. Although most asci broke easily, it was sometimes necessary to use the shaft of the micro-loop for stubborn cases.

All asci isolated from the same perithecium were trans-

ferred to a petri dish of complete medium and after 2 days at 37°C, typed as selfed or crossed (Fig. 8).

Before the start of the experiment, an initial random sample of ascospores from 70-100 perithecia from the cross was taken, to find the initial ratio of the nuclei of the two strains, as follows:

Table 25. Cross y x Y.

Proportion of yellow and green nuclei obtained from a random plating of ascospores.

Total number of yellow ascospores	=	982
" " " green "	=	811
Total	=	1,793
Proportion of yellow nuclei (p)	=	0.54
" " green " (q)	=	0.46

Classification of perithecia.

On examining asci from 52 perithecia, three main types of perithecia were found, all containing asci of only one type: either selfed y or selfed Y or crossed y x Y (Table 26).

A smaller, fourth type of perithecium occurred in which a mixture of types of asci were found. These perithecia were called "twins", and were thought to be due to a fusion of two perithecia or the development of one inside the other. From cytological work in the laboratory, evidence was found in support of the hypothesis. The occasional fusion of two perithecia has been reported in A.herbariorum by Fraser

and Chambers, (1907). The other peritheoia were typed according to the type of ascus they contained, i.e. selfed yellow, selfed green and crossed. The following table shows the numbers of peritheoia of different types:

Table 86. Cross y x Y (one gene segregating).

Classification of peritheoia from a random sample of 7-10 asci per peritheoium.

	Ascus content				
	All selfed yellow	All selfed green	All crossed	"Twins"	Total peri-thecia
No. of peritheoia	18	14	13	7	52

Peritheoia containing a mixture of types of ascus, i.e. "Twins", were further analysed. In none of the 7 "twin" peritheoia were more than two types of ascus found. Peritheoia containing three types of asci, however, might be expected to be rare.

Table 27. Cross y x Y.

Analysis of types of asous in 7 "Twin" peritheoia.

	Types of Asci			
	self yellow and self green	self yellow and crossed	self green and crossed	self yellow, self green and crossed.
No. of peri-theoia	1	4	2	0
	6 self yellow + 1 self green	2 crossed + 7 self yellow	5 crossed + 3 self green	
		x 7 crossed + 1 self yellow	5 crossed + 4 self green	
		2 crossed + 6 self yellow		
		1 crossed + 7 self yellow		

self = asci of selfed origin.

x There is a slight bias in favour of typing crossed peri-theoia as "twins" due to misclassification in cases where one selfed and a number of crossed asci are found. The so-called "selfed" asous may be a crossed asous in which ascospores of only one type have germinated.

Section II. Reliability of the ascus-squash method.

(a) The results of the ascus-squash method were tested to find whether they were comparable with the results obtained from a plating of a random sample of ascospores. It had been shown that no differential germinability existed between yellow and green ascospores, and as the proportion of yellow and green ascospores was found to be 0.56 and 0.44 respectively (Table 28), not significantly different from 0.54 and 0.46, obtained from the initial plating of a random sample of ascospores (Table 25).

From Table 28 it is apparent that no bias operated between selfed yellow and green and crossed perithecia and the mean number of asci sampled per perithecium was similar.

Further tests were carried out to ascertain the ascospore content of perithecia, using a different method, i.e. by plating ascospores from different perithecia.

(b) Classification of perithecia by a plating technique.

With the ascus-squash technique mentioned in the previous section, not more than 7-10 whole asci are sampled per perithecium: a type of ascus present in small proportion might thus pass undetected.

A further investigation to check the ascospore content of individual perithecia was carried out using a plating method, sampling 200 or more ascospores from individual perithecia and classifying the resultant colonies for colour.

Table 28. Cross y x Y Analysis of results obtained by the ascus-squash method.

	Selfed Yellow	Selfed Green	Crossed	"Twins"		Total
No. of perithecia	18	14	13	7		52
No. of asci from these perithecia	160	125	114	29 yell 8 gn. 22 crossed	59	458
Proportion of selfed yellow to selfed green asci	0.56	0.44				
Mean number of asci sampled per perithecium	9.0	9.0	8.8	8.4		8.8

200 colonies per dish was a convenient number to count, though it was still possible to count twice this number. The standard errors are given on p.125.

Conidial contamination.

As one of the objects of the plating technique was the detection of a small proportion of one type of nucleus occurring in a perithecium, it was important to ensure that perithecia were freed of conidia, as far as possible, before preparing squashed perithecium suspensions for plating, to obviate contamination with conidia that might contain nuclei different from those in the perithecium. To free perithecia of conidia, the former were rolled on the surface of a dried agar plate before crushing and preparing the ascospore suspension. That this method proved adequate is demonstrated by the low average figure for conidial contamination in the ascospore suspension as detectable under the microscope: 1.7 and 1.5% conidial contamination was obtained from Cross Y x + (Table R), and Cross y bi x w lys (Table S) respectively. On the assumption that equal numbers of conidia from the two strains in a cross, are present, the contamination due to either type of conidia would be half the percentage found under the microscope.

