ASPERGILLUS NEWS LETTER

No. 2

Spring 1961.

Department of Genetics,
The University,
Sheffield,
England.

Dear Colleague,

Again I apologise for the errors and omissions which this number undoubtedly contains. However, I hope it is still of a certain value as an informal report for news of publications, addresses, etc.

Suggestions relating to content and presentation of future numbers would be appreciated.

J. A. ROPER.

Please return

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CURRENT RESEARCH PROJECTS

IKEDA and TAKEICHI.

1. Effects of ultraviolet light on diploidization and segregation in A. oryzae and A.sojae: The effect of UV upon diploidization is being studied in connection with the concept of relative heterothallism.

2. Anomalous segregation observed in diploid strains of A. sojae: A heterozygous diploid produced between a white and a yellow mutant of A.sojae gave rise to white, yellow and brown progeny. The mechanism is being studied.

SERMONTI and FRATELLO

Relative viability of the parental and the cross-over chromosome in diploid segregants of Aspergillus.

ARDITTI-MUTCHNICK and STRIGINI

Analysis of diploids derived from ascospore plating.

STRIGINI

Coincidence of non-disjunction and cross-over in induced somatic segregation.

FRATELLO

Analysis of the locus (or loci) y.

STRIGINI

Action of P^{32} on haploid and diploid Aspergillus.

MORPURGO

Automatic selection of segregants by means of recessive resistance to analogues and screening of crossing-over inducing substances.

MORPURGO

Studies on analogue-resistant mutants.

a) mapping.
b) physiology of resistance.

ROBERTS

Metabolism of galactose by A. nidulans.

KAFER-BOOTHROYD and colleagues

1. Segregation in diploids after high doses of X-rays as compared to spontaneous (especially chromosomal) segregation.

2. Analysis of translocations.

3. Frequency of spontaneous and induced mitotic segregation.

PUBLICATIONS

ASPERGILLUS

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PENICILLIUM

G. PONTECORVO and G. SERMONTI. Recombination without sexual reproduction in Penicillium chrysogenum. Nature, 172, 126 (1953)

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PRELIMINARY NOTES ON CURRENT RESEARCH

C. TAKEICHI.

By using mutants of Aspergillus sojae it was demonstrated again that the heterokaryons and the heterozygous diploids irradiated by ultraviolet light gave rise to diploids and somatic segregants respectively in higher frequencies. These two effects of ultraviolet light were compared with the so-called inactivation (killing) effect of UV. The haploid conidia inactivated by ultraviolet light were reactivated by visible light, whilst visible light gave no significant effect upon diploidization and segregation. It was concluded, therefore, that the mechanism of UV function on inactivation is different from those on diploidization and segregation. Differences between two functions of UV, on diploidization and on segregation, are not known.

RITA ARDITTI MUCHNIK.

Induced somatic segregation and chromosome mapping in Penicillium chrysogenum.

A research project was started in order to map more loci in P. chrysogenum by means of induced somatic segregation of heterozygous diploids. Diploids were synthesized between strains of our type-collection marked with several mutations, some of which already mapped by Sermonti (1957).

Unexpectedly, all of the synthesized diploids gave segregation of practically only the parental types even when previous information indicated that some of the markers were located in different chromosomes. A heterozygous diploid (XVI) which segregated recombinants as well as parental types about five years ago, was now unable to give rise to an appreciable number of recombinant types. The hypothesis was put forward that the unexpected segregation was the result of chromosomic aberrations due to either ageing of the strains, or to mutagenic treatment during the preparation of the parent haploids. Starting with a green prototrophic strain (Wis 47.1564) new mutations have been U.V.-induced and multiple marked strains have been prepared. Diploid strains were synthesized after every single new mutation was added and the behaviour of the markers was thus controlled by studying the segregation of the diploid. Diploids with five heterozygous loci have now been produced which segregate a high proportion of recombinant phenotypes after nitrogen mustard treatment. The work is in course.

RITA ARDITTI MUCHNIK and G. SERMONTI.

"Fixation" of induced somatic segregation in Penicillium chrysogenum.

