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J. H. CROFT.

Control of perithecial production

The greater proportion of homokaryotic haploid wild isolates of A. nidulans display a dual nature concerning perithecial production. Colonies grown from conidia of monospore cultures segregate into perithecial (p) and aperithecial (ap) forms. A low proportion of mosaic p - ap colonies is also present. Conidia from p and ap colonies repeat the segregations in subsequent generations, ap colonies giving a higher proportion of ap colonies in their progenies than do p colonies. The actual proportions vary with the genotype. For example, conidia from ap colonies of Birmingham isolate 37 give 95% ap progeny while those from isolate 114 give 5%.

Similar segregations also occur in non-mendelian proportions among ascospore progenies of homokaryotic isolates (see also Mahoney and Wilkie 1962). Within any one isolate ascospores give rise to constant proportions of p to ap colonies, whatever the phenotype of the parental colony.

Analysis of the progenies of sexual crosses show that the proportions of p to ap segregants among the conidial progenies of p colonies, ap colonies or among ascospore progenies is under a multifactorial control in each case, as also is the proportion of mosaic colonies present.

Within isolate 37 a heterokaryon transfer test has indicated the participation of an extrachromosomal factor in the control of the difference between the p and ap states, though the nature or action of this factor is not yet known. Further, resting ap conidia gradually convert to the p state, about half assuming the p state without loss of viability over a 30 day period at 25°C. Genetic control of this rate of conversion within the resting spore has also been demonstrated.

Reference: Mahoney, M. and D. Wilkie (1962). Proc. Roy. Soc. B. 156, 524-532.

J. H. CROFT

Recovery of unstable variants after acridine treatment

Unstable variants have been shown to arise spontaneously in wild isolates of A. nidulans with a frequency of 10^{-2} to 10^{-3} (Upshall 1966). Similar variants have been recovered in some of these isolates with a very high frequency (up to 90%) after treatment with acridines. Growth on solid medium, containing acriflavine (5 to 100 mg./l) gave rise mainly to the 'minute type a' phenotype, whereas that containing proflavine, 5-amino acridine acridine orange or acridine yellow gave mainly the 'crimson' phenotype, though the other phenotypes described by Upshall were also recovered with a low frequency. Growth in liquid medium containing acridines gave all phenotypes, but mainly the 'crimson' phenotype. A low proportion of

stable variants were also recovered. It is not yet known if these represent point mutations.

Meagre evidence is available suggesting that these unstable variants are slightly more tolerant to acridines than the wild-type. If this is so we cannot rule out the possibility that acridines increase the yield of these unstable variants partly or wholly by selection rather than by induction. Work is continuing to clarify the nature of these variants.

Reference: Upshall, A. (1966). Nature 209. 1113-1115.

A. C. BUTCHER

Crossing ability in A. nidulans.

Wild isolates of A. nidulans fall into distinct heterokaryon compatability groups on the basis of their ability to produce heterokaryotic conidial heads with each other (Grindle 1963). However, it is possible to produce hybrid perithecia between isolates falling into different groups (Jinks et al 1966). The following experiment has been carried out to investigate crossing ability between isolates of different heterokaryon compatibility groups. Seventeen isolates, representing seventeen groups, have been crossed together in all possible combinations with reciprocals. No auxotrophic markers were used in these investigations, a wild-type isolate being crossed with a second isolate containing only a w or y conidial colour marker in each case. Using a standardized crossing technique, the proportion of hybrid to selfed perithecia was scored for each cross. There was considerable variation between isolates in their ability to produce hybrid perithecia and the main point to emerge from the analysis was that the ability of an isolate to cross (i.e. A x B) is closely related to the ability of that isolate to self (i.e. A x Ay). It is not yet known if crosses are more readily produced between members of the same heterokaryon compatibility group than between members of different groups.

References: Grindle, M. (1963). Heredity 18, 397-405.
Jinks, J.L., C.E. Caten, G. Simchen and J.H. Croft (1966). Heredity 21, 227-239.

A. UPSHALL

Persistent multiple somatic segregation.

After ultra-violet irradiation of conidia of the "Red" cytoplasmic variant of A. nidulans, (Arlett et al. 1962), a colony was recovered which, on propagation by conidia, segregated into four phenotypically distinct

classes: A, B, C and D. Successive conidial propagations of colonies of each class has shown that each persistently segregates to produce varying proportions of the four classes. It has been observed that the persistent segregation follows a consistent pattern in that the most frequent segregant among the progeny has the phenotype of the parental class. Furthermore, the relative proportions of the four classes follow a definite sequence depending on the parent sampled, for example, from the B parent, the relative sequence of classes is always A < B > C > D.

The following table gives the percentage frequencies of the four classes obtained from conidial samples of the different parents and illustrates the features described above.