The plating technique used was as follows: perithecia were picked at random, cleaned of conidia, each perithecium transferred to a small centrifuge tube containing 0.1 ml.

of calcofene solution and crushed to liberate the ascospores. A check on conidial contamination was made by counting the number of conidia in a sample of approximately 200 ascospores, using a haemocytometer. Suitable dilutions of the ascospore suspension was made to obtain 200 or more colonies on plating 0.1 ml. of the final dilution on complete medium. To ensure that a suitable plate for counting was obtained and also to act as a check, a second plating was made of 0.1 ml. of suspension from a suspension ten times more concentrated.

The same "mixed-inoculum" cross of y x Y as used in the whole-ascus squash method was sampled.

Results of the plating technique and their interpretation.

From results of the ascus-squash method (Table 26), it was obvious that most perithecia were composed of one type of ascus only and a small proportion, "twins", contained two types of ascus.

The question was whether:

(1) by using the plating technique similar twends could be distinguished and

(2) whether perithecia composed of crossed asci could be readily distinguished and differentiated from those containing a mixture of asci (i.e. "twins").

(1) From the table of results (Table 4) it was obvious that platings from some perithecia gave all yellow ascospores, from others all green ascospores were obtained and from a

Table 29. Cross y x Y

Frequency distribution of ascospores from platings of pseudothecial preparations, showing percentage yellow ascospores obtained.

% yellow	100-90	90-80	80-70	70-60	60-50	50-40	40-30	30-20	20-10	10-0	Total
No. of pseudothecia	14	1	2	4	7	7	1	0	0	14	50

third group, varying proportions of yellow and green ascospores.

A frequency distribution table was made of the percentage yellow ascospores, grouping results into classes of 10%, as this gave a standard error of 3.5 on the mean value of the class 40-50%, with a minimal ascospore count of 200. The standard error of other classes was less than 3.5. With minimal counts of 100, 150 and 300 the corresponding standard errors would have been 5.0, 4.1 and 2.8 respectively. A minimal count of 200 was considered to be reasonably accurate.

The frequency distribution see Table 20 shows that the perithecia fall into three main groups; those giving all yellow ascospores; those giving all green (i.e. 10-0% yellow) and those giving yellow and green; the majority of the latter type yield 40-60% yellow ascospores.

Having found that approximately equal numbers of perithecia of three types were obtained (Table 20), those perithecia yielding yellow and green ascospores were further analysed.

A crossed ascus contains equal numbers of yellow and green ascospores, thus a perithecium containing crossed asci only would be expected to yield yellow and green ascospores in a ratio not significantly different from 1:1, assuming no differential germination. The 22 perithecia containing a mixture of ascospores were tested to find whether the ratio was significantly different from 1:1 and the following results

were obtained:

Table 30. Cross y x Y.

Plating of a random sample of more than 200 ascospores from each of 50 perithecia.

Types of colonies produced by each perithecium:

	only yellow	only green	yellow and green in ratios(x) of 1:1	yellow and green in ratios(x) different from 1:1	Total
No. of perithecia	14	14	9	13	50

(x) significant level taken as 0.05.

Those perithecia showing a ratio of yellow/green ascospores significantly different from 1:1 were probably "twins", and those in ratios of 1:1, probably crossed. Although perithecia containing equal numbers of selfed yellow and green asci would be indistinguishable from crossed, it is unlikely that in a "twin" from selfed yellow and green, equal numbers of selfed and green asci would be present.

A proportion of $\frac{13}{50}$ "twins" was found by the plating method compared with $\frac{7}{52}$ by the ascus-squash technique.

In so far as checking the results of the ascus-squash technique, the plating method proved satisfactory as it confirmed that about 80% perithecia contain only one type of ascus.

An estimation of the total number of asci in a perithecium.

A by-product of the plating method, was the estimation of total asci per perithecium for the cross y x Y (Table R). A known volume of each ascospore suspension was counted and as whole perithecia were crushed in a known volume of suspending fluid, the total ascospores and hence the total asci, could be calculated.

Table 31. Cross y x Y.

Mean number of ascospores per perithecium.

No. of perithecia analysed.	Mean number of ascospores per perithecium.	σ	Mean number of asci per perithecium.
39	99,600	279	12,450

Data were obtained from Table R appendix Part II.

Conclusion.

It was found, using two methods (ascus-squash method p.116. and the plating method p. 120.) that the majority of perithecia contain one type of ascus only, selfed of either type or crossed.

This, and the fact that most perithecia containing crossed asci contain only crossed asci, is compatible with the assumption that perithecia originate prevalently from two nuclei. The results also make it inescapable to deduce that the two nuclei either:

(a) fuse at the beginning of the perithecium and multiply as diploid, or

(b) enter into conjugate divisions at the beginning of the perithecium and then fuse in each ascus primordium.