Conidia from a heterozygous diploid of Penicillium chrysogenum treated with nitrogen mustard (HN-2) show a high rate of colour segregants (up to 70% of the surviving colonies). When plating is made on a medium supplemented with manganous chloride (40 mM) both the killing and the segregating effects are partially reversed. An experiment was carried out in order to establish how long the reversibility period lasted. When conidia were allowed to germinate on a liquid complete medium before plating on $MnCl_2$ both the killing and the segregating effects started to become irreversible after about 13 hours of culture and were completely "fixed" within the 24 hours. This interval presumably corresponds to the germination period.

O. SIDDIQI.

Mutagenic Action of Nitrous Acid in Aspergillus nidulans.

The first attempts to induce mutations with nitrous acid were made in Aspergillus by Thom and Steinberg (1939). They produced a number of morphological mutants in A. niger and A. amstelodami by incorporating 0.2% $NaNO_2$ in the growth medium. The mutagenic action was attributed to the deamination of gene protein. Recent revival of interest in nitrous acid mutagenesis stems from the fact that it has been shown to deaminate individual bases in DNA. Several authors have interpreted the mutagenic action of nitrous acid in molecular terms. In view of this possibility the effect of nitrous acid on A. nidulans was investigated.

Conidia harvested from a six days old culture are vigorously agitated in suspension to break up the chains. They are treated with $NaNO_2$ in acetate buffer at pH 4.4 in a water bath at 37°C. The treatment mixture consists of the following:

Conidia	about $1X10^8$
Acetate buffer	0.1 molar at pH 4.4
$NaNO_2$	0.017 molar
Volume	20 ml.

The reaction can be instantaneously stopped by transferring samples at required intervals of time to pH 7 phosphate buffer at room temperature. The treatment increases the frequency of both morphological and nutritional mutants.

The dosage-effect curves for nitrous acid treatment were obtained by screening for suppressors of methionine requirement. This system for assaying mutation rates has been devised by Dr. L.J. Lilly (unpublished). Strain meth 1 reverts to prototrophy by a number of different suppressor mutations. Three of these which are phenotypically distinguishable from each other, tentatively designated as su meth1A, su meth 1B and su meth1C have been used in determining the effect of treatment on mutation rates. Figure 1 shows the lethal effect on strain meth1 bi while figure 2 represents the increase in the frequency of su meth1 A after treatment. Both the lethal and the mutagenic effects of the treatment are pH specific and fall rapidly with increase in pH.

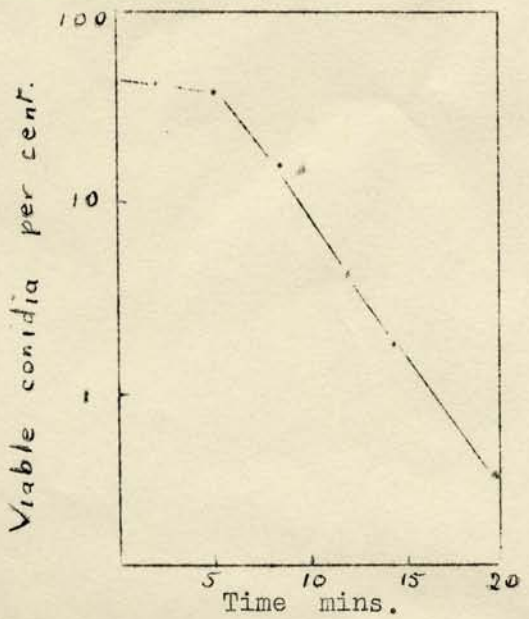


Fig.1. Survival of meth 1 bi strain after treatment.

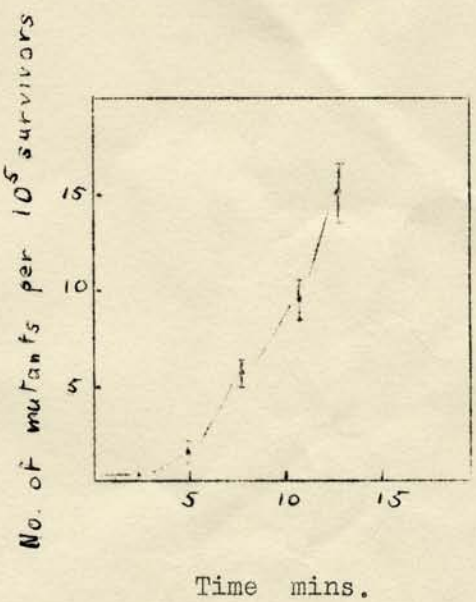


Fig.2. Increase in mutation rate of su meth1 A against treatment time.

