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CURRENT RESEARCH PROJECTS

G. MORPURGO.

Effects of monofunctional and bifunctional mustards in inducing mitotic crossing-over. The purpose is to test the hypothesis that the very high frequency of mitotic crossing-over induced by treatment with the bifunctional mustard methyl bis (β chloroethyl)-amine is due to the cross-linking of non-homologous chromatids and hence to effective exchange of parts. First results strongly support this hypothesis.

M.G. PETRELLI, R. RICCI and G. SERMONTI.

Study of suppressor mutations of pfp-r (p-fluoro-phenylalanine resistance).

PRELIMINARY NOTES ON CURRENT RESEARCH AND NEW LINKAGE DATA

GIORGIO MORPURGO.

Somatic segregation induced by p-fluorophenyl-alanine (p-FP)

Conidia of a diploid strain of A. nidulans heterozygous for colour and nutritional markers were incubated 12 hours in a liquid minimal medium plus 0.04 mM p-FP and then plated on complete medium. The treatment with p-FP produced a lethality of about 70% of the conidia. On the complete medium treated conidia gave origin to two types of colonies about equally frequent; 1) normally growing colonies. 2) extremely dwarf, non-sporulating colonies.

The colonies belonging to the latter type were transferred on fresh medium. After a few days most of them produced healthy sectors, green or segregant for colour markers. All the sectors were further analyzed for nutritional requirements. The analysis showed that all the segregant sectors were haploid or non-disjunctional for one or more chromosomes.

The same treated conidia can frequently produce haploid and non-disjunctional sectors being so analogous to what Käfer calls "breakdown types."

In addition to the preceding types p-FP can produce also colonies without conidia and unable to produce sectors. These types can occasionally grow at a normal rate. Aconidial types and dwarf sectoring colonies are also produced by treating haploid strains with p-FP.

p-FP does not produce any increase in somatic crossing-over frequency or in the coincidence of haploidization with crossing-over and does not produce any kind of "point mutation" after treatment of haploid strains.

LORNA J. LILLY

A possible technique for the simultaneous measurement of forward mutation rate in several cistrons.

The reversion from methionine requirement ($meth_1$) to prototrophy in A. nidulans occurs with very high frequency (approximately 10^{-5}). The reversions can be shown to be due to suppressor genes, all those so far tested being unlinked to $meth_1$. The suppressors have various visible phenotypes ("A", "B", "C", etc.). If each phenotype is determined by a single cistron then these suppressors could be used for the simultaneous visible scoring of forward mutation in several cistrons.

Preliminary investigations have been made on some of the phenotypes occurring with the highest frequency. The results of crossing one strain of type "A" with one of type "B" indicate that each strain carries a single suppressor and that the two suppressors are unlinked. Crosses between strains of type "A" show that at least two unlinked loci can independently determine this phenotype.

It is interesting that Siddiqi, who used these suppressors when studying nitrous acid induced mutations, found that mutation to type "A" occurred with 2.5 times greater frequency than mutation to either "B" or "C". He suggested that this might be due either to a larger target size or to

differences in sensitivity to nitrous acid. The evidence that at least two cistrons control phenotype "A" may make the latter theory unnecessary. Attempts are currently being made to cross other strains. The location of the suppressors is in progress.

D.J. COVE

Unlinked loci asscciated with nitrate reductase in A. nidulans

In order to get an homogeneous sample of auxotrophic mutants for gene-enzyme studies, conidia from a prototrophic strain of A. nidulans were treated with U-V light. Of the colonies which developed from treated spores, 30,000 were tested on minimal medium and about 1,200 were no longer able to grow. Of these, 135 could grow when the $NaNO_2$ in the minimal medium was replaced by NH_4Cl, and 40 of these could also utilise $NaNO_2$ as a nitrogen source. Heterokaryon complementation studies on these latter mutants divide them into nine groups. Linkage studies on representatives of these groups indicate that at least five and probably seven loci are involved, none of which are closely linked. All 40 mutants have been shown to lack the enzyme nitrate reductase, when grown under conditions inducing appreciable activity in wild. The enzyme may be assayed either by following the oxidation of reduced triphosphopyridine nucleotide spectrophotometrically, or by measuring NO_2 production colorimetrically. The enzyme is inducible and it is hoped that the system will be useful for the study of genetic control mechanisms.