PART IIB. PERITHECIUM ANALYSIS.

PART IIB. PERITHECIUM ANALYSIS

Introduction.

The finding that a high proportion of perithecia which contain crossed asci, contain only or almost only, asci of this kind, opened the way to a new method of genetical analysis for a homothallic organism like A.nidulans.

If one could identify perithecia with only (or practically so) crossed asci, random sampling of ascospores from perithecia of this kind would yield gametic ratios comparable to those obtained from a cross in a heterothallic organism. These ratios would therefore be utilisable for mendelian analysis in a homothallic organism without need of recombinant selection.

A technique was developed by means of which perithecia containing only (or practically so) crossed asci were identified and samples of ascospores from these perithecia classified for segregating characters. The technique was called "Perithecium analysis."

Section I Selection of perithecia for analysis.

It was important to:

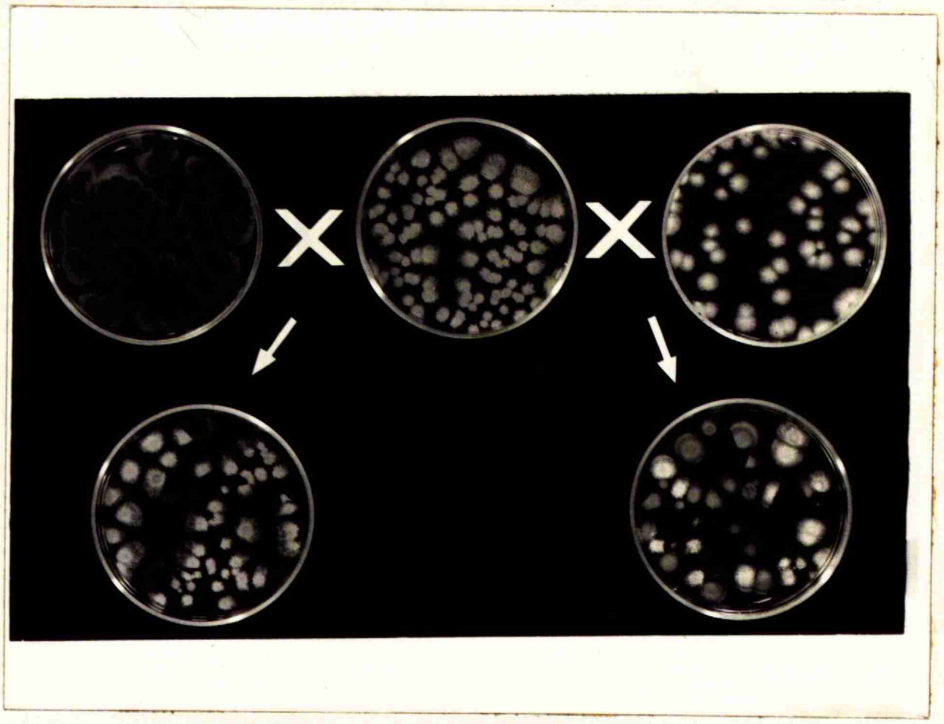
a) find a way to identify perithecia containing crossed asci (for short: "crossed" perithecia);

b) identify, among these, those which contained only, or practically only, crossed asci.

For the preliminary identification of "crossed" perithecia the plating technique previously described was employed but to avoid unnecessary large-scale platings of selfed perithecia, suspensions of ascospores from individual perithecia were first assayed, by plating a small aliquot on a non-selective medium and keeping the remainder of the suspension in the refrigerator. After incubating the assay plating for 48 hrs. those perithecia which gave colonies of more than one type could be identified as "crossed". Further platings from the remainder of the suspension could be carried out if there were an insufficient number of colonies for further classification on the assay plates.

This identification of "crossed" perithecia is most easily done if genes for colour of conidia are used as "markers", otherwise a laborious classification of many colonies for nutritional requirements has to be carried out, before finding, in a high proportion of cases, that the perithecium analysed is not of the right type.

In a Cross y x Y, with one gene for colour segregating, colonies of two colours are expected from "crossed" perithecia.



W Y x W y x w Y

green and yellow colonies (one gene segregating)

Green, white and yellow colonies. (two genes segregating)

Plate 9. Types of colonies formed from Crosses with one and two genes segregating.

In a Cross W y x w Y, with two genes segregating, colonies of four genotypes are expected with three phenotypes: yellow, white and green. Plate 9 shows the two types of colony obtained from a "crossed" perithecium of Cross y x Y and the three types obtained from a "crossed" perithecium from Cross W y x w Y.

From a plating of ascospores on a non-selective medium from perithecia containing only crossed asci, a ratio of 1:1 yellow and green colonies is expected from the Cross y x Y and a ratio of 1:2:1 for white: yellow: green colonies from the Cross W y x w Y.