Parental Phenotype	Relative proportions of the four classes			
	A	B	C	D
A	66	26	6	2
B	9	71	18	2
C	1	5	73	21
D	0	0	10	90

This behaviour can be explained by assuming a mutation in a second cytoplasmic factor which segregates in a similar fashion to, but independently of, the factor responsible for the "Red" variant. Whereas the cytoplasmic basis of the "Red" variant is well established, that of the second factor has yet to be proven.

Reference: Arlett, C.F., M. Grindle and J. L. Jinks. (1962). The "Red" cytoplasmic variant of Aspergillus nidulans. Heredity 17, 197-209.

A. P. J. TRINCI and C. WHITTAKER

Stimulation of spore germination in Aspergillus nidulans by carbon dioxide.

We have been studying the autoinhibition of spore germination in A. nidulans at high spore densities (ca. 1 x 10^7 spores/ml. of culture medium). Aeration of dense spore suspensions, either by vigorous agitation with magnetic stirrers or by bubbling air through them, was found to relieve this inhibition and stimulate spore germination. When carbon dioxide free air, however, was used to aerate spore suspensions the percentage of spores which germinated was considerably reduced as shown below.

See over.

Aeration	Percentage of spores which had germinated after 6 hours at 37°C
1. Air bubbled through spore suspension in test-tube	74%
2. Carbon dioxide free air bubbled through suspension at the same rate as in 1.	14%

The suspending medium employed was Minimal medium with 0.5% glucose at pH 6.5 and the spore density was 1×10^7 spores/ml. There were two replicates per treatment and at least 300 spores per replicate were counted.

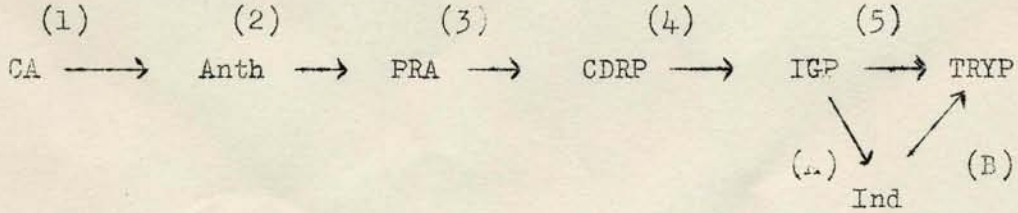
The incorporation of low concentrations (1×10^{-3}M) of sodium bicarbonate in the medium also stimulated germination at high spore densities.

Carbon dioxide is known to stimulate the germination of some bacterial spores and it also appears to influence the duration of the lag phase of yeast cultures. Carbon dioxide fixation by fungal spores has been demonstrated in at least two species. Stimulation of spore germination by carbon dioxide may in time be shown to be a common phenomenon in heterotrophic organisms.

C. F. ROBERTS, R. A. HUTTER, J. DEMOSS

Analysis of the tryptophan path in A. nidulans

The path of tryptophan synthesis in a number of micro-organisms may be represented (see Bonner, Demoss and Mills, p. 305, Evolving Genes and Proteins, Ed. Bryson and Vogel, Academic Press, 1965).



A group of 154 tryptophan responding mutants were isolated by filtration after UV irradiation and yield the following results on genetic and biochemical analysis.

Comp. group	No. of mutants	Growth response	Accumulation	Enzyme defect	Location
A	32	$Anth^-$ Ind^+	None	Anth synthetase (1)	II
B	31	$Anth^-$ Ind^-	Anth IG	Tryp. synthetase (5B)	I
C	2	$Anth^+$ Ind^+	None	Anth. synth. (1)	VIII
	57	$Anth^-$ Ind^+	+/- Anth	Anth. synth. (1) P.R. isomerase (3) IGP synthetase (4)	
D	18	$Anth^-$ Ind^+	Anth	P.R. transferase (2)	II
E	14	$Anth^+$ Ind^+	None	Not detected	VI

The A and D loci are loosely linked (15%)

The C mutants exhibit heterogeneity in accumulation of anth. and also

prove heterogenous with respect to their enzyme defects. Of the mutants examined so far three lack all three activities (1), (3) and (4), 1 only lacks IGP synthetase (4).

Examination of extracts of the wild type on sucrose gradients has shown that activities (1), (3) and (4) sediment together but separate from (2) and (5). Thus the proteins synthesized by the A and C genes form an aggregate which has three enzymic functions. It appears, therefore, that the genetic and biochemical relationships of the tryptophan pathway are very similar, perhaps identical, in Aspergillus and Neurospora.

The fifth group of mutants (E) which respond to tryptophan are not defective in any step between CA and Tryp, and do not respond to nicotinic acid or nicotinamide. They show response to quinic acid in the presence of phenylalanine and tyrosine and maybe leaky aromatic mutants with a partial block before CA.

E. PEES

Lysine, histidine and isoleucine mutants.

Following UV irradiation, 220 lysine, 122 histidine and 2 isoleusine mutants have been isolated with a heat-shock selection method (Pees and Eldridge A.N.L. 5, 1964).