D. APIRION

Phenotype of paba1 with succinate as only carbon source

By replacing glucose with succinate as only carbon source, paba1 is no longer p-aminobenzoic requirer. In a medium containing 0.5% glucose and 0.5% succinate (w/v) the requirement for p-aminobenzoic acid is like on glucose. This suggests either an inhibition by glucose of the utilisation of exogenous PABA, but not of endogenous PABA, or two alternative pathways of PABA synthesis, one of which is inhibited by glucose (in the wild type as well as in paba1) and the other is blocked in paba1.

G.A. VAN ARKEL

Sodium arsenate as an inducer of somatic reduction

The recovery of haploid segregants from heterozygous diploids of Aspergillus has been greatly facilitated by the discovery of the p-fluoro-phenylalanine treatment (Morpurgo, Aspergillus News Letter 2, 10, 1961).

It was found, however, that when a diploid is heterzygous for the phenylalanine requiring mutant phen2, only $phen2^+$ recombinants are produced. Consequently, in the analysis of new markers only half of the possible information is obtained for linkage group III, when in a tester strain phen2 is used as the marker for that chromosome.

This complication can be overcome by plating conidia from a heterzygous diploid on CM supplemented with sodium arsenate to a final concentration of 0.05 M. Isolations made of vigorously growing sectors yield haploid strains of all possible segregant classes, indicating that interaction with nutritional markers is absent. Spontaneous resistant colonies occur rather frequently and resistance turns out to be gene-controlled.

J. FOLEY and N.H. GILES

Studies with adenine-requiring mutants of A. nidulans.

A series of adenine-requiring mutants of A. nidulans is being isolated for the following purposes. 1) A comparison of adenine biosynthetic pathways and their genetic control in A. nidulans, S. cerevisiae, and N. crassa. 2) Comparative studies of interallelic complementation with adenine-specific mutants in diploids and in heterokaryons.

Adenine-requiring mutants are being isolated following ultra-violet irradiation, utilizing the filtration-concentration technique of Woodward et al. (P.N.A.S. 40: 192-200, 1954). The mutants thus far obtained fall into several categories: adenine specific types, hypoxanthine utilizers which do not secrete purple pigment, and those which do. The mutants are still being isolated and none has as yet been located genetically.

Histidine-requiring mutants of A. nidulans.

During the above mutant hunt, four histidine-requiring mutants were obtained from plates supplemented with both adenine and histidine. The fact that they are all inhibited to a greater or lesser extent by complete medium probably explains why this type of mutant has not been reported previously in Aspergillus.

M. GRINDLE

A comment on the heterokaryon compatability of Aspergillus nidulans

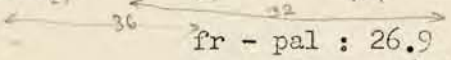
It has long been our experience that not all strains of Aspergillus nidulans would form heterokaryons with one another. We now have evidence that whereas marked strains of the same wild isolate of A. nidulans almost invariably form heterokaryons with each other, marked strains of different wild isolates seldom do so. If anyone has experience of this apparent incompatability system, as affected by different markers and by different isolates, we shall welcome their comments.

G. DORN

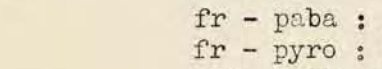
A revised map of the right arm of chromosome IV

Previous data suggested that paba22, which Siddiqi (Thesis, 1961) had located distally to pyro4, was in fact, located between pyro4 and fr1.

A new mutant, palC-4, splits this interval, and the order is now as follows: fr1——palC-4——paba22——pyro4. The recombination fractions percentage in a cross: y; fr1 paba22 pyro4 x bi1; palC-4, with 279 classified ascospores, were:



fr - pal : 26.9
fr - paba : 36.0
fr - pyro : 38
pal - paba : 27.2



pal - pyro : 32.6
paba - pyro : 10.4
(free recombination in all other cases except y——bi)

C. DE PALMA and G. MORPURGO.

The mutation pfp-1 for p-fluoro-phenylalanine resistance has been located on the left arm of the first chromosome of Aspergillus nidulans between the centromere and ribo-1, 0.2 units from ribo-1. Ten other independent resistance mutations are allelic with pfp-1.