"Twin" perithecia would usually give ratios different from these although when only one gene ($y/_Y$) is used as a "marker", the possibility of a "twin" perithecium giving a 1:1 ratio cannot be excluded. Using both colour genes there are further safeguards. In the latter case, green colonies can never constitute more than 25% of the total (as they are recombinants) but the ratio of white to green, or yellow to green, will be less than 2:1 or 1:1 respectively, if the perithecium contained a mixture of selfed of one parental type and crossed asci. ("triplets", containing selfed asci of both kinds and crossed, will be sufficiently rare to be disregarded). In addition, the sum of the proportion of yellow and green ascospores should approximate that of the white.

For perithecium analysis a "mixed-inoculum" cross was made of y bi x w lys, involving four loci; y/Y and bi/BI were closely linked and the other two, w/W and lys/LYS, showed independent segregation from each other and the first two loci. The two "colour" loci (w/W and y/Y) were used first to identify "crossed" perithecia and then to identify among these, perithecia which contained only crossed asci.

Assay platings of a random sample of ascospores from 50 perithecia (see Appendix II Table S) showed that in ten cases, colonies of three colours were produced. As a sufficient number of colonies were obtained, further plating was obviated. An analysis of these ten was made to find in which of these perithecia the ratio of yellow: white: green was 1:2:1, indicating that the perithecium was composed of practically all crossed asci and could be used for perithecium analysis (see Table 32).

The results show that in six perithecia (Nos. 3, 9, 24, 33, 41 and 46) the ratio of yellow: white: green was not significantly different from 1:2:1. There was, however, an excess of yellow in two perithecia and a shortage of white in two others.

Perithecium 12 showed 104 yellow, 5 green and 15 white ascospores. The ratio of green/white ascospores agrees with the expected 1:2 ratio, but the yellow ascospores far exceed the expected number and the sum of the yellow and green ascospores is far in excess of the white ascospores. Similarly, in Perithecium 44, the green/white ratio is as expected but an excess of yellow ascospores is found, though not so marked as

Table 32. Cross y bi x w lys

Analysis for colour of colony from a plating of a random sample of ascospores from individual perithecia.

Perithecium number.	Colonies Yellow No.	White No.	Green No.	Total Colonies	χ^2 for hypothesis of 1:2:1 ratio	p.	Perithecia with Yellow:White: Green ratio significantly different from 1:2:1 (Significance level 0.05)
3	64	99	63	226	3.480	0.16	
9	49	115	47	211	1.74	0.41	
11	129	195	121	445	7.086	0.03	Shortage of white
12	104	15	5	124	74.1	0.001	Excess of yellow
24	92	189	103	384	0.724	0.70	
33	59	139	63	261	1.222	0.53	
41	25	47	35	107	3.449	0.18	
44	190	293	147	630	8.940	0.01	Excess of yellow
46	142	240	140	522	3.394	0.18	
48	98	141	102	341	10.65	0.005	Shortage of white

in Perithecium 12. Thus we conclude that Perithecia 12 and 44 are "twins", composed of selfed yellow and crossed asci.

Perithecia 11 and 48 showed a shortage of white although the ratio of yellow: green was 1:1 as expected. The shortage of white cannot be accounted for by "twinning"; it might perhaps be due to a mutation, closely linked with white and reducing viability, that had occurred in those two cases.

Section II. Genetical analysis of selected perithecia.

a) Classification of colonies for nutritional requirements.

Four perithecia out of the six showing the correct colour ratios mentioned in the preceding pages, were further classified for the requirements or otherwise of lysine and/or biotin, since in this cross (y bi x v lys) two "nutritional" genes were segregating, besides those for colour.

Minimal agar plates of two kinds were poured: i.e., containing either 0.01 γ/ml desthiobiotin or 0.4 mg/ml L-lysine mono-hydrochloride. 10-15 duplicate point-inoculations were made, from each of the colonies to be tested, onto a plate of each kind, marking with the same number on the reverse of the plate, the position of the inocull of each pair. Growth of one member only of a pair of inocull, on the biotin plate, indicated that the colony required biotin but not lysine.; growth only on the lysine plate indicated requirement for lysine; growth on neither plate indicated requirement for both and growth on both plates indicated no requirement (Plate 10). As biotin is excreted into the medium by the colonies which do not require it, it is important to space the colonies well apart, to classify the results as soon as possible (say 48 hrs) and retest those colonies that grew later than the majority on biotin-free medium, as they might be requirers that were fed by adjacent colonies.