The lysine mutants can be divided in six complementation-groups:

1. lys-3 (AL), on chr. VI (allelic to lys-2).
2. lys-16 (BL), on chr. V (allelic to lys-5)
3. lys-6 (CL), on chr. VII. allelic ? lys 7 & 11
4. lys-7 9DL), on chr. VII. allelic ? lys-18, 6
5. lys-10 (EL), on chr. V. allelic ? lys 231
6. lys-51 (FL), on chr. I. allelic ? lys 88

Two lysine mutants are unable to form heterokaryons.

The histidine mutants can also be divided in six complementation groups:

1. his-100(AL), on chr. II.
2. his-14 (BL), on chr. VIII or I.
3. his-8 (CL), on chr. IV.
4. his-115 (DL), on chr. VIII.
5. his-122 (EL), on chr. VII.
6. his-2 (FL), not yet located.

The two isoleucine mutants (ile) are allelic with each other, and were mapped on chr. VII.

Crosses have been carried out for a further mapping of these loci:

Chr. I lys-51 (FL) is linked to pro-1 (6%) and paba-2 (4.5%), and located between these loci (1070 ascospores analysed).

Chr. II. his-100 (AL) shows free recombination with w and Acr-1, and is

unknown at which side of fr-1 the histidine mutant is located.

Chr. V. lys-5 and lys-10 (EL) are not meiotically linked. Crosses between lys-10 (EL) and ribo-3 or ribo-5, give 36.7% and, respectively, 35.1% recombination between these mutants; lys-5 is not linked to ribo-3 or ribo-5.

Chr. VI. lys-2 is not meiotically coupled with nic-10 and lac-1.

Chr. VII. It turned out, that the loci lys-6 (CL), lys-7 (DL), his-122 (EL) and ile-1 (AL) all are located on this chromosome. lys-6 (CL) shows free recombination with lys-7 (DL), ile-1 (AL), cho-1, nic-8 and mal-1. The mutant ile-1 (AL) recombines freely with lys-6 (CL) and nic-8. The loci mal-1, lys-7 (DL), his-122 (EL) and cho-1 are linked as follows:

crosses	% recomb.	asocospores analysed	control	
mal-1 x lys-7 (DL)	14.2	323	bi - y	8.6
his-122 (EL) x lys-7 (DL)	12.9	1623	paba - y	16.9
cho-1 x lys-7 (DL)	43.2	469	bi - y	8.1
his-122 (EL) x cho-1	18.7	193	paba - y	17.6
mal-1 x cho-1	48.2	143	bi - y	8.4

It is most likely that the sequence of these four loci is:

mal-1 — 14.2 — lys-7(DL) — 12.9 — his-122(EL) — 18.7 — cho-1

Further localization studies are in progress.

A. J. CLUTTERBUCK AND U. K. SINHA

N-methyl-N'-nitro-N-nitrosoguanidine (NTG) as a mutagen for Aspergillus nidulans

The method of Adelberg et al. (1965), Biochem. Biophys. Res. Comm. 18, 788-95) for mutagenesis by NTG has been successfully applied to Aspergillus.

Conidia harvested from fresh slopes are suspended in 2 ml. Tris-Maleic buffer (each at a final concentration of M/20) adjusted to pH 6.0 with NaOH. 6.0 mg NTG mg NTG is dissolved in 10 ml. Tris-Maleic buffer and after warming, the solutions are mixed and incubated at 37°C with occasional agitation. Thus the treatment mixture consists of the following:-

Conidia - c. 10^8/ml.

TM buffer (pH 6.0) - 12.ml.

NTG - 6.0 mg. i.e., 0.5 mg./ml.

Treatment is stopped by spinning down the conidia and washing at least twice in water.

The percentage survival depends on the time of treatment, e.g.

time in minutes:-	5	10	15	20	25	30
Percentage survival	29	14	6.5	3.3	2.3	1.3

Even with viabilities as high as 50% a high yield of mutants is obtained. In practice we have aimed at a viability of 25% for the isolation of rare mutants. At this level the proportion of visible mutations is high and a considerable fraction of isolates are double mutants.

Under identical conditions of treatment there is no appreciable difference in percentage survival of bi1, bi1; w3, bi1; phen3 and bi1; w8; phen3 strains. Treatment of ascospores from aconidial strains is more effective after re-incubation in liquid CM for 1 hour.

As NTG is known to be a carcinogen great care is needed in handling it, and in the disposal and cleaning of glassware.

NTG may be obtained from Aldrich Chemical Company, Inc.

C. HERMAN AND A. J. CLUTTERBUCK

A method for selection of auxotrophs by means of "spidery" growth.