Glasgow list of located, or partially located, mutants of Aspergillus nidulans

(Supplementary to the lists in Advances in Genetics, 1953 pp 202-203 and 1958 pp 108-113)

Symbol of locus	Phenotype	Linkage group and position*	Alleles	Origin: Strain	Mode	Year	Reference(x)
	1. Nutritional						
ab	-amino butyrate	IIR		bil	UV	1957	Forbes 1959
an2	aneurin	II		bil; AcrI w3	"	1956	Pontecorvo & Kafer
arg2	arginine	IIIL meth2		bil	"	1957	Forbes 1959
arg3	arginine	VIIIR		bil	"	"	"
frI	fructose⁻	IV-R		y2; pyro4	"	"	Roberts
gal I	galactose⁻	III-L	gal6	bil; w3	"	"	"
gal2	"	VI or VIII		bil; w3	"	"	"
gal3	"	IIL		bi; w3	"	"	"
gal4	"	VIII	gal7	bi; w3	"	"	"
gal5	"	IL	gal8	bi; w3	"	"	"
lac I	lactose⁻	VI	lac, 2, 4, 6, 7	y2; pyro4	"	"	"
lac 3	"	IIR	lac5	bil; w3	"	"	"
lu	leucine	IL		bil	"	"	Forbes
mal I	maltose⁻	VII		y2; pyro4	"	"	Roberts
meth2	methionine	IIIL arg2		bil	"	1956	Forbes 1959
orn8	arginine/ ornithine	VIIIL	orn7, 9	bil	"	1957	Forbes 1959

Symbol of locus	Phenotype	Linkage group and position*	Alleles	Origin: Strain	Mode	Year	Reference (x)
paba22	p-aminabenzoate	1VR palC-4-pyro4	paba21	bil	UV	1960	Siddiqi 1962
palA-1	inability to split α-naphthyl phosphate at pH 8	111L arg2-gal 1	2, 3, 6, 12, 14	bil rA-1	"	1962	Dorn
palB-7	do	V111R cha		"	"	"	"
palC-4	do	1VR frl-paba22		"	"	"	"
palD-8	do	V11 nic8		"	"	"	"
palE-11	do	11		"	"	"	"
rA-1	enhanced ability to split α-naphthyl phosphate at pH 8	1L gal5		bil	"	"	"
pac-A-1	inability to split α-naphthyl phosphate at pH 5	1V		bil rA-1	"	"	"
pac-B-2	do	11 ribo6		bil	"	"	"
pac-C-3	do	V1 or V111		bil	"	"	"
ribo1	riboflavine	1L	ribo7	bil	"	1950	Macdonald
ribo2	"	V111	ribo4	bil; Acrl w3	"	1956	Pontecorvo & Kafer
ribo3	"	V		y; pyro4	"	1957	Forbes & Sundaram
ribo5	"	V		y; pyro4	"	"	" "

Symbol of locus	Phenotype	Linkage group and position*	Alleles	Origin: Strain	Mode	Year	Reference (x)
ribo6	riboflavine	ll		bil; w3	UV	1958	Forbes & Sundaram
sb3	sorbitol⁻	Vl	sb5	bil; w3	"	1957	Roberts
sul-paba22	suppressor of paba22	lVR paba22	su-4paba22	bil; paba22	spont	1962	Luig 1962
	2. Visible						
bll	blue ascospores non autonomous	ll	bl2, 3, 4	y; w2; sl2	NO2	1961	Apirion 1963
cl4	white ascospores non autonomous	lVL		y; w2; sl2	NO2	1961	Apirion 1963
cl6	do	lL ribol	cl1, 3, 5	"	UV	"	" "
	3. Resistant						
apl	aminopterin resistant	Vlll (R?)		bil	spont	1962	Forbes
f101	resistant to fluoroacetate, unable to grow on acetate	Vlll R arg3-ribo2		w3; pyro4	spont	1962	Apirion
f102	do	Vlll		w3; pyro4	"	"	"

TRANSLOCATIONS

Strain	Chromosomes involved
pabal	Vl-Vll
bil; w3	Vl-Vll (also possibly lll-Vlll)
bil; w2	lll-Vlll
bil and MSD	no translocation

* Two loci indicated in this column mean that the mutant has been located meiotically between them; one locus means meiotic linkage with locu

(x) Unpublished, when no indication of the year.

E. FORBES.

A Strain with all Chromosomes marked for use in haploidisation.

For assigning any new mutant to its linkage group, or for identifying translocations producing pseudo linkage between two mutants known to be on different linkage groups, a master strain with a marker on each of all eight linkage groups is desirable. Such a strain has been in use in this laboratory for about three years. It was obtained by crossing "Tester Strain A" (Forbes, Heredity, 1959, 13, 67-80) to su1-ad20 y ad20; Acr1; s3; ribo2, and isolating a recombinant (Master Strain D, abbreviated M.S.D.):

1	11	111	1V	V	V1	V11	V111
su1ad20 y ad20	Acr1	phen2	pyro4	lys5	s3	nic8	ribo2

The method of selection of haploids of the 1959 paper can be used with M.S.D. in the same manner as it was with the previous two Tester Strains (A & B), the advantage being that only one diploid is necessary for scanning all eight linkage groups; the limitation is still that distinction between linkage groups 1 and 11 cannot be made.

