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With 1 *plate and* 1 *text-figure*
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A system generating spontaneous intrachromosomal changes at mitosis in *Aspergillus nidulans*

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1. INTRODUCTION

Strains of *Aspergillus nidulans* with standard haploid nuclei show the expected stable growth. However, strains which have a chromosome segment in duplicate (one segment in normal position and one translocated to another linkage group) are highly unstable at mitosis (Bainbridge & Roper, 1966; Nga & Roper, 1968*a*). Colonies of duplication strains have a reduced linear growth rate and a characteristic 'crinkled' morphology; they produce frequent sectors of two broad classes. One class of variants show phenotypic improvement and, in varying degree, approach wild type in growth rate and morphology. The second class, observed rarely but regularly, have a deteriorated phenotype. Improved variants arise by loss of a variable part, perhaps sometimes all, of one or other of the segments carried in duplicate. The loss is sometimes, perhaps always, interstitial. Deteriorated variants probably result from new tandem duplications located, in the few cases analysed, on one or other duplicate segment. Loss and apparent gain of chromosomal material probably occur by intrachromosomal processes since both classes arise without evidence of genetic exchange between duplicate segments.

There remains some uncertainty about the genetic basis of deteriorated variants, but analysis of many improved variants is definitive. In respect of this latter class we can question the roles of the translocation, duplication and other genetic factors in mitotic instability. In particular, the duplication poses the following question. It might be supposed that there are frequent spontaneous losses of chromosome segments from normal haploid nuclei; the resulting hypo-haploid nuclei would be inviable. However, in a duplication strain, nuclei with loss from either duplicate segment would remain viable. As opposed to this passive role of the duplication, it might be suggested that an imbalance of chromosomal material would actively provoke instability. This, and other questions, can be approached by study of the relative stabilities of haploid and diploid strains with and without translocations and duplications, and with differences in residual genotypes.

2. METHODS

Media. Minimal medium (MM) was Czapek–Dox medium with 1% (w/v) glucose. Complete medium (CM) was a complex medium containing yeast extract,

hydrolysed casein, hydrolysed nucleic acid, vitamins, etc. Solid media contained 2% agar.

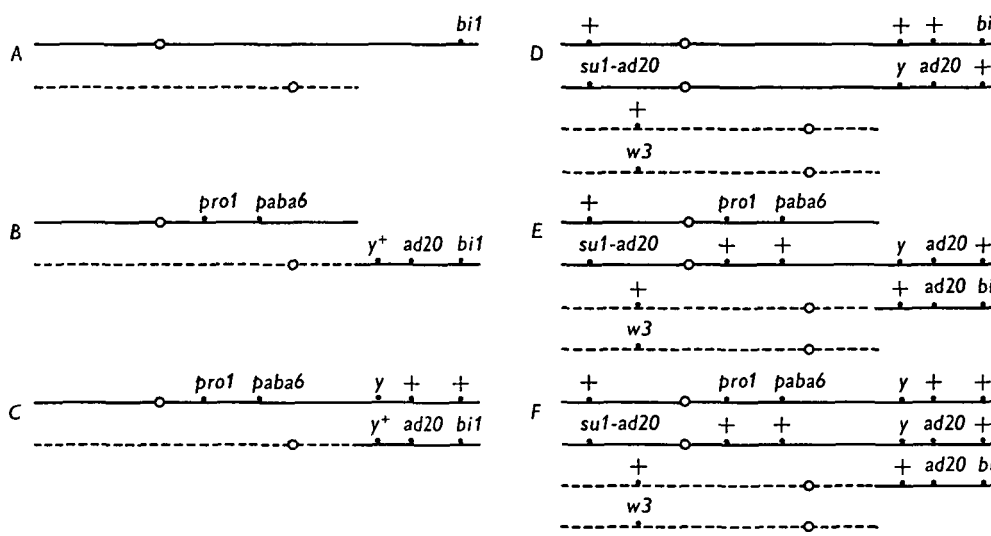
Organisms. Strains of *A. nidulans*, all derived from Glasgow stocks, were kept at 5° C on CM slopes. They were purified before use by single colony isolation and tested by auxanography. Except where otherwise specified, all strains were free from translocations. 'Master' strain E (MSE), carrying markers on all eight linkage groups, was that of McCully & Forbes (1965). Mutant alleles of particular importance in this work are given in the legend to Fig. 1.