G. MORPURGO

Resistance to antimetabolites in Aspergillus nidulans.

1) Resistance to 8-aza-guanine (8AG).

Growth of A. nidulans is practically suppressed by 8AG added to Czapek-Dox at a concentration of 1.5 mM. The inhibitory action of 8AG is reversed by guanine or adenine, not by xanthine. No spontaneous resistant appeared after plating of about 10^6 conidia, although a light background growth was obtained. After U.V. treatment at a dose to give 0.5% survival up to 2 resistants per 1,000 surviving conidia were obtained. In heterokaryons resistance turns out to be gene-determined and recessive. Resistant mutants grow at a concentration of 8AG 200 times as much as that which inhibits the wild type. 8AG-resistance provides a remarkable test of forward-mutation in Aspergillus. Similar results have been obtained in Penicillium chrysogenum by Dr. Rita Arditti, of our laboratory.

2) Resistance to p-fluoro-phenylalanine (PFP)

PFP suppressed growth at a concentration of 1 mM, and its action is reversed by phenylalanine, and less effectively by tryptophan and phenylpyruvic acid. No spontaneous resistant was observed (about 2.10^6 conidia plated); background growth was present. After U.V. treatment (see above) up to 2 resistants per 1,000 surviving conidia were obtained, although this frequency is highly variable from strain to strain, probably due to the interaction of the nutritional markers. In heterokaryons resistance turns out to be gene-determined, and various relations of dominance have been observed with different mutants.

Some PFP-resistant strains have a partial requirement of tyrosine or phenylalanine and are selectively inhibited by indole. It is, therefore, possible to select PFP-sensitive strains from PFP-resistant mutants in recombination experiments.

P. STRIGINI and G. MORPURGO.

Biotin requirement by wild type Aspergillus nidulans and Neurospora crassa.

Wild-type conidia of A. nidulans show a basic requirement for biotin in a synthetic medium (Czapek-Dox) where glucose is the source of carbon and sulphate the source of sulphur.

This requirement is not apparent if large inocula are made on the same medium. The biotin needed is 10^{-6} γ/ml, that is, 1/10 of the minimum amount required by the biotinless mutant bi1.

The biotin requirement is bypassed by replacing the source of carbon with intermediates in Embden-Meyerhof pathway (fructose, pyruvate, acetate), or in pentose pathway (gluconate, ribose) or in Krebs cycle (succinate, citrate).

Total replacement of the source of sulphur by reduced sulphur sources (sulphite, thiosulphate, sulphide or cysteic acid, cysteine, but not methionine) allows a delayed growth in the absence of biotin. Substitution of glucose with fructose or sulphate with sulphide allows Neurospora to grow without biotin. Germination and growth of Penicillium chrysogenum is not affected by biotin. The biotin requirement of Aspergillus is partially supressed in a parathiotrophic strain (S_{12}) while this is not the case with a methionineless mutant (meth.1).

We wonder why the biotin requirement has not been noticed by other researchers in Aspergillus. We would appreciate any information available in other laboratories on this point.

G. MORPURGO.

Somatic segregation induced by p-fluoro-phenylalanine (PFP)

PFP, added to the complete agar medium, is an effective inducer of somatic segregation in diploid Aspergillus. The segregation involves only whole chromosomes, probably through a mechanism such as non-disjunction. 8-azaguanine and 5-bromouracil are uneffective.

T. SEARASHI

Genetics studies on amylase in A. oryzae.

Fifty amylase mutants have been independently obtained from Asp. oryzae by U.V. irradiation, using filter method in which minimal solution was supplemented by starch or amylopectin as C source. These mutants do not grow or stand in growth on the medium containing starch, but grow on glucose.

Genetic investigation on amylase production was carried out through "parasexual cycle" (Pontecorvo et al, 1953). Twenty-five out of fifty mutants were marked by differences in nutritional requirements and in colour of the conidia and they were used in this work. All twenty-five mutant genes (ae1 - ae25) are recessive to normal one. None of the heterokaryons and heterozygous diploids synthesized between twenty-four non-producing mutants (ae1 - ae24) did clearly restore amylase production, whereas, in the case in which ae1 - ae24 strains were combined with ae25 strain, all heterokaryons and heterozygous diploids produced as much amylase as the normal strain.