Since 1960 however, routine haploidisation has been done in this laboratory by the Morpurgo (Aspergillus News Letter, Spring, 61.) and Lhoas (Nature, Lond. 1961, 190, 744) technique of growing diploids on DL p - Fluorophenylalanine (FPA) 0.12g/2 litres of CM.

Selection of haploids on FPA has the complication that since there is competitive inhibition between phenylalanine and FPA, only very few of the haploids carrying phen2 are able to grow i.e. only two of the possible four classes in respect of Chromosome 111 and any other chromosome are available.

One obvious improvement would be to have a master strain with chromosome 111 marked by a marker other than phen2. Another improvement would be to dispense with the su-ad20 ad20 system since only one marker is required on each chromosome when using the FPA technique.

D. APIRION, G. DORN and E. FORBES.

The V111 linkage group.

This linkage group has turned out to be large. The loci so far identified are:

co, ribo2, cys2*, cha (Kafer 1956, 61)

orn8, arg3, ap1 (Forbes 1959 & unp.)

f101, f102 (Apirion, unp.)

palB (Dorn, unp.)

gal2* and gal* (Roberts 1961)

The mutants with an asterisk arose in strains later known to carry translocations.

Meiotic analysis gave the following results:

Cross	Total analysed	Recombination fractions in chromosome V111
paba 1; w3; f101 x bil; orn7 ribo2	102	orn - f:39; f - ribo: 4.8 orn - ribo:38
bil; arg3 x bil; orn7 ribo2	54	arg - ribo:26

bil; arg3 x w3; paba1; f101 99 arg - f:20; others: 50;

bil; w3; gal7 x paba1; 194 orn - f:40; f - ribo:3

orn7 f101 ribo2 orn - ribo; 39; others: 50;

Continued ....

Meiotic analysis (continued)

Cross	Total analysed.	Recombination fractions in chromosome V111.
bil; arg3 x bil; f101 ribo2	200	arg - f:23; f - ribo:3.5; arg - ribo:22
bil; orn9 cha x bil; arg3 f101 ribo2	202	arg - f:18; f - ribo:4.5; arg - ribo:21; others:50;
bil; arg3 x paba1; w3; orn7 f101 ribo2 gal7	204	orn - arg:40; arg - f:22.5; f - ribo:3; arg - ribo22 others: 50
bil; orn9 cha x bil; arg3 f101 ribo2	selected ribo+ f- 156	arg - f:34
paba1 x bil; arg3 f101 ribo2	selected ribo+ f- 259	arg - f:34
bil; arg3 x bil; f101 ribo2	selected ribo+ f- 104	arg - f:20
ad23; cha x w3; orn7 ribo2 gal7	selected w+ 101	all:50
bil; ad23; palB-7 cha x paba1; w3; orn7 ribo2	selected 95 b1+; w+ ad+;	cha - pal:8 others:50

From these data we conclude that there are two or possibly three groups of meiotically linked loci:

1) co and orn8, very closely linked (Forbes unp.)

2) arg3 - f101 - ribo2, in this order (left to right) showing about 20% and 5% recombination, respectively.

3) cha and palB showing less than 10% recombination, with palB probably distal.

With the information provided by Kafer (1961) on mitotic crossing over in the right arm we can draw a map with group 1 to the left, group 2 in the middle and group 3 to the right. The most probable order for the last two groups is arg3 - f101 - ribo2 - cha - palB.

B. J. KILBEY.

Yellow conidial mutants of Aspergillus nidulans strain 16643*

In preliminary work with this strain of Aspergillus nidulans three independent mutations of the gene Y were isolated. These mutants have yellow conidia instead of the green conidia of the wild-type; their symbols are y^{5}, y^{13} and y^{26}.

Spectrophotometric analysis showed that each mutant possessed the same conidial pigment. The conidial colour was unchanged by variations in the temperature of incubation (25°C - 37°C), the pH of the medium (6.8-5.4), or by alterations in the levels of chloride and copper ions in the medium (cf. Takagi, 1957). No revertants of these mutants were found in 10^{5} - 10^{6} conidia and the three mutants were non-complementary when combined pairwise in the diploid conidium.