From the cross y bi x v lys previously examined for

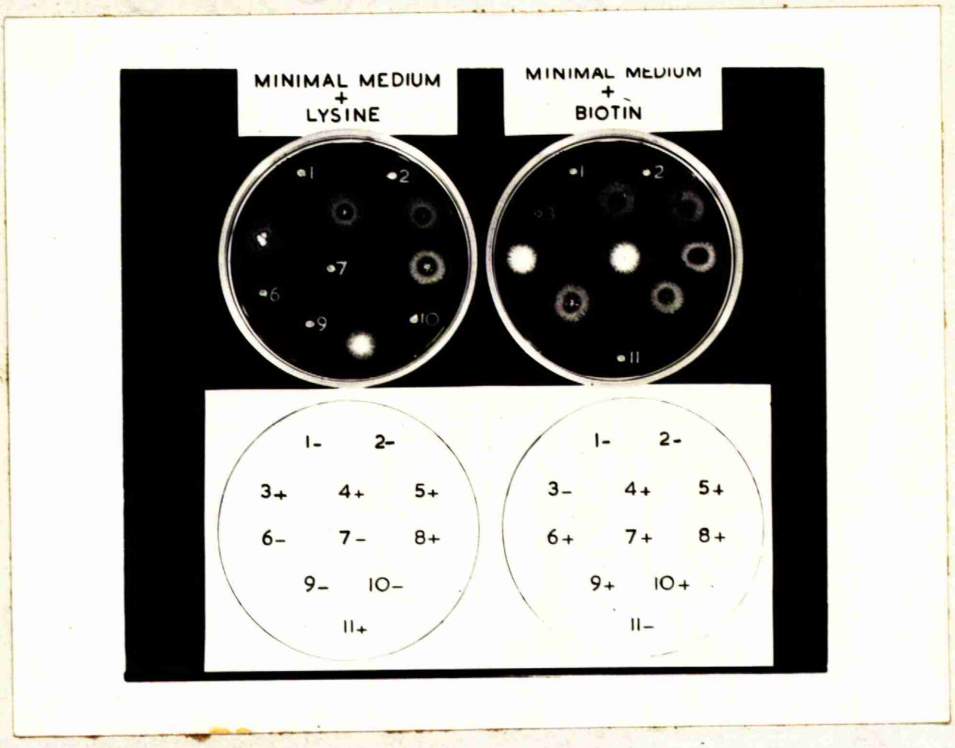


Plate 10. Cross w lys x y bi.

Classification of colonies for lysine and biotin requirements on minimal medium + lysine and minimal medium + biotin.

Table 33. Cross y bi x w lys

Classification of sample of colonies from plating on complete medium a sample of ascospores from 4 perithecia known to carry only, or almost only, crossed asci.

	Yellow					White					Green					Total
No. of Perithecium	BI lys	BI LYS	bi lys	bi LYS	Total	BI lys	BI LYS	bi lys	bi LYS	Total	BI lys	BI LYS	bi lys	bi LYS	Total	
3	1	1	5	15	22	4	15	11	13	43	5	15	0	1	21	86
9	0	0	3	3	6	9	2	6	3	20	2	4	1	1	8	34
11	0	0	7	14	21	9	15	6	12	42	11	14	2	0	27	90
24	0	1	7	9	17	4	7	3	12	28	2	7	0	1	10	55
	1	2	22	41	66	26	39	28	40	133	20	40	3	3	66	265

colour ratios (Table 33), further analysis for nutritional requirements (biotin and lysine) was carried out on a total of 265 colonies from four "crossed" perithecia (Nos. 3, 9, 11 and 24) which had given correct allele ratios of the colour "markers" (Table 33). The analysis was carried out on colonies from plates of the second dilution of the assay plating, except in the case of perithecium 24 where a proportion of the colonies from the first dilution was used, testing all colonies from one sector of the plate. (see Table 8, Appendix Part II).

The 4 perithecia were homogeneous (p = 0.5 to 0.7) so the results could be pooled, as shown in Table 34.

Table 34. Cross y bi x w lys (four gene segregation, y and bi linked; other loci free recombination)

Peritheciun Analysis.

Classes obtained from plating on complete medium a sample of ascospores from 4 perithecia each known to carry crossed asci.

	BI lys	BI LYS	bi lys	bi LYS	Total
Yellow (W y)	1	2	22	41	66
Green (W Y)	20	40	3	3	66
White (w Y and w y)	26	39	28	40	133
Total	47	81	53	84	265

Allele ratios.

w/W : 133/132 bi/BI : 137/128

y/Y : 66/66 lys/LYS : 100/165

Recombination fraction y-bi : 0.068 $\pm$ .02

On the pooled results of the four peritheoia (Table 34) tests for linkage were carried out:

b) Tests for linkage

1. y/Y and bi/BI

	y	Y	Total
bi	63	6	69
BI	3	60	63
	66	66	132

Parental types = 123

Recombinant types = 9

Recombination fraction = $\frac{9}{132}$

= 0.068 ± 0.02

2. w/W and bi/BI

	w	W	Total
bi	68	69	137
BI	65	63	128
	133	132	265

Parental types = 134

Recombinant types = 131

3. lys/LYS and bi/BI

	lys	LYS	Total
bi	53	84	137
BI	47	81	128
	100	165	265

Parental types = 131

Recombinant types = 134

4. lys/LYS and w/W

	w	W	Total
lys	54	46	100
LYS	79	86	165
	133	132	265

Parental types = 140

Recombinant types = 125

χ^2 = 0.85 p = 0.3 to 0.5

5. lys/LYS and y/Y

	y	Y	Total
lys	23	23	46
LYS	43	43	86
	66	66	132

Parental types = 66

Recombinant types = 66

With 4 loci segregating independently, whichever two are selected, the other two should give a 1:1:1:1 ratio or, in the case of epistasis, a modified 1:2:1 ratio. When ratios significantly different from 1:1:1:1 (or 1:2:1) are obtained, linkage and/or differential viability may be the cause.