Attempts to isolate suppressors of putrescine requirement in the strain bi1; pu2 (pu2 is a new allele of pu1) by point inoculation onto solid MM + biotin + limiting putrescine (2.7×10^{-7}M) have led to the isolation of faster growing sectors with a spidery growth form which prove to require nitrite in addition to their former requirements.

From growth tests described below it has been concluded that the auxotrophy for nitrite in the absence of that nutrient induces a change from the compact slow growth of the parent strain on limiting putrescine to the rapid but sparse growth of the spidery mutant. Since there is probably little difference in the mass of growth per plate produced by the two growth forms, both can be supported equally well by limiting concentrations of putrescine.

The tests were as follows:

	Medium	Parent strain	"Spidery" strains
a.	MM + biotin + limiting putrescine	minute	spidery
b.	MM + biotin	no growth	no growth
c.	MM + biotin + optimum putrescine	normal	spidery
d.	MM + biotin + optimum putrescine + nitrite	normal	normal
e.	MM + biotin + limiting putrescine + nitrite	minute	minute
f.	nitrateless MM + biotin + limiting putrescine	spidery	
g.	sugarless MM + biotin + limiting putrescine	spidery	

These tests show:

a. MM + limiting putrescine supports spidery growth of the mutants but only slow compact growth of the parent.

b. The spidery mutants still require putrescine.

c. They exhibit spidery growth even on optimum levels of putrescine.

d. and e. The induction of spidery growth in the mutants is abolished by addition of nitrite to the medium so that, like the parent strain, they show normal growth on optimum putrescine and slow compact growth on limiting putrescine.

f. and g. Spidery growth can be induced in the parent strain by deficiency of nitrogen or carbon sources, and such spidery growth can be supported by limiting concentrations of putrescine.

Following up this last point, an attempt was made to isolate lactose mutants on the same principle. Nitrosoguanidine treated conidia (see Sinha and Clutterbuck, this issue) of bi1; pu2 were point inoculated onto MM + biotin + limiting putrescine + lactose as sole carbon source. The sectors isolated included one lactose non-utiliser, 6 nitrite requirers and 4 ammonium requirers.

A survey of existing auxotrophs showed that spidery growth was produced at certain limiting concentrations of the appropriate nutrient by auxotrophs for aneurin, arginine, lysine, methionine and adenine, whereas paba, putrescine and pyridoxine requirers gave only compact growth. It should theoretically be possible to select for any auxotroph of the first group by starting with any one from the second. The method would involve point inoculation onto medium containing limiting concentrations of both nutrients. Addition of an ammonium salt, or preferably an amino acid such as proline, to the medium could be used to eliminate nitrite and ammonium requirers, if these are not wanted.

E. KÄFER

Multiple mitotic crossing over in the centromere region or diffuse centromeres?

In a "control" diploid with supposedly normal chromosome constitution the marker pro1 has been found to segregate as if pro1 was located on the left arm of linkage group I. It was suggested that this might be due to a pericentric inversion (see ANL 5, p. 11, 1964). To test this hypothesis the pro1 strain from this diploid was crossed to various "standard" strains and recombinants from these crosses, as well as several ancestor pro1 strains, were combined with several different tester strains to check for meiotic and mitotic segregation of pro1. The following results were obtained.

1) Meiotic crossing over in crosses between these pro1 strains and various tester strains appears to be "normal" and does not confirm the presence of an inversion involving pro1 and the centromere region.
2) Mitotic crossing over is consistently the same in all 12 diploids analyzed to date and shows the following unexpected segregation for pro1: in either case, whether the selective marker is on the lefr or the right arm, homozygous pro segregants are obtained which are homozygous for all the markers on the same arm as the selected marker and heterozygous for all markers on the other arm. In detail the results to date are the following:
a) When su1ad20 (IL) is used for selection (from diploids carrying su1ad20 in coupling with ribo1 pro1 paba1 y ad20) "suppressed" ribo pro segregants, still heterozygous for paba1 and y were regularly found with a frequency of about 9% among suppressed green diploid segregants (observed total 219/2360 from 12 diploids giving an average frequency of 9.3± 1.1%).
b) When y (IR) is used to select yellow diploid segregants from these same diploids, yellow pro paba segregants, still heterozygous for su1ad20 and ribo1 were found with a frequency of about 2%, while "nondisjunctionals", yellow suppressed ribo pro paba segregants, showed a frequency of about 3% (observed total of pro paba segregants 29/1247 from 8 diploids giving an average frequency of 2.3± 0.15%).

Any other types of unexpected segregants were at least 10 times less frequent and presumably due to coincidence of two independent events of recombination.

These results are not in agreement with a simple pericentric inversion in pro1 strains except if multiple exchanges within the inversion are unexpectedly frequent. Another, rather unlikely, possibility would be the existence of diffuse centromeres. However, the fact that earlier results were found to be consistent with the position of pro1 on the right arm (e.g. among 144 "suppressed" diploid segregants from diploid 2, none were proline requiring only, Pontecorvo and Käfer, Adv. Genet 9, p. 91, 1958) makes such a general hypothesis unlikely and suggests that only some pro1 strains contain whatever genetic factor causes the contradictory segregation od pro1. It is hoped that tests of further pro strains will provide an answer to this problem.