Green spored colonies were found to arise with low frequencies among the progeny from crosses between different yellow mutants. None were found when each mutant was self fertilised. This fact suggested a recombinational origin for the green progeny. Support for such a conclusion was obtained from the genotypes of the green isolates.

B. J. KILBEY.

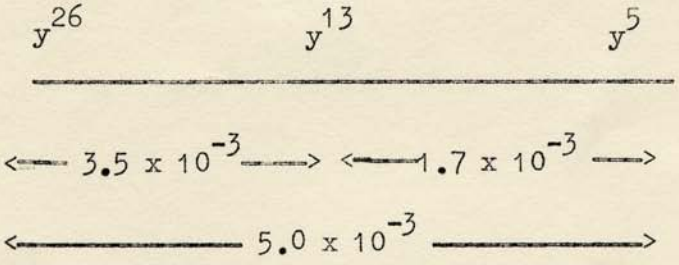
In every case except one (12/13) the occurrance of the green phenotype was associated with recombination among the controlling markers bi, pro (biotin and proline requirements linked on either side of Y) and leuc (leucine requirement unlinked to Y). Since less than 10% of the ascopores in these crosses originated from heterozygotic products of fertilization such a result is unlikely to be obtained by random mutational events. In any case, no revertants have been found in comparable numbers of conidia.

Estimates of recombination percentages between these three mutants have been calculated together with the 95% limits of expectation for the true value (based on the Poisson distribution). These figures are given in Table 1. and summarised in a tentative map of the region.

Table 1.

% Recombination between the mutants y^{5} y^{13} and y^{26}

Cross between	% Recombination	95% Limits
$y^{13} \times y^{5}$	1.7×10^{-3}	3.1×10^{-4} - 2.3×10^{-3}
$y^{5} \times y^{26}$	5.0×10^{-3}	2.3×10^{-3} - 9.4×10^{-2}
$y^{26} \times y^{13}$	3.5×10^{-3}	1.6×10^{-3} - 6.4×10^{-3}



Reference: Takagi, Y. (1957) J. Gen. appl. Microbiol., 3, 125.

* Commonwealth Mycological Institute collection.

This is a part of work done during the receipt of an M.R.C. Research Studentship.

E. KAFER-BOOTHROYD.

Origin of eight translocations in stock strains of A. nidulans.

Most of the translocations repeatedly encountered in stock strains of A. nidulans have now been traced back to their origin and a large number of strains which have translocations in their pedigrees have been tested. Of the eight translocations which were found in various strains, five appear to have been induced by treatment with X-ray, three with UV.

a) T(I-V11), UV-induced in bil, at the same time as cho. Analysis is completed and published (in Genetica 33, p.62, 1962).

b) T(V1-V11), X-ray induced in bil at the same time as pabal (pedigree in MGB 19, p.13, 1963). T(V1-V11) is present in bil;w3 and pabal y; Acr1; co and has spread so widely through most pedigrees that a complete analysis is impossible. 21 further strains which are recombinants from eight crosses heterozygous for T(V1-V11) have been tested: 11 were found to carry T(V1-V11), 10 to be without it. The widely used strains ad14 prol pabal y; w3 (from cross ad14 pabal y x prol bil; w3) and its offspring ribo1 ad14 prol pabal y = /841/ were both found to be without T(V1-V11).

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c) T111-V111) and T(111-1V-V111): T(111-1V-V111) appears to consist of two translocations, one of them being T(111-V111); they were induced by x-ray in the original wildtype strain at the same time as $y(=y^2)$. T(111-1V-V111) is found in y ad2, y; thi1 and most likely in y; w2 ad1. T(111-V111) is present in bil; w3 (and presumably in its parent y; ad1) and, therefore, in all mutant strains derived from this strain. It has been identified in bil; ad23 w3, in bil; w3 pu, in an offspring of bil; w3; "cys2" and in one of two offspring of bil; w3; sb3. T(111-V111) has also been found in two further strains, one of them a 5th generation descendent of bil; w3 ni3, the other a 6th generation descendent of y; thi1 in the "panto" pedigree. (Complete pedigrees available to anyone interested). A further aberration appears to be present in the bil; w3 pu strain. However, results to date have not been conclusive, since analysis of diploids containing several translocations is often complicated by a high frequency of mitotic segregants which are "abnormal", that is, e.g. sectoring like aneuploids but not hyperhaploid, or stable and hperhaploid, or segregant for several chromosomes and diploid. Determination of the ploidy of mitotic segregants, therefore, becomes more difficult and measurement of conidia may often be necessary.