There was no significant departure from 50% recombination in the cases of $w/_{W}$ and $bi/_{BI}$; $lys/_{LYS}$ and $bi/_{BI}$ and $lys/_{LYS}$ and $w/_{W}$. In the case of $y/_{Y}$ and $bi/_{BI}$ there was a defect of recombinant types. Allele ratios for $bi/_{BI}$ and $y/_{Y}$ were not significantly different from 1:1, so there was no evidence of lowered viability of either pair of alleles and linkage was evident between $bi/_{BI}$ and $y/_{Y}$; the recombination fraction being 0.068 ± 0.02. This figure is not significantly different from $0.05 \pm .004$ (Ponteocorvo 1952a).

Considering the alleles at the lysine locus, we find a ratio of lys:LYS of 100:165. That the defect of the lysine allele is due to decreased viability and not to linkage with w/W is shown by the checkerboard diagram No. 4. Taking the W allele, W LYS is the parental type and the deficit of W lys, the recombinant, could be due either to linkage or to viability effects. In the w class we find an excess of the recombinant type (w LYS): obviously linkage cannot be the cause of the shortage. The viability of the lys allele can be provisionally estimated from the data as 0.60.

Section III. Perithecium analysis and recombinant selection.

In perithecium analysis, a random sample of ascospores from a selected perithecium ("crossed") is used for genetical analysis, whilst in recombinant selection, certain classes of ascospores only are selected, and segregation of the other alleles studied in these classes. Though it might appear that by using the former method, much labour of plating and selection is saved, on closer examination it is apparent that the advantages and disadvantages of the two techniques depend on the circumstances of each specific case. The choice of technique depends, as follows, on the Cross under examination, the relationship of the loci studied and on certain other details:

1. Suitability of cross under examination.

Use can only be made in perithecium analysis of perithecia containing only (or practically only) crossed asci. It is thus a waste of time to apply the technique to a cross in which only a very small proportion of "crossed" perithecia arise. Recombinant selection from a plating of a large random sample of ascospores will then be much more expeditious. Mr.A.W.J. Button, in this laboratory, found that in certain crosses a proportion of "crossed" perithecia in excess of 50% and even up to 100% may occur. Under these conditions obviously, the technique of perithecium analysis may be preferable, providing the genes are not closely linked. In crosses where a suitably high proportion of "crossed" perithecia occurs, and only loose linkages have to be estimated, a most economical use of data is

made by perithecium analysis, as all ascospores can be classified for nutritional requirements from only one plating and onto a single type of medium (non-selective). As shown in Table 34 all three colour classes can be used, whilst with recombinant selection, based on colour "markers" only the green recombinant class could be further tested.

In the case of a perithecium proving to be a "twin", a certain amount of information may still be obtained, as certain classes may be picked and the segregation of other alleles studied in these classes, as in recombinant selection. The proportion of "twins" is, then, another factor when deciding on choice of technique.

2. Linkage.

With very close linkage, recombinant selection should be used, as with perithecium analysis it may be impossible to classify the high number of colonies required to obtain a sufficient number of recombinants among them. With low recombinant fractions, of the order of $\frac{1}{1000}$ or less, as in the case of pseudo-alleles, there is no alternative. Insufficiency of data when working with linked genes is shown in Table 34, Cross w lys x y bi: only 9 recombinants between y/Y and bi/BI were found from a total of 132 colonies tested.

3. Effect of media.

As a non-selective medium is used in perithecium analysis, the complication that the viability of certain types of segregant differs on different media is avoided; also the suppression

of prototrophs by auxotrophs, when very high densities of platings are used, as found by Roper (1950) Grigg (1951) and discussed by Jinks (1952).

4. The distinguishing of linkage from viability effects.

Using recombinant selection, in certain cases it is difficult to distinguish between linkage and viability effects unless more than one selection is carried out on crosses in coupling and repulsion.

From the very same data of peritheclum analysis (Table 34) we can see how this can be done. If we take only the W Y class (as we might have done in the case of recombinant selection), the data are as follow:

Cross W lys x y bi

Recombinant selected: W Y

Segregation at other loci:				
BI lys	BI LYS	bi lys	bi LYS	Total
20	40	3	3	66

We find an excess of LYS over lys (43 to 23). Since in this cross, W and LYS come from one parent and w and lys from the other, we might deduce that selection for W brings about an excess of LYS, and that therefore the loci are linked. This is not so, as shown before (p 141), and lysine has 60% viability: the results of recombinant selection are thus entirely accounted for by viability and not by linkage in this case. This shows that:

a) viability may simulate linkage

b) viability may also mask linkage.