Incubation was at 37 °C.

Methods of genetic analysis and strain preparation. General techniques were those of Pontecorvo *et al.* (1953). Mitotic haploidization of diploids was used to assign genes, deletions and duplications, to their linkage groups, and for chromosome substitution.

3. RESULTS

Six classes of strains were used (Text-fig. 1). *A* was a haploid without translocation or duplication. For comparative purposes a particular strain with translocation, but no duplication, was needed. Unfortunately, because of the origin of the strain *C* (Pritchard, 1956, 1960), the corresponding translocated, unduplicated strain did not exist. *B*, obtained as a mitotic variant from *C* (Nga & Roper, 1968*a*), was the nearest available. It was wild type in morphology and growth



Text-fig. 1. Linkage groups I (unbroken line) and II (broken line) of the classes of strains tested. Mutant alleles determined the phenotypes: *y*, yellow conidia (as opposed to wild-type green); *w3* (epistatic to *y* and y^+), white conidia; *ad20*, *bi1*, *paba6* and *pro1*, requirement respectively, for adenine, biotin, *p*-aminobenzoic acid and proline; *su1-ad20*, suppressor of *ad20*. *D*, *E* and *F* were heterozygous also for the following MSE markers: *gal1* (linkage group III) and *facA* (V), inability to use, respectively, galactose and acetate; *pyro4* (IV), *s3* (VI), *nic8* (VII) and *ribo2* (VIII) requirement, respectively, for pyridoxin, thiosulphate, nicotinic acid and riboflavine. Centromeres are designated by an open circle.

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rate and probably had little, if any, duplication of linkage group I material. *C* was a haploid with a duplicate (right, terminal) segment of linkage group I terminally attached to linkage group II. *C* was just one representative of many chromosomally similar strains studied. Others of this class carried various combinations of linkage groups I and III–VIII derived by substitution from different strains; in yet other, similar, duplication strains, genes on linkage group II and on the duplicate segments had been substituted by outcrossing. *D*, *E* and *F* were the diploids obtained by combining *A*, *B* and *C* respectively with MSE. As for *C*, *F* was one representative of a class whose members differed in residual genotype.

Table 1. *Sectors produced by strains A to F*

Strain	No. of colonies examined	Sectors		Total sectors	Sectors per 100 colonies
		Yellow	Green		
A	210	0	0	0	0
B	250	0	0	0	0
C	149	47*	20*	67	45
D	135	1	0	1	0·7
E	176	0	0	0	0
F	120	601*	0	601	501

Sectors differing from the parent in conidial colour and/or morphology were scored. *D*, *E* and *F* yielded, in addition, a few white sectors. Improved green sectors from *C* were detected readily by their growth advantage; improved green sectors, if any, from *F* would have escaped ready detection.

* Non-parental morphology.

Conidia of each strain were plated on CM to give about 5 colonies per dish; colonies were inspected when they had approximately identical mean diameter and instability was measured by a count of sectors which were non-parental in conidial colour and/or morphology. Counts were open to subjective error but differences between strains far exceeded such possible error.

Diploids heterozygous for a gene determining conidial colour produce segregants expressing mutant colour following haploidization, mitotic crossing-over or non-disjunction. Although they were relatively infrequent, it was desirable to distinguish these segregants from variants arising by intrachromosomal instability. In small colonies the former generally occur as patches rather than as distinct sectors and, accordingly, only sectors were scored. Although this drastically underestimated the degree of intrachromosomal instability of certain strains, it provided a more valid comparative assessment. Furthermore, intrachromosomal instability yielded sectors which were almost always non-parental in morphology, while the sectors produced by mitotic crossing-over or haploidization were of parental morphology. Finally, and most important, representative sectors were analysed genetically and this gave unequivocal evidence of their mode of origin. The frequencies of sectors, from representatives of the six classes of strains, are summarized in Table 1.