Vegetative segregants from heterozygous diploids involving amylase mutant genes were distinguishable haploid from diploid and further analysed their genotypes on the basis of their phenotypes, according to the conclusion of vegetative segregation in A. nidulans by Pontecorvo et al. and the model in Penicillium chrysogenum by Sermonti (1956). Twenty-four mutant alleles, ae1 - ae24 which are negative in dextrinizing power and saccharifying power, are at one of the loci and linked to w, ad and me (belong to I linkage group).

One mutant allele, ae25 which is normal in dextrinizing power but not in saccharifying power, is at the other locus and linked to lys and thi (belong to II linkage group).

Two loci on different chromosomes concerned with the capacity to produce amylase in A. oryzae, have been presumably identified.

E. KAFER-BOOTHROYD.

Culture Media:

Complete Medium: The complete medium used in Montreal (Advances in Genetics 9, p. 107, 1958) is supplemented with adenine, methionine, riboflavin, putrescine ($\frac{1}{2}$ of the amount used for supplementing minimal medium) and lysine (twice the amount used for MM) when the corresponding mutants are used. It inhibits the growth of the pantothenic acid requiring mutant (panto).

Yeast Extract Medium (devised by Dr. R. W. Barratt) is used for panto and supports most other strains very well, giving dense conidiation and colour. It contains only 0.5% Difoo yeast extract, 2.5% glucose, 1.25% agar (no adjusting of pH needed). For optimal growth of ad, meth, phen, ribo and pu additional supplements ($\frac{1}{2}$ amount used for MM) are needed.

Interaction on Supplemented Minimal Medium:

phen - nic: Test for nicotinic acid requiring mutants (nic8, nic2) is complicated, when a phenylalanine requiring mutant (phen2) is also segregating. On MM with normal (or suboptimal) amounts of phenylalanine (lacking nicotinic acid) strains carrying nic and phen2$^+$ will not grow at all, while strains carrying nic and phen2 will grow partially. Tryptophane, to which nic 8 normally responses, seems to have rather an inhibitary effect under these conditions. Extra amounts of phenylalanine make clear cut tests possible.

meth - paba: Normally p-aminobenzoic acid requiring mutants do not adapt at all on the MM used. It was found, however, that optimal (or higher) concentrations of methionine permit growth of paba1 to practically normal size. The paba-mutant is only recognizable by the reduced amount of conidiation.

Strains:

y; thi1 (Glasgow strain) contains aberrations involving linkage groups III, IV and VIII.

y; thi1; panto (Glasgow strain) contains further aberrations; the original strain has not yet been analysed; a panto - recombinant tested contained aberrations involving linkage groups, I and V, and III, VII, VIII. Other panto - recombinants contained less rearrangements, but panto has not yet been found separate of the III - VIII - translocation. Meiotic linkage of panto to s0 (linkage group III) and cha (linkage group VIII) is found. Localization of panto on linkage group III (Advances in Genetics 9, pp. 117, 127, 138, 1958) is, therefore, still doubtful. Strains for a detailed analysis are being prepared.

bil; w3; cys2 (Glasgow strain): cys2 was crossed to various standard strains. In early crosses growth response of "cys2" - recombinants varied a great deal, some growing on normal concentrations of thiosulphate, others requiring higher concentrations or cystine. Recombinants responding to thiosulphate adapt like sulphite mutants; in cross-feeding tests it was found that alleles of s1 and s3 crossfeed these recombinants but not alleles of s0 (=s_d =s12 in Glasgow). Diploids Q_1 and Q_2 used for localization of "cys2" (Advances in Genetics 9, p. 121, 1958) have been reanalysed. Complete linkage of "cys2" to ribo2 (in diploid Q_2) was confirmed; using crossfeeding tests, "cys2" was distinguished from s3 in haploids from diploid Q_1 and complete linkage of "cys2" with linkage group III was identified. The "cys2" - strain used therefore contains a rearrangement involving linkage groups III and VIII and possibly a modifier influencing the requirement of "cys2"; "cys2" is likely to be an allele of s0.