~~PRELIMINARY NOTES~~

C. MORPURGO AND C. CALVORI

Variable frequency of back mutation in different genomes

In a previous paper from Calvori and Morpurgo a strain, 16N, with abnormally high rate of true back mutation, has been isolated.

Variability of the rate of back mutation in the same gene has been studied in strains derived from the cross 16N x 35; the genetic constitution of strain 16N is: su1ad20, ribo1, pfp-2 pro1, ad20, bi1, pyro4; and that of strain 35is paba1, an1, y, meth1, nic2, nic8, s12.

Rate of back mutation can vary by a factor not less than 40 simply by changing the genome.

Rate of back mutation for pfp (16N) in various diploids is reduced to one third of the original frequency.

A note on the same argument is in publication in English in the "Annali Istituto Superiore di Sanit..'.

G. MORPURGO AND L. VOLTERRA

Fine analysis of mitotic intracistronic crossing-over in Aspergillus nidulans.

A diploid heteroallelic for two different mutation in the cistron which determine PFP resistance in Aspergillus nidulans has been synthesized.

Fifty-eight strains, recombinants in the pfp-cistron have been selected and the causes of the processes leading to intracistronic recombination studied. Results can be summarized as follows: about half of the recombinants are due to gene conversion; the other shows recombination for the external marker ribo-1. In these cases the presence of a "linker" distal to ribo-1 has been established. Moreover, results can be explained only assuming that crossing-over is unequal in the majority of cases producing modification in the gene structure.

A note on the same argument is in publication in English in the "Annali Istituto Superiore di Sanitá."

A. M. MILLINGTON-WARD

A Replicator for Random Colonies.

Velveteen is stuck permanently on one side of numerous Gas-jar Covers.

(Plain glass discs 3" in diameter). These "Replicators" are sterilised in petri dishes in the autoclave.

They may be picked up with a rubber suction pad, (e.g. from a child's toy bow and arrow). After use the pile is cleaned with a soft nail brush, if necessary. It is better if the suction pad is not too efficient, otherwise it is more difficult to flick off the used "replicator" and pick up a new one.

The method, media, etc. are the same as described by Mackintosh and Pritchard (Genet. Res. 1963).

E. FORBES AND U. K. SINHA.

Location of some temperature sensitive mutants.

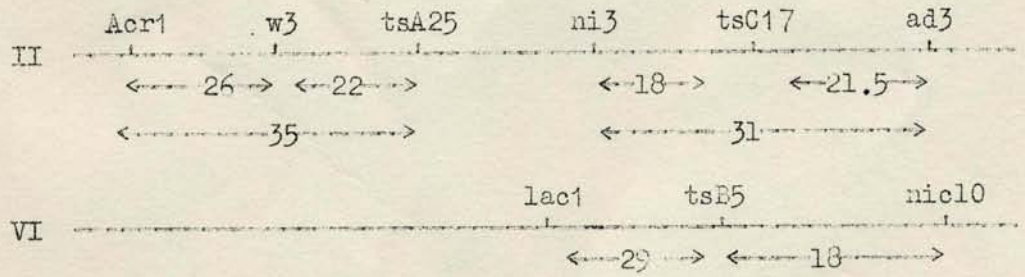
A large number of temperature sensitive mutants was isolated from a translocation-free bi1 strain following U.V. treatment. Those investigated at present do not grow even on C.M. at 37°C, but grow like bi1 at 25°C.

Ten mutants were assigned to linkage groups by haploidisation with master strains E or F. They were located as follows:

Linkage group	No. of mutants	Translocation
II	3	Free
III	3	Free
VI	1	Free
VIII	2	Free
III or VIII	1	III/VIII

None of them has been tested for allelism with any other.

Two mutants (tsA25 and tsC17) out of the three on Chromosome II and one (tsB5, on Chromosome VI were located meiotically; each maps at a different locus. The maps with the relevant markers are as follows:



All ten ts mutants investigated are recessive and both heterokaryons and diploids grow at 37°C.

Crosses can be made at 37°C, and then plated at 25°C to recover ts recombinants without any lowering of viability. Selection of ts^+ recombinants is made by plating at 37°C.

B. W. BAINBRIDGE, H. DALTON AND J. H. WALPOLE

Identification of the arginosuccinase gene

The growth responses of a variety of arginine requiring strains to intermediates in the arginine biosynthetic pathway have been known for some time. arg1, arg2 and arg3 respond to arginine. orn2 and orn7 respond to ornithine and arginine. pro1 and pro3 respond to proline/ ornithine/arginine and orn4 and orn5 to glutamic acid/proline/ ornithine/ arginine. Response to citrulline is difficult to classify unlike results reported in Neurospora.