Strain A. This was included as a formal control and it was hardly to be expected

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that it would yield sectors with a frequency detectable in a small sample. This result is entirely in accord with general experience.

Strain B. No sectors were observed. This was consistent with extensive experience of haploid strains carrying different, sometimes multiple, translocations. The only possible exceptions to this are the strains studied by Ball (1967) and Clutterbuck (1968). In the former it was likely that there was, in addition to the translocation, a small duplication; the latter case was interpreted on a physiological basis rather than as a case of genetic instability.

Table 2

Linkage group	Haploids from a yellow sector of *D**			Haploids from a yellow variant of *F*†		
	Genes	Yellow	White	Genes	Yellow	White
I	*ad*+	7	8	*pro*+*paba*+	9	10
	ad	6	5	*propaba*	4	3
III	*gal*+	6	6	*gal*+	6	6
	gal	7	7	*gal*	7	7
IV	*pyro*+	8	9	*pyro*+	7	6
	pyro	5	4	*pyro*	6	7
V	*fac*+	4	5	*fac*+	5	4
	fac	9	8	*fac*	8	9
VI	*s*+	7	5	*s*+	10	8
	s	6	8	*s*	3	5
VII	*nic*+	5	7	*nic*+	7	5
	nic	8	6	*nic*	6	8
VIII	*ribo*+	10	6	*ribo*+	5	5
	ribo	3	7	*ribo*	8	8

* All the haploids were biotin-independent. The diploid yellow sector was adenine-requiring; this, and the phenotypes of the haploids, confirm that it was homozygous for *ad20* and heterozygous for *su1-ad20*.

† All the haploids had normal or near-normal morphology and were adenine and biotin independent.

Strains of class C. All strains of this class showed high instability and analysis of the resulting variants has been given by Nga & Roper (1968*a*). The duplication, rather than features of the residual genotype, either permitted expression of, or provoked, the instability. This was shown by the fact that instability persisted following the almost complete substitution of the residual genotype of *C*, including that of the duplicate segments, by the gene content of a normal stable haploid.

Strains of classes D *and* E. These showed the usual low incidence of mitotic segregants derived from mitotic crossing-over and haploidization. Extensive analyses of other such diploids, over many years, has revealed no segregant which should be ascribed to intrachromosomal instability rather than to mitotic crossing-over, haploidization or non-disjunction. This permitted two conclusions. If deletions were common in a normal, diploid genome, some should be expressed. For example, deletion of a dominant allele for conidial colour might have yielded a sector of incompletely diploid tissue with mutant colour. Alternatively,

haploidization might have followed deletion but, again, this would have given tissue with mutant colour. It may be concluded that deletions are uncommon in a normal diploid genome. Since strains of classes *D* and *E* were similar in behaviour it can be concluded also that a translocation, in heterozygous condition, does not provoke deletions. *E* gave no sectors in this sample; *D* gave one diploid, yellow sector which was shown, by haploidization, to have resulted from mitotic crossing-over (Table 2).

Strains of class F. In haploids, the duplication reduced the linear growth rate and sectors with deletions showed a growth advantage. The duplication was expressed only slightly in diploids and deletion variants had far less selective advantage over their diploid parent. Thus it was all the more significant that the instability of *F*, measured by sector production, was so such higher than that of *C* (Plate 1).

It was important to be sure that variant sectors from *F* arose by intrachromosomal instability. Eight yellow variants of independent origin and normal morphology were analysed. All were still diploid by the criterion of conidial size (Roper, 1952). Twenty-six haploid segregants from each yellow variant were classified for markers of the diploid parent. In every case both members of all eight linkage groups were recovered in these haploid segregants. However, since all the segregants had normal or near-normal morphology it could be concluded that most or all of the translocated duplicate segment had been lost. The deletion certainly included the y^+ locus but its precise extent could not be determined without outcrossing, and this was not undertaken. The genotypes of the yellow variants showed unequivocally that they arose by deletion and not by mitotic crossing-over, haploidization or non-disjunction. Analysis by haploidization of one yellow sector, typical of all eight, is given in Table 2.