With additional selections, even without additional crosses in coupling and repulsion the trouble is avoided. Extracting data

from the same Table 34 and picking another recombinant, bi lys, no linkage effects between the lys/$_{LYS}$ and w/$_{W}$ loci are shown.

Cross y bi x w lys

Recombinant selected: bi lys	Segregation at other loci: W y	w Y	w y	W Y	Total
	22	28		3	53

In this Cross, selection for lys does not bring about a defect of the recombinant type, W and lys, in regard to the parental class, w and lys, as the ratio of the two respectively is 25/28.

Conclusion.

The technique of "Peritheciun analysis" was exemplified in one Cross with 4 genes segregating. It was shown how genetical analysis can be carried out on a random sample of ascospores if peritheeia containing only, or practically so, crossed asci are selected. The technique is most convenient in crosses in which the following conditions are fulfilled:

1) the proportion of "crossed" peritheeia is high;

2) the loci, between which recombination has to be estimated, are not linked or only loosely linked.

This work was the start for a further study and Mr. A.W.J. Button on continuing the investigation discovered "relative heterothallism" in A.nidulans.

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Cross XII y x + (4/4 segregating. y and + strains isolated from Asco 9, for backcross of y bi x +. See Table M for details of asco-dissection of Cross VII)

Types of ascospore colony obtained on plating random samples of ascospores from perithecia from a "mixed-inoculum" cross.

Perithecium No.	Haemocytometer count				Ascospores per perithecium ×10³	Type and number of colonies from ascospores				% yellow ascospores.
	No. of fields †	Conidia		Ascospores		First dilution		Second dilution		
		No.	%	No.		Yellow	Green	Yellow	Green	
I D	?	0		9		approx. equal		90	78	53·6
II	?	0		5		–	> 200	–	> 200	–
III D	?	0		4		approx. equal		61	73	45·5
IV	?	1		10		> 200	–	> 200	–	100
V	?	0		6		> 200	–	97	–	100
VI D	?	0		4		approx. equal		47	48	49·5
VII D	?	–		–		excess of green		11	52	17·5
VIII	?	–		–		> 200	–	248	[illegible]	100
IX	?	–		–		–	> 200	–	200	–
X	?	1		6		–	> 200	–	63	–
1a	?	1	3·3	30		184	153	61	56	54·7*
2a	?	1	2·0	49		188	182	8	8	50·8*
3a	?	1	1·5	62		303	20	12	1	93·8*
4a	?	1	2·0	44		–	221	–	10	–
5a	?	0	< 1·0	46		9	486	2	80	1·82*
6a	?	2	3·0	66		122	151	80	71	44·7
7a	?	1	4·0	24		920	–	174	–	100
8a	3	1	1·0	109	56·8	296	208	212	224	48·6
9a	2	0	< 1·0	136	121	approx. equal		216	247	46·7
10a	2	1	< 1·0	127	113	–	> 200	–	> 200	–
11	2	1	< 1·0	136	121	> 200	–	> 200	–	100
12	2	2	1·4	139	124	> 200	–	> 200	–	100
13	3	0	< 1·0	107	55·8	approx. equal		191	197	51·9
14	3	2	2·0	101	52·6	approx. equal		254	267	48·8
15	3	1	< 1·0	175	91·1	approx. equal		239	196	54·9
16	3	3	3·0	105	54·7	–	> 200	–	> 200	–
17	3	3	3·0	115	59·0	–	> 200	–	> 200	–
18	2	1	< 1·0	134	120	> 200	–	> 200	–	100
19	2	1	< 1·0	141	126	> 200	–	> 200	–	100

Perithecium No.	Haemocytometer count. No. of fields.	Conidia No.	Conidia %	Ascospores No.	Ascospores per perithecium. $\times 10^3$	Type and number of colonies from ascospores. First dilution Yellow	First dilution Green	Second dilution Yellow	Second dilution Green	% yellow ascospores.
20	2	3	2.4	80	71.4	approx. equal		247	161	60.5
21	2	2	2.0	93	83.0	approx. equal		229	215	51.6
22	3	4	3.0	151	78.6	–	> 200	–	> 200	–
23	2	3	3.0	101	90.3	> 200	–	> 200	–	100
24	2	2	1.5	170	152	approx. equal		211	180	54.0
25	2	1	< 1.0	174	156	approx. equal		318	167	56.6
26	2	3	3.0	108	96.4	> 200	–	> 200	–	100
27	2	1	< 1.0	140	125	718	154	53	15	82.4*
28	2	4	4.0	122	109	548	239	120	57	69.6*
29	2	0	< 1.0	111	99.1	approx. equal		196	270	42.2
30 D	2	1	< 1.0	153	137	no data		91	29	75.8
31	2	2	2.0	97	86.6	> 200	–	> 200	–	100
32	2	1.5	< 2.0	140	125	–	> 200	–	> 200	–
33	2	3	3.0	123	110	–	> 200	–	> 200	–
34	2	0	< 1.0	164	146	> 200	–	> 200	–	100
35	2	0	< 1.0	135	121	338 approx	248 approx	154	73	67.8
36	2	2	2.0	102	91.1	> 200	–	> 200	–	100
37	2	0	< 1.0	119	106	–	> 200	–	> 200	–
38 D	2	5	3.0	144	128	approx. equal		97	91	51.6
39	3	3	3.0	123	64.1	352 approx	624 approx	78	156	33.3
40	2	4	4.0	111	99.1	–	> 200	–	> 200	–
41	2	2	2.0	97	86.6	approx. equal		181	115	61.2
42	2	6	6.0	117	10.4	approx. equal		141	176	44.5
43	2	2	2.0	112	299	808 approx	444 approx	249 approx	98	71.7 approx
44	3	1	1.0	102	53.1	520 approx	130 approx	183	62	74.7
45	2	3	3.0	97	86.6	–	> 200	–	> 200	–
46	2	3	3.0	98	87.5	approx.	equal	163	213	43.4