From these auxanographic results it was apparent that only arg1, arg2 and arg3 responded to arginine but not to ornithine. The three genetic loci are genetically distinct being located in linkage groups VI, III and VIII respectively. It seemed likely, therefore, that the three loci corresponded to the three enzymes in the pathway, ornithine ⟶ citrulline ⟶ arginosuccinate ⟶ arginine. The three strains were tested in an attempt to identify the terminal enzyme of the pathway, arginosuccinase:

arginosuccinate ⟶ fumarate + arginine

The methods used were essentially those of Fincham and Boylen (1957) J. gen. Microbiol. 16, 438. Hot water extracts from wild type, arg1, arg2 and arg3 strains were analysed chromatographically using phenol-ammonia as solvent. An amino-acid spot with an R of 30 corresponding to arginosuccinate was obtained with all 4 extracts. However, the spot obtained with the arg1 strain was noticeably more intense than the other three spots.

Crude dialysed enzyme extracts of the four strains were incubated at 37° for 2 hours with arginine (50μM), sodium fumarate (50μM), and sodium phosphate buffer (20μM pH 7.4). The reaction mixtures were analysed chromatographically as before. Spots corresponding to arginosuccinate were observed with wild type, arg2 and arg3 enzyme extracts. No spot or a very faint spot was observed with arg1 extracts. No arginosuccinate spots were obtained in a control series in which the enzyme extracts had been boiled prior to incubation.

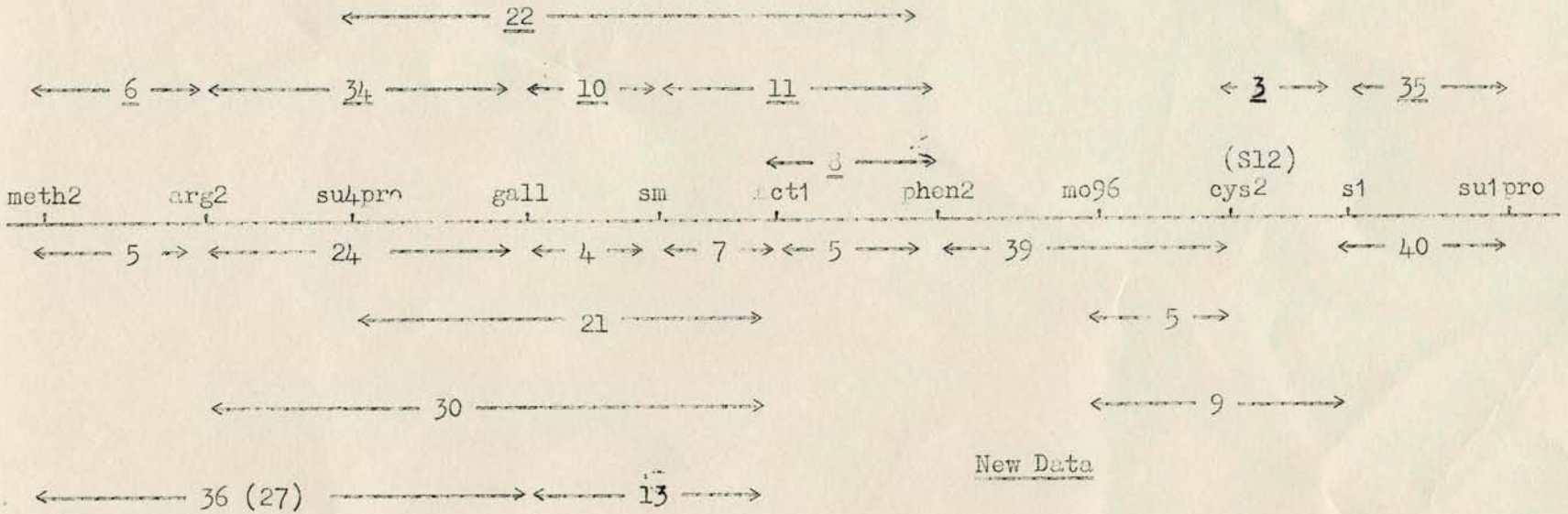
B. W. BAINBRIDGE. TABLE OF LOCATED OR PARTIALLY LOCATED MUTANTS

LOCUS SYMBOL	PHENOTYPE	LINKAGE GROUP	LINKED MARKERS	STRAIN OF ORIGIN	MUTAGEN	YEAR	REFERENCES
mo1	slow growing, abnormal conidial heads, orange pigment	1 R	pro1 3 paba1 7	su1 ad20 an1 ad20 bi1; Acr1 w2	UV	1963	Bainbridge
mo9	fluffy aerial mycelium, small conidial heads	V111 R	cha 14	bi1; w3; cys2	UV	1963	Bainbridge
mo87	Slow growing, abnormal conidial heads. brown pigment.	V1 (haploidisation)		su1 ad20 an1 ad20 bi1; Acr1 w2	UV	1963	Bainbridge
mo96	Compact, grooves conidiation 3 days at 37°	111	s12 4 s1 9	y; ad3; s1	β-propio lactone	1963	Bainbridge
arg1	lacks argino succinase - requires arginine	V1 (haploidisation)		bi1	X rays	1949 1966	Roper Bainbridge
Act1	resistant to 1.2 g/l Acti-dione	111	sm 7 phen 5	ribo1 y; nic8	UV	1963 1966	Warr Bainbridge