4. DISCUSSION

Intrachromosomal instability at mitosis has been detected in all three duplication strains of *A. nidulans* tested and seems likely to be a property of all duplication strains. We propose the term 'mitotic non-conformity' for this instability phenomenon; the term avoids the possibly misleading concept of mitotic error and is intended to convey that parent and daughter nuclei may not conform in genotype.

Two conclusions about mitotic non-conformity can be drawn from this study. First, it is provoked by an imbalance of chromosomal material. There is increasing evidence that this may be true for other organisms and various instances have been discussed by Nga & Roper (1968*a*). In most of these cases the duplicate segments are probably in tandem, instead of 'opposed' as in our system. This may emphasize the fact that imbalance alone is the determining factor. The second conclusion is that genotypic changes are confined to the segments which themselves provoke change. The latter conclusion needs a small, but possibly important, qualification. Nga & Roper (1968*b*) detected mitotic variants with what were probably single

deletions of part of the translocated duplicate segment together with the distal part of the chromosome arm to which it was attached.

The immediate, basic problem is to explain how chromosome segments in imbalance provoke their own instability while the rest of the genome remains immune. Haploid duplication strains suffer deletions from either the translocated or untranslocated segment (Bainbridge & Roper, 1966; Nga & Roper, 1968*a*) and this excludes any explanation based simply on an abnormal association of chromosome regions. Current knowledge of DNA replication neither predicts nor offers explanation for mitotic non-conformity, and other areas of study offer only tenuous leads towards a theoretical exploration. One possible, partial parallel with our findings is presented by heterochromatin, which sometimes shows a lower mitotic stability than does euchromatin. To give just two examples, S. Beermann (1966) showed loss of interstitial heterochromatin during development in *Cyclops* and Burns & Gerstel (1967) showed mitotic instability of a block of heterochromatin in *Nicotiana*. However, without cytological or other evidence, it would be unjustified to propose a heterochromatic state for one or other duplicate segment; furthermore, with the present imperfect knowledge of heterochromatin, the proposal would offer little advance in understanding.

From a study of chromatid segregation at mitosis in *A. nidulans*, Rosenberger & Kessel (1968) proposed segregation units which carry DNA strands of the same age. Further, to explain the distribution in the hypha of nuclei of different age, they suggested membrane sites to which segregation units are attached; the attachment sites are presumed to initiate replication and to be limited in number. If such proposed sites initiated replication of chromosome segments, rather than of whole chromosomes, then replication errors might result from an imbalance of segments; furthermore, the errors might be confined to those particular segments. Other observations in *Aspergillus*, though disjointed, could be relevant. Diploid nuclei of *A. nidulans* undergo rare but regular spontaneous haploidization at mitosis, by successively losing chromosomes until a stable haploid state is reached (Käfer, 1961). Haploidization is accelerated by *p*-fluorophenylalanine (Morpurgo, 1961; Lhoas, 1961), and Lhoas (1968) has shown that the mean volume of diploid, but not of haploid, nuclei is reduced by this inhibitor. Perhaps the reduction in nuclear volume makes attachment sites less accessible and so leads to failure of replication of one or other chromosome, with consequent eventual reduction to haploidy. Growth of duplication haploid strains of *A. nidulans* is inhibited by a level of *p*-fluorophenylalanine which hardly affects normal haploids and extension of the above argument may explain this finding. Perhaps duplication haploid strains have a mean nuclear size greater than that of normal haploids; we have no evidence on this, but it is probably significant that the mean conidial size of the duplication haploid, *C*, falls well into the range normally found only for diploids.