D = results discarded as insufficient data.

* = calculation from first dilution

† volume of suspension per field = $0.064 mm^3$.

TABLE 3.

Cross w lys x y bi (y/y, w/w, bi/Bi and lys/LYS segregating.)

"Mixed-inoculum" cross prepared 29/6/51, platings commenced 13/8/51, finished 20/8/51. Perithecia all taken from one dish.

Types of ascospore colony obtained on plating random samples of ascospores from perithecia.

Perithecium No.	Haemocytometer count.				Type and number of colonies from ascospores.					
	No. of fields. †	Conidia		Ascospores	First dilution			Second dilution		
		No	%	No.	Yellow	White	Green	Yellow	White	Green
1	3	2	2	102	204	-	-	50	-	-
2	2	2	2	97	564	-	-	29	-	-
3	2	3	3	110	64	99	63	23	43	23*
4	2	1	1	106	524	-	-	70	-	-
5	3	1	1	111	436	-	-	128	-	-
6	2	1	1	111	492	-	-	106	-	-
7	3	4	4	116	248	-	-	130	-	-
8	2	2	2	114	276	-	-	80	-	-
9	2	0	0	124	49	115	47	10	20	8*
10	2	0	0	115	440	-	-	52	-	-
11	2	1	1	102	129	195	121	27	46	27*
12	3	1	1	97	104	15	5	39	-	-
13	3	0	0	117	494	-	-	156	-	-
14	2	2	2	105	420	-	-	104	-	-
15	2	2	2	104	564	-	-	169	-	-
16	2	3	3	99	394	-	-	116	-	-
17	2	2	2	107	840	-	-	60	-	-
18	2	3	3	110	249	-	-	140	-	-
19	2	3	3	106	412	-	-	108	-	-
20	3	1	1	99	508	-	-	109	-	-
21	2	1	1	100	604	-	-	128	-	-
22	2	1	1	109	720	-	-	103	-	-
23	1	0	0	123	724	-	-	151	-	-
24	2	0	0	101	92	189	103*	6	25	8
25	2	1	1	102	760	-	-	83	-	-
26	3	0	< 1	129	730	-	-	207	-	-
27	2	1	1	110	388	-	-	71	-	-
28	3	1	1	98	294	-	-	62	-	-
29	2	1	1	113	604	-	-	136	-	-

Perithecium No.	Haemocytometer count.				Type and number of colonies from ascospores.					
					First dilution.			Second dilution.		
	No. of fields †	Conidia		Ascospores.						
		No.	%	No.	Yellow	White	Green	Yellow	White	Green
30	?	1	2	49	117	-	-	68	-	-
31	2	0	< 1	114	582	-	-	116	-	-
32	2	1	1	118	664	-	-	70	-	-
33	2	0	< 1	112	59	139	63	14	30	19
34	4	2	2	113	784	-	-	128	-	-
35	3	2	2	98	375	-	-	106	-	-
36	3	2	2	105	520	-	-	258	-	-
37	?	1	1	100	680	-	-	290	-	-
38	3	2	2	108	528	-	-	167	-	-
39	?	1	1	109	472	-	-	480	-	-
40	3	0	< 1	109	640	-	-	168	-	-
41	2	1	1	105	Plate overcrowded			25	47	35
42	3	0	< 1	106	664	-	-	124	-	-
43	2	4	4	116	784	-	-	392	-	-
44	2	3	3	110	190	293	147	34	77	25
45	2	4	4	95	552	-	-	118	-	-
46	2	0	< 1	110	142	240	140	15	26	19
47	2	3	3	113	856	-	-	107	-	-
48	2	3	3	98	98	141	102	20	32	16
49	2	2	2	112	240	-	-	191	-	-
50	2	1	1	113	768	-	-	118	-	-

? : no data

* For further analysis for nutritional requirements, see Table 33. Part II B.

† Volume of suspension per field = 0·064 mm^3.