The Wild Type Strain (Glasgow) of A. nidulans and Results of Backcrossing:

The strain adl4 pro1 paba1 y; w3 (Glasgow) has been backcrossed eight times to obtain standard strains, with various markers, close to the original wild type for meiotic analysis of small rearrangements. The frequency of crossed perithecia (on MM + ad + paba) was usually high, often 100%. More than usual variation in colony-size and development-time was observed in these crosses as well as in other crosses using the new backcross strains. This may indicate that modifiers are present in the original A. nidulans strain which are selected against when recombinants for stock are selected from crosses. The viability of the mutant - alleles (as measured by allele ratios of mutant: wildtype) was generally lower at the beginning (especially for adl4 which seemed to be associated with a reduction in colony size in some backcrosses) and increased later. No significant change in crossing-over frequency

was observed. Fluffyness (as observed in wildtype, but not in most other Glasgow strains) segregated in the first few crosses (later all recombinants were fluffy). It seems to be controlled by a single gene (smooth colonies usually being in slight excess over fluffy ones). It is not easy to recognize an MM or thin CM. No meiotic linkage has been found in crosses to markers on linkage group I, w3, pyr04, s3 and nic8. No attempt at localization in diploids have been made so far, and no gene-symbol has been decided on, as it is not known whether "fluffy" or "smooth" is dominant.

Conidial Colour Mutants:

Bw: Further crosses of this mutant (to markers meth1, nic2, cho and cha) have not yet revealed any meiotic linkage. Influence of temperature on its expression has been found: parts of a colony grown at 35°C shows the "brown" phenotype, while parts grown in room temperature show the conidial colouring determined by the residual genotype. 10 different colour combinations can be distinguished.

cha: A recent spontaneous colour mutant (light green = chartreuse) cha has been isolated from a sector in the cho-bil T (I - VII)-strain. There is a slight reduction in size of the conidial heads; new growth of y cha is very light yellowish green, but colonies turn purple on continued incubation. The mutant cha is independent of T (I - VII) and has been located on linkage group VIII, not meiotically linked to ribo2 or co. The probable order of the three mutants (as judged from the type of cha/cha - sectors obtained from a diploid with all three markers in coupling) is cha - ribo2 - centromere - co.

Linkage Group VII

Further analysis of the T (I - VII) found in the original cho bil - strain indicates that nic8 and cho may be on the same chromosome arm, nic8 being more proximal. The breakage point of the translocation is located distal to cho.

A.TECTOR.

Genetic analysis of irradiated diploids.

Conidia from a diploid genetically marked at least once on each linkage group were given various doses of irradiation with Cobalt-60. Normal-appearing colonies growing from these were selected for analysis by mitotic haploidization.

Among those analysed, a third of the strains showed random segregation of unlinked markers, as found in the parental diploid. For the different doses, these were: 0/2 from 50,000 r, 4/14 from 40,000, 2/3 from 35,000 and 3/9 from 30,000.

Of the others, 6 showed complete linkage between the markers of two linkage groups, probably caused by a translocation. The groups involved were I - V, I - VII, II - VII, II - VIII, III - V and IV - VIII.

Three others had multiple associations of various sorts, one between linkage groups I - IV - VIII, another between IV - VII - VIII, and the third between II - VIII and V - VI - VII. Another strain was aneuploid (2n + 2) and also appeared to have rearrangements involving I, II, III and VII.

In each of two strains, a mutation requiring either nicotinic acid or

trytophan was found, located in linkage group VII, but not yet tested for allelism with nic8. Both strains carry further aberrations.

Three of the remaining diploids are still being analysed; each has one translocation and may have either a lethal or another rearrangement. The other three strains are not analysable by the selective methods available.

Survival of haploid and diploid conidia.

The survival curves of conidia irradiated with doses of Cobalt-60 X-rays from 6,000 to 60,000 r were remarkably similar for haploid and diploid strains. Both were straight lines with similar slopes on semi-log paper. The survival at 60,000 r was about 0.1%.

Control and irradiated conidia of haploid and diploid strains were plated at several densities. Crowding caused a decrease in survival that was apparent even when there were fewer than 100 colonies per plate and counts were made 18 to 24 hours after plating. The drop in survival frequency became more marked as the density increased. This effect was found in both haploids and diploids, and had no apparent relationship with the irradiation dose used.

C. F. ROBERTS

The genetic analysis of sugar mutants.