d) T(1-V): Recent results indicate, that the original mutant strain bil; sn; lys5 may contain a translocation T(1-V), possibly induced by the UV-treatment which caused mutation to sn and lys5. The T(1-V) reported earlier (A.N.L. 2, p.11, 1961) in one of the panto strains could be identical with this translocation, since this panto strain is a 6th generation offspring of bil; sn; lys5.

e) The panto-translocations. The mutant panto was induced by x-rays in y; thi1 T(111-1V-V111). Panto recombinants have been isolated from 8 different generations. Of seven tested strains six carry a T(111-V11-V111) from which T(111-V111) has been separated in one case (mentioned under c) above). One of the early offspring of the original panto strain shows an even larger complex, T(11-111-V11-V111). The original y; thi1; panto strain is therefore now assumed to contain a complex (11-111-1V-V11-V111). There would be two newly induced translocations, one involving linkage group 11 and one of 111, V11 or V111, the other V11 and either 111 or V111. As in the case of the original y strain, treatment with x-rays would, therefore, have induced two translocations in the same nucleus in which the mutant panto was induced.

f) T(1-V111), UV induced in bil, at the same time as phen2 (bil; phen2 strain lost). T(1-V111) was first discovered in a diploid designed to map ve. As shown in the diagram, T(1-V111) has been traced back through four generations to the strain ribo1 ad14 y; phen2 /962/, which is a recombinant from a cross between /841/ without a translocation (see paragraph b) and bil; phen2. T(1-V111) in repulsion to y in a diploid completely inhibits formation of yellow diploid conidia, whereas T(1-V111) in coupling with y, as in /962/, produces many y/y - segregants which, on isolation, look like "compact" and produce normal sectors on complete medium. These findings can be explained most easily, if it is assumed, that T(1-V111) is either an unidirectional translocation or a reciprocal one with the translocated segments differing greatly in size (a large segment of linkage group V111 translocated to the y-arm of 1).

Pedigree of Translocation T(1-V111)

ribo1 ad14 prol pabal y /841/ x bil phen2 (†) T(1-V111)?

↓

ribol ad14 y; phen2 /962/ x bil; sm; lys5 /905/

an1 bil; w2; pyro4; s3 /887/ x /961/ bil; phen 2; lys5

ribol y bil; Acr1; phen2; s3; nic8 /889/ x /888/ bil; w2; pyro4; lys5; s3

pabal y; ve$^+$ BCV /466/ x /78/ ribol bil; Acr1; pyro4; s3; nic8

/706/ ribol bil; ve$^+$

Strains with translocations: _____ T(1-V111);
Strains tested, but no translocations found: not underlined.
(1 or 2 linkage groups not marked in diploids testing strains /887/ and /889/)
† = Strain lost; ? = Test not completed
/ / = Montreal stock number (all strains in stock in Glasgow, except /466/ & /706/)

Translocations in Glasgow strains as indicated by complete linkage of linkage groups in mitotic haploids:

T (1 - V111) : bil; Acr1; pyro4; s3; nic8
bil; w2; pyro4; lys5; s3
bil; phen2; lys5.

T (111 - V111): bil; w3 pu
bil; ad23 w3
bil; w3 sb3
bil; w3 "cys2"

T (111-1V-V111) y ad2
y; thil

Origin and pedigree of T (V1-V11) have been presented in MGB 1963.

T. B. BOAM

Preliminary studies on the genetics of Aspergillus rugulosus

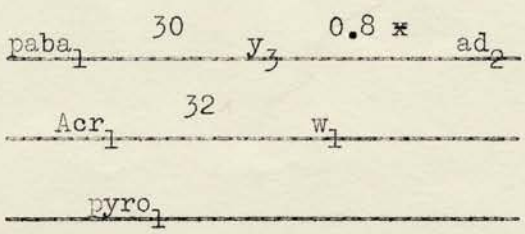
In preliminary investigations into the genetics of A. rugulosus a member of the A. nidulans group - the following points have so far emerged.

1. Techniques and analytical procedure (both mitotic and meiotic) are in general like those for A. nidulans. Growth is good up to 37°C., but heterokaryons grow best and yield most perithecia at 30°C.

2. The spectrum of U.V. induced mutants closely resembles that of A. nidulans. The only conspicuous difference in an individual mutant is the response of the proline mutant which responds to proline or arginine, but not to ornithine or citrulline. Growth of the mutant on arginine is competitively inhibited by lysine.

3. Heterokaryons frequently yield more than 50% hybrid perithecia. In several instances heterokaryons formed between strains which each carry only two mutant alleles gave 100% hybrids.