It remains to be seen whether we are dealing with an entirely abnormal process. Duplications of the type used here may be rare in nature; but duplications, possibly tandem, have been proposed for a number of higher organisms on various experimental and theoretical bases. Perhaps such duplications provoke purposeful

mitotic non-conformity. If this were so, *A. nidulans* would provide a useful system for studying further elements of the process.

SUMMARY

Previous studies had shown that haploid strains of *Aspergillus nidulans* which have a chromosome segment in duplicate are unstable at mitosis. Through the study of various haploid and diploid strains, with and without translocations and with balanced and unbalanced genomes, it has been shown: (1) that imbalance of chromosome segments is responsible for instability, and (2) that the chromosomal deletions produced are confined solely or largely to the segments which provoke instability.

The term 'mitotic non-conformity' has been proposed for this instability phenomenon. An explanation for it has been sought in terms of attachment sites, limited in number and specific for chromosome segments, at which replication is initiated.

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REFERENCES

Bainbridge, B. W. & Roper, J. A. (1966). Observations on the effects of a chromosome duplication in *Aspergillus nidulans*. *J. gen. Microbiol.* **42**, 417–424.

Ball, C. (1967). Chromosomal instability related to gene suppression in *Aspergillus nidulans*. *Genet. Res.* **10**, 173–183.

Beermann, S. (1966). A quantitative study of chromatin diminution in embryonic mitoses of *Cyclops furcifer*. *Genetics* **54**, 567–576.

Burns, J. A. & Gerstel, D. U. (1967). Flower color variegation and instability of a block of heterochromatin in *Nicotiana*. *Genetics* **57**, 155–167.

Clutterbuck, A. J. (1968). A variegated position effect in *Aspergillus nidulans*. *Heredity* **23**, 165.

Käfer, E. (1961). The processes of spontaneous recombination in vegetative nuclei of *Aspergillus nidulans*. *Genetics* **46**, 1581–1609.

Lhoas, P. (1961). Mitotic haploidization by treatment of *Aspergillus niger* diploids with *p*-fluorophenylalanine. *Nature, Lond.* **190**, 744.

Lhoas, P. (1968). Growth rate and haploidization of *Aspergillus niger* on medium containing *p*-fluorophenylalanine. *Genet. Res.* **12**, 305–315.

McCully, K. S. & Forbes, E. (1965). The use of *p*-fluorophenylalanine with 'master-strains' of *Aspergillus nidulans* for assigning genes to linkage groups. *Genet. Res.* **6**, 352–359.

Morpurgo, G. (1961). Somatic segregation induced by *p*-fluorophenylalanine. *Aspergillus Newsletter* **2**, 10.

Nga, B. H. & Roper, J. A. (1968*a*). Quantitative intrachromosomal changes arising at mitosis in *Aspergillus nidulans*. *Genetics* **58**, 193–209.

Nga, B. H. & Roper, J. A. (1968*b*). Mitotic instability in duplication strains of *Aspergillus nidulans*: lethal deletions. *Heredity* **23**, 626–627.

Pontecorvo, G., Roper, J. A., Hemmons, L. M., Macdonald, K. D. & Bufton, A. W. J. (1953). The genetics of *Aspergillus nidulans*. *Adv. Genet.* **5**, 141–238.

Pritchard, R. H. (1956). A genetic investigation of some adenine-requiring mutants of *Aspergillus nidulans*. Ph.D. thesis, Glasgow University.