Wild type utilises a number of carbohydrates as carbon sources for growth and mutant strains lacking the ability to grow with specific sugars as sole carbon source were isolated by replica plating following U.V. irradiation. The mutants are called sugar mutants, they are classified on standard minimal medium in which glucose is replaced by the relevant carbohydrate (1% w/v.).

The mutant phenotypes segregate 1:1 in crosses to wild type. Analysis by recombination and complementary tests reveales that the 27 mutants isolated represent 10 loci, 9 of which have been located in linkage groups and 7 of these accurately mapped. The loci and allelic relationships of the mutants are shown in Table 1.

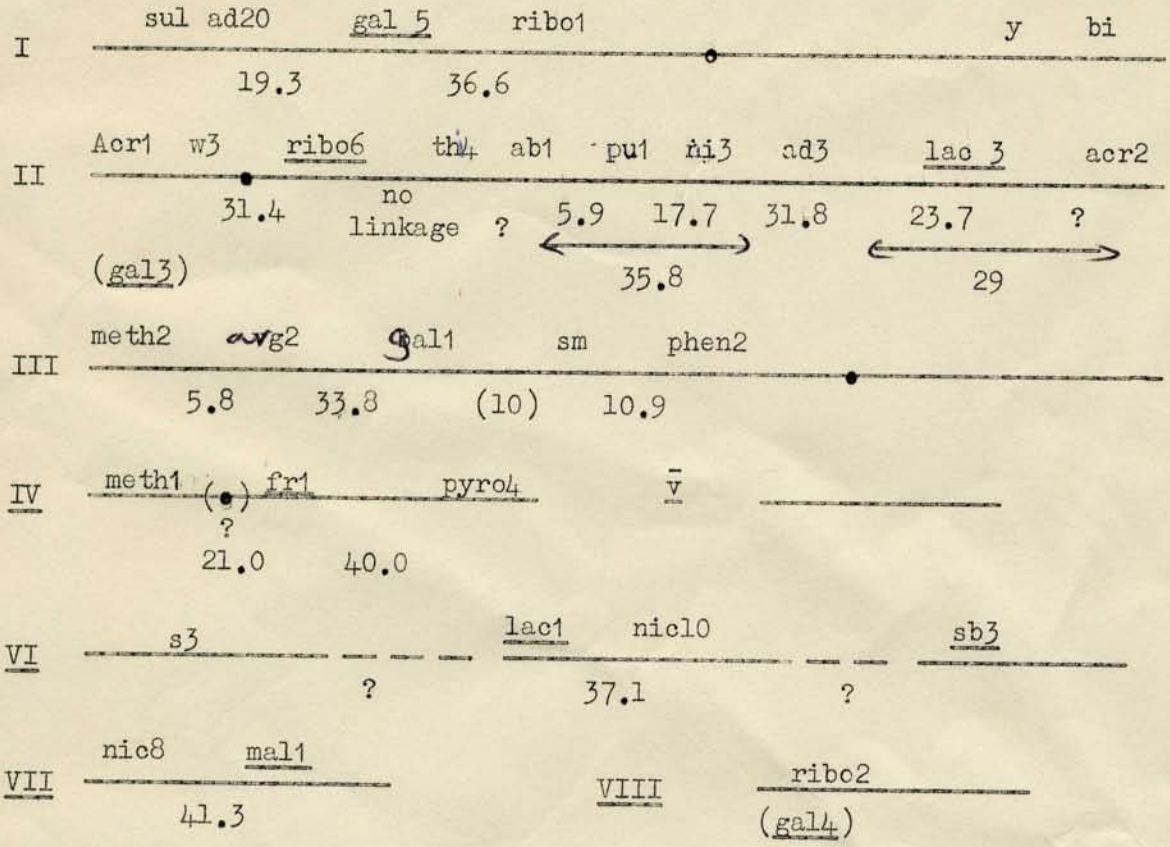
Table 1. The Genetic Analysis of Sugar Mutants

Locus	Phenotype	Linkage Group	No. of alleles	Allelic mutants
fr	Fails to utilise fructose	IV	3	fr 1 - 3.
sb3	" " sorbitol	VI	7	sb3 - 9.
lac 1	" " lactose	VI	5	lac 1, 2, 4, 6 and 7.
lac 3	" " lactose	II	2	lac 3, 5.
mal 1	" " maltose	VII	2	mal 1, 2.
gal 1	" " galactose	III	2	gal 1, 6.
gal 2	" " galactose	VI or VIII	1	-
gal 3	" " galactose	II	1	-
gal 4	" " galactose	VIII	2	gal 4, 7.
gal 5	" " galactose	I	2	gal 5, 8.