4. Meiotic and mitotic analyses have so far shown the following linkages.



* It is not yet established whether ad_2 is to the left or right of y_3.

B.W. BAINBRIDGE

A. Genetic analysis of morphological mutants

A number of visible changes have been induced in Aspergillus nidulans by treatment of conidia with ultra-violet light or β-propio-lactone. Four of these have been studied in detail.

mo_1 produces an orange pigment and has a slow irregular growth form. The conidial heads are abnormal in lacking a vesicle and the heads resemble Penicillium spore heads. The mutant gene producing this effect is located between pro_1 and $paba_1$, 3 units from pro_1 and 7 units from $paba_1$.

mo_{87} is characterised by the production of a deep brown pigment and a smooth grey-brown appearance. mo_{89} was isolated independently in another strain, but resembles mo_{87} very closely. The mutants may be allelic as they fail to complement in the heterokaryon. mo_{87} has been provisionally located to chromosome VI by haploid analysis.

mo_{96} is similar in general appearance to "co" but has a more ridged appearance. Conidiation occurs 24 hours later than in the wild type strain. mo_{96} is located to the left of s_{12}, approximately 4.6 units from s_{12} and 5.3 units from s_1.

B. Study of a translocation affecting morphology

It has been observed that crosses between phenotypically normal strains often gave rise to morphological abnormal forms. Some of these were due to the spontaneous mutation of a gene affecting morphology and the expected 1:1 ratio was obtained. In other crosses, however, a morphologically abnormal type segregated to give a ratio significantly different from a 1:1 ratio. The observed ratio was, in fact, a 2:1 normal to morphological type. This type could be readily distinguished by a reduced growth rate accompanied by radial grooves which give the colony a crinkled outline. The following strains consistently gave rise to crinkled colonies when crossed with appropriate strains:
bi_1; w_3. bi_1; w_3; an_5. bi_1; w_3; $pyro_4$. bi_1; w_3; $pyro_{12}$. y; ad_1.

Two hypotheses can be formulated to explain these results. It is possible that two different gene pairs in the parental combinations (a b, a^+b^+) give normal morphology whereas in the recombinant association they interact to produce a crinkled class ab^+ and an inviable class a^+b. Alternatively, an unequal or non-reciprocal translocation would be expected to give, in the products of meiosis, the parental association of chromosomes resulting in normal morphology and the recombinant association of chromosomes resulting in a duplication of a section of chromosome in one class and a deficiency of the same section of chromosome in the other. These classes would correspond to the crinkled and the inviable class respectively.

Because of the known prevalence of translocations in Aspergillus stock strains (Käfer 1962) it was suspected that the second theory was more likely to be correct.

An examination of the pedigrees of the strains concerned showed that they were derived from bi_1; w_3, which is known to have T(III-VIII) and T(VI-VII) (Käfer this Aspergillus News Letter). T(VI-VII) could be eliminated from this study as an appropriate cross between translocated and untranslocated strains did not produce crinkleds. One of the parents of bi_1; w_3, i.e. y; ad_1, gave rise to crinkled on crossing.

Preliminary ascus analysis has shown the occurrence of the expected three types of asci, i.e. (a) 8 normal (b) 4 normal : 2 crinkled and (c) 4 crinkled.

It should be noted, however, that crosses for ascus analysis must be carefully selected, as ascospore viability may be very low if a number of translocations are present. In the cross quoted above, 68% viability was obtained which shows good agreement with the theoretical 75%.

Crosses between crinkleds and translocated strains gave the expected 1:1 ratio in most cases, but occasionally gave rise to abnormal ratios when the crinkled had reverted. So far crosses between crinkleds and untranslocated strains have failed to yield 1:1 ratios but this again could be explained by reversions of crinkled. A detailed analysis of these reversions is being carried out at present.

The evidence presented gives reasonable support to the theory that the crinkled effect is produced by an unequal translocation and that this translocation is T(III-VIII). As the translocation is present in bi_1; w_3 and all strains derived from it, it is remarkable that the phenomena has not been reported previously.

A.J. CLUTTERBUCK

Studies in heterokaryosis

Attempts are being made to investigate the relationship between conidial ratios and hyphal nuclear ratios in Aspergillus nidulans heterokaryons, using the difference in size of diploid and haploid nuclei to distinguish between nuclei from the two component strains. The nuclei are observed by means of the fluorescent staining technique described below.