B. A. BAINBRIDGE. A REVISED MAP OF LINKAGE GROUP III

CROSS	PROGENY	RECOMBINATION FRACTION IN LINKAGE GROUP III	ORDER
pro1 paba 1 y; phen2 x bi1; w3; Act1 cys2	75	phen-Act 8 others 50	
gal1; pyro4; facA303; s3; nic8; ribo2 x y; meth2 Act1	186	meth-gal 36 gal-Act 13	meth-gal-Act
ad20 bi1; phen2 Act1 x bi1; arg2 meth2	237	meth-arg 5 arg-Act 30 Act-phen 4	meth-arg-Act-phen
bi1; arg2 meth2 x gal1 meth2 Act1; pyro4; facA303; nic8	45	arg-gal 24 gal-Act 5	arg-gal-Act
pro1 paba1 y; Act1 x pro1 bi1; Acr1 w3 ni3; su4 pro1 s12; meth1	126	Act-su4 22 others 50	
bi1; w4; sm; lys5 x gal1 meth2 Act1; pyro4 facA303; nic8	165	meth-gal 27 gal-sm 4 sm-Act 7	meth-gal-sm-Act
pro1 paba1 y; phen2 cys2 x pro1 bi1; Act1 su4 pro	156	su4-Act 21 Act-phen 4.5 phen-cys 39	su4-Act-phen-cys
bi1; w3; phen2; pyro4; nic8; ribo2 x pro1 paba1 y; ad3; s1 su1pro	102	s-su1 40 others 50	

Underlined data from other workers



New Data

References: Roberts (1963) Jour. gen. Microbiol. 31, 45.
Warr and Roper (1965) ibid 40, 273
Forbes (1956) Micro. Gen. Bull. 13, 9.
Käfer (1958). Adv. Genet. 9, 105.
Bainbridge (1963) Aspergillus News Letter 4, 20.

J. A. ROPER

Culture temperature and biotin requirement in Aspergillus

Strigini and Morpurgo (A.N.L. No.2) have already reported a requirement for biotin in strains of Aspergillus not carrying any bi mutation. This requirement can generally be ignored; chemicals and glassware presumably carry sufficient biotin as contaminant.

At 42° many, perhaps all, strains are very exacting; spread conidia show no growth in the absence of added biotin. This has been noted particularly for diploids.

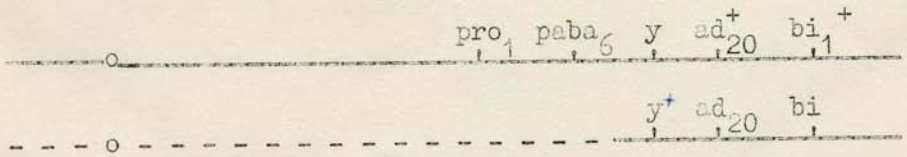
The concentration of biotin customary for bi strains restores full growth at 42°.

B. H. NGA

Vegetative Instability Associated with Chromosome Duplication in Aspergillus nidulans.

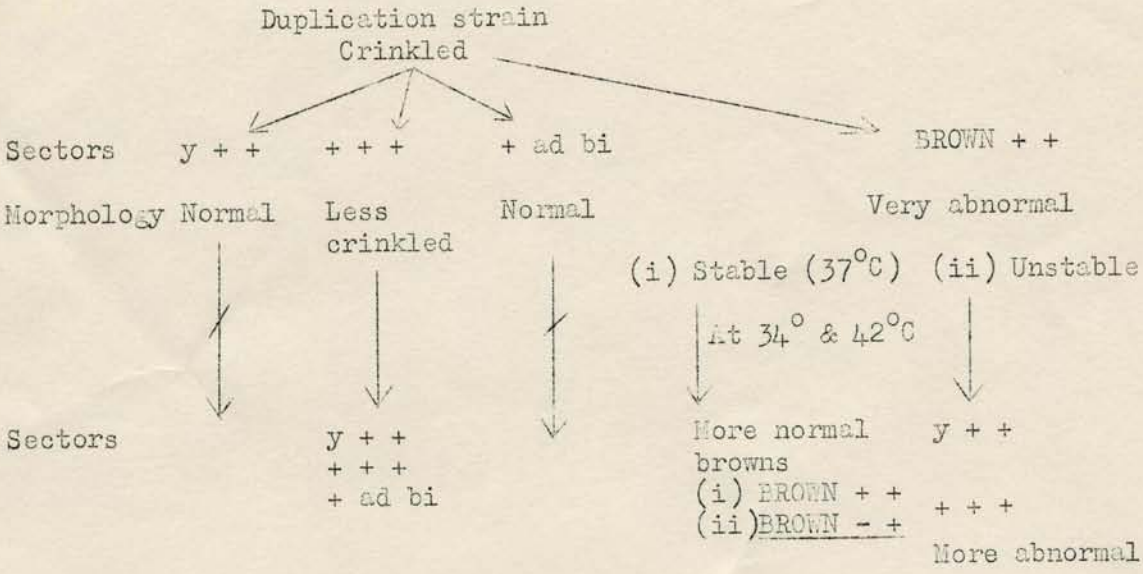
Vegetative instability has been observed in a strain with a duplication of the right arm of chromosome III (Bainbridge, A.N.L. 4 and 5, and Bainbridge and Roper, J. gen. microbiol. 42.)