The location of the sugar markers is shown in Diagram 1. The linkage groups are after Käfer (1958) and a number of new nutritional markers (ribo 6, riboflavin, abl, γ amino butyric acid, arg2, arginine) isolated and located in linkage groups by other workers have also been mapped in the course of the analysis. Stocks of all these mutants are held at Glasgow, while the sugar mutant stocks are also held in Oxford.

Diagram 1

Location of sugar markers.



Distances in meiotic recombination fractions (%)

The markers in parenthesis have not been located accurately.

In general the sugar mutants provide satisfactory markers with good viability and not interacting in any way. They may be used in selection experiments though often heavy background growth is experienced. This is particularly so with the partial mutants. There is no reason against extending the range of markers available in nidulans (or any other colonial mould) by use of further carbohydrates.

The genetic control of galactose utilisation.

In a series of 12 different U.V. irradiation experiments a further 28 mutants failing to utilise galactose were isolated from 16,517 colonies tested by replica plating. Qualitative tests for recombination with gal1, gal5 or gal7 have grouped 18 of these mutants as probable alleles of the loci while 10 mutants are unlocated. The present state of the analysis of the galactose mutants is shown in Table 1.

Table 1. The genetic analysis of galactose mutants

Locus	Non-complementary mutants	Closely linked mutants	Maximum number of alleles
gal 1	2	10	12
gal 2	-	-	(1)
gal 3	-	-	(1)
gal 4	2	3	5
gal 5	7	-	7
Unlocated mutants			10
		Total mutants	36

1. Mitotic gene conversion at the gal5 locus

A fine analysis of the gal5 locus using four alleles (gal5, 8, 10, 13) was attempted via the parasexual cycle. Diploids homozygous for each of the alleles were synthesised and pairs of diploids heterozygous for the alleles taken in all combinations and with reciprocal arrangements of the outside markers su1 ad20 and ribo1 were also synthesised.

su1 ad20 (gal5 +) ribo 1 leu 1 + + y ad20 +

\+ (+ gal8) + + po1 pabal + ad20 w3

Written gal5/ gal8

su1 ad20 (+ gal8) ribo 1

\+ (gal5 +) +

Written gal8/gal5

When conidia of the diploids are plated on galactose medium with an excess of adenine slow growing colonies develop, some of which produce diploid sectors growing like the wild type on galactose.

Frequency of sectoring

The homozygous diploids produce sectors at very low frequency whereas certain pairs of heterozygous diploids produce sectors at a much higher frequency (Table 2). The average number of sectors per colony (m) is estimated from the proportion of colonies without sectors (Po).

$$Po = e^{-m}$$

Generally the heterozygous diploids with reciprocal arrangement of the alleles form sectors at frequencies of the same order of magnitude.

Distribution of the distal marker.

The genotypes possible for the distal marker are distinguishable phenotypically. All 172 gal$^+$ diploid sectors isolated were of the same phenotype as the original diploid (su1 ad20/ +) and had apparently arisen

without crossing over in the interval ribo1 - su1 ad20. On selection of strains arising by mitotic haploidisation from one series of 20 gal$^+$ diploids (10 from 5/13 and 10 from 13/5) it could be shown that the diploids had arisen without crossing over and apparently by reversion of one or other of the alleles.

There results may be interpreted as mitotic gene conversion of the gal5 alleles.

Table 2. Diploid gal$^+$ sectors produced from diploids of gal5 alleles

Diploid	No. of colonies plated	No. of sectors	Mean no. of sectors per colony $m \times 10^{-3}$
5/5	235	1	4.7
8/8	370	0	0
10/10	251	1	14.1
13/13	310	0	0
5/8) 8/5)	1835	62	34.4
5/10) 10/5)	718	2	2.8
5/13) 13/5)	1770	99	57.5
8/10) 10/8)	282	1	5.5
8/13) 13/8)	510	3	5.9
10/13) 13/10)	1245	5	4.0