Slides, poured with very thin layers of agar (< 10μ thick) were inoculated with hyphae. They were then covered with a cellophane membrance and a further layer of agar 3-4 mm. thick, since contact with a relatively large bulk of medium appears to be essential for the growth of heterokaryons. The cellophane and agar overlay is stripped off before fixing.

Unfixed hyphae may be stained directly, but many of the nuclei then appear elongated and impossible to measure. Acetic fixatives cause bursting of the hyphae unless special measures are employed. Professor D. Lewis has found that degassing the preparation with a vacuum pump before fixing gives satisfactory results. Fixing with chloroform followed by Carnoy's fluid can also be used. Both these methods leave the nuclei relatively large and diffuse as seen under the fluorescence microscope, so a method has been devised which destroys all signs of mitotic figures, etc., and produces compact, round, clearly staining nuclei which are ideal for measurement. After removal of the cellophane, the slides are placed, face upwards, on a metal plate kept at -20^{0}C; when the material is frozen, they are removed and dried rapidly in a current of warm air. The preparation is then fixed by adding a drop of Carnoy's fluid which is allowed to evaporate before staining.

Preparations are stained with a 50 mg/litre solution of acridine orange in buffer (pH 7.5), and rinsed and mounted in the same buffer. For staining, pH is not critical, staining time is. The exact time required is dependent on the thickness of the agar on the slide. Correctly stained DNA fluoresces green, RNA red; however, if understained, the RNA is green also, while if overstained, the DNA is not distinguishable at all.

The fluorescence is observed with a microscope fitted with an Ilford 110 orange secondary filter. A high-pressure mercury vapour lamp with an ammoniacal copper sulphate cell as primary filter, in conjunction with a dark-ground condenser gives the best illumination, but something can be seen using an ordinary condenser and an over-run filament microscope lamp.

Observations have been confined to the hyphal tip cells since experiments have shown that in A. nidulans, unlike Neurospora, these are almost autonomous in growth: if a hypha is cut across 0.5 mm. behind the tip, the growth rate is not affected. The growth of a short tip cell, however, may be augmented by translocation through the septal pores.

At 37°C on minimal medium a heterokaryon tip cell may be up to 0.5 mm. in length and contain 25-70 nuclei; homokaryon tips are frequently longer. Cells behind the tip contain an average of about seven nuclei.

Preliminary observations of heterokaryons indicate that the conidial ratio is close to the overall nuclear ratio, at least in the particular heterokaryon studied. However, the nuclear ratio varies considerably from one tip cell to another, supporting the hypothesis of regulation of nuclear ratios by interhyphal natural selection rather than by intrahyphal adjustment.

J. R. WARR

Resistance to toxic agents in Aspergillus nidulans

Mutations in A. nidulans conferring increased resistance to either iodoacetate, actidione, p. flourophenylalanine, malachite green or teoquil have been isolated. In each case resistance is due to a single gene. Several attempts have also been made to obtain an increase in resistance by a mechanism not involving mutation such as Hinshelwood and his school have observed in a number of micro-organisms. This has not so far proved successful.

Actidione resistance

A dominant gene for actidione resistance, (Act_1) is located on chromosome III. Heterokaryons between Act_1 and wild type have been subcultured on increasing concentrations of the drug. This leads to an increase in the proportion of resistant conidia produced by the heterokaryon up to a maximum. At this stage, equilibrium is presumably reached between the advantage conferred by the resistant nuclei in the presence of actidione and the necessity for the nutritional deficiencies of these nuclei to be compensated by the sensitive strain.

p.Flourophenylalanine resistance

Recessive genes for p.f.p.a. resistance have been mapped on chromosome I, close to $ribo_1$ (pf_{21}, pf_{22}). They appear to act as suppressors of the nic_8 gene, possibly by the increased production of a common precursor of both nicotinic acid and phenylalanine (shikimic acid?) in the resistant strain.

Iodoacetate resistance

Strains resistant to iodoacetate are not cross-resistant to flouroacetate and can use acetate as a sole carbon source. Genes conferring resistance to this substance (Iod_1, Iod_2) are located on chromosome II.

Teoquil resistance

In the course of early work on teoquil resistance, confusing results were obtained. This has now been shown to be due to the considerably greater degree of resistance of hyphae than conidia, leading to non-reproducibility of the classification of resistance. A recessive mutation for teoquil resistance is located on chromosome III (te_6).

Malachite green resistance

A malachite green resistant mutant (mg.) is cross resistant to acriflavine to the same extent as acr_2. Preliminary biochemical studies have shown that riboflavin reduces the inhibitory effect of malachite green on wild type and resistant strains.