This vegetative instability has now been observed in strains with other duplications. The process has been analysed in a strain (kindly supplied by Professor R. H. Pritchard) with a duplication of the right arm of chromosome I carrying the three markers, y, ad, bi. The strain is "crinkled" in appearance.



A diagrammatic representation of the duplication strain. Unbroken and broken lines represent chromosomes I and II respectively.

When stabbed on to CM + ad plates and incubated at 37°C, this strain sectored readily at 5 - 7 days to give various types.



Pedigree of sectoring of Duplication strain

(↓ Indicates do not further sector).

Diploid of a stable brown with M.S.F. was made and "brown" was located to chromosome I and "Y" to II.

The sectoring behaviour of the sectors shows that, at least in some instances, the chromosomal loss is not terminal. If one mechanism alone is responsible for the instability it can only be unequal sister-strand exchange.

Such sister-exchange might also give types with yet further duplication. Preliminary meiotic and mitotic analyses, as well as instability patterns are consistent with tandem duplication in the brown abnormal types.

The present study is directed at confirming the above and to determine the genetic nature of the duplication.

C.BALL.

Further studies on instability associated with gene suppression.

1. Further properties of a mutant previously described (A.N. no. 6)

(a) Selection

An attempt was made to isolate "stable" suppressors and "stable" auxotrophs from a conidial population of this mutant. This was successful in part since a suppressor type designated "grey", which showed stability as judged by colony size and germination on selective medium (37°C three days) was recovered. However, after longer incubation all colonies of this type produced sectors. The degree of suppression exhibited by

these sectors on subculture to selective medium was identical to that shown by the original "unstable" parent. Attempts are now being made to define the genetic nature of "grey".

(b) Chemical effects.

Incorporation of streptomycin in high concentration into medium lacking methionine increased the number of colonies produced. This was not found for $meth_1$ strains lacking the suppressor gene.

2. Induction of instability by chromosome translocation.

A variant designated "US", selected initially for methionine independence in an untranslocated $meth_1$ strain, was found to be vegetatively unstable. Repeated subculture on CM yielded a variety of morphological types differing in degree of conidiation and mycelial pigmentation. Among these were found a low percentage of normal conidiating types arising as whole colonies or as sectors.

(a) Analysis of "US"

Crossing "US" to a variety of untranslocated strains yielded the following results:

(i) Proportion of "US" in progeny > proportion of normal phenotypes in progeny.

(ii) "US" and suppressor of $meth_1$ segregated together.

(iii) In a cross to the MSE strain, certain "US" types centre gal^+_1, produced normal sectors gal^+_1 or gal^-_1.

(b) Analysis of normal conidiating types

(i) These types exhibited properties resembling those of the type described in section (a) and A.N. no. 6, but differed with respect to the intensity with which any one property was expressed.

(ii) By selecting normal conidiating types with dark pigmentation for crossing it was possible to recognise this phenotype in the progeny of crosses to normal strains. Ratios of 2 dark pigmented: 1 normal were obtained. There was no instance of the "US" phenotype arising, but 50% of the dark pigmented category were found to have more reduced growth rate and in most cases produce sectors after prolonged incubation.

(iii) Suppressor of $meth_1$ and dark pigmentation segregated together.

(iv) Haploidisation analysis of a diploid (normal dark x MSE) indicated the presence of III - V translocation. In addition pigmentation and suppression of $meth_1$ segregated in a manner expected.

The data is thus consistent with translocation of the left arm of chromosome III being translocated to chromosome V in both "US" and normal conidiating types; the 1/3 unstable duplicated types among the meiotic segregants being expected.

The overall phenomenon is most simply interpreted in terms of spontaneous translocation giving rise to both suppression of $meth_1$ and vegetative morphological instability. Reversion of "US" to normal conidiating types taking place by mutation in the same region of the genome.