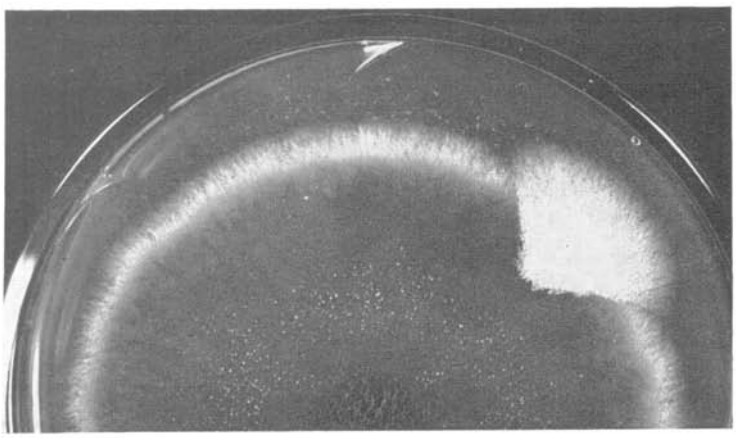
Pritchard, R. H. (1960). The bearing of recombination analysis at high resolution on genetic fine structure in *Aspergillus nidulans* and the mechanism of recombination in higher organisms. In *Microbial Genetics. Symp. Soc. gen. Microbiol.* **10**, 155–180.

ROPER, J. A. (1952). Production of heterozygous diploids in filamentous fungi. *Experientia* **8**, 14–15.

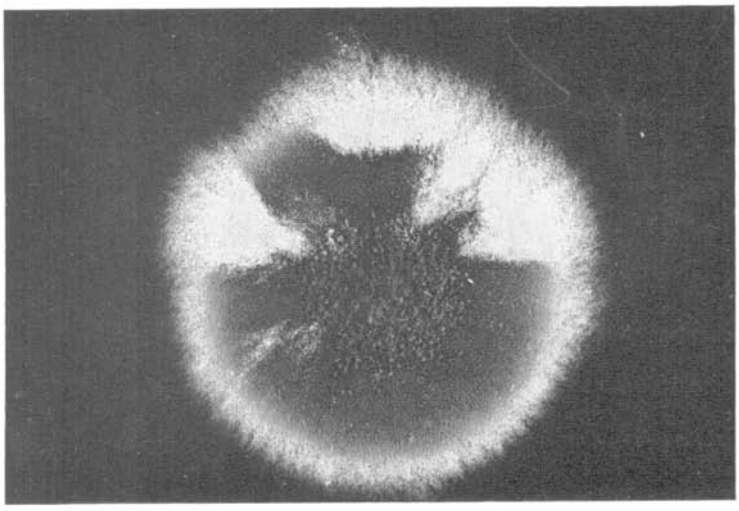
ROSENBERGER, R. F. & KESSEL, M. (1968). Nonrandom sister chromatid segregation and nuclear migration in hyphae of *Aspergillus nidulans*. *J. Bact.* **96**, 1208–1213.

EXPLANATION OF PLATE

A colony of haploid *C* (*a*) and two colonies of diploid *F* (*b* and *c*), showing yellow sectors. Magnification: (*a*), × 1·5; (*b*) and (*c*), × 3. For purposes of illustration the haploid colony was allowed to grow fully so as to produce this single, more readily visible, sector.



(*a*)



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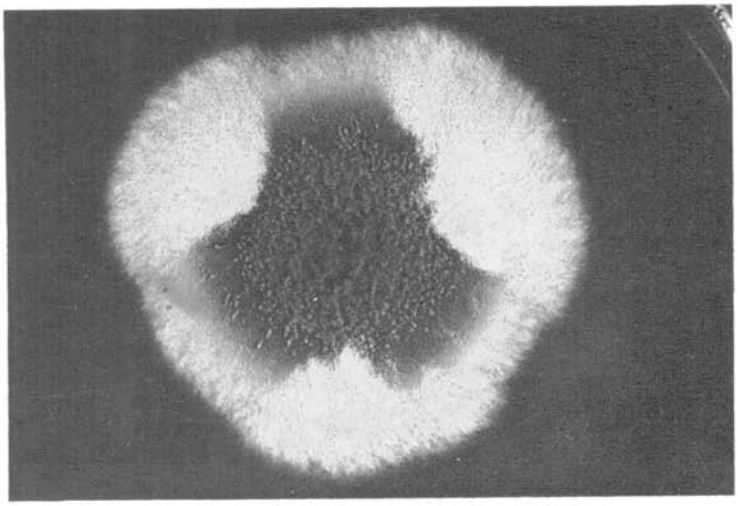

(*c*)