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Phenotypic specificity of aneuploid states in *Aspergillus nidulans*

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SUMMARY

Hyperhaploid and hyperdiploid colonies of *Aspergillus nidulans*, disomic or trisomic for one or more chromosomes respectively, are abnormal in phenotype and somatically unstable. It has been shown for Glasgow strains that a different specific phenotype arises, depending upon which chromosome is additional (Pollard, Käfer & Johnston, 1968). This report extends this system and shows that irrespective of the residual genetic background of the isolate, the system of phenotypic specificity applies.

1. INTRODUCTION

Colonies of *Aspergillus nidulans* with extra chromosomes are abnormal in phenotype and somatically unstable, this latter characteristic being due to the loss of the additional chromosomes at mitosis. Consequently, faster-growing sectors arise at the periphery of the aneuploid colonies (Käfer, 1961; Upshall & Croft, 1967). Phenotypic specificity of these hyperhaploid disomic or hyperdiploid trisomic states in *A. nidulans* has been reported by Käfer (1961) and Pollard *et al.* (1968) have classified homozygous non-segregating unstable colonies as aneuploid on the basis of phenotype. All of this published work, however, has been carried out upon mutant derivatives of the original Glasgow isolate of *A. nidulans* (Yuill, 1939), so that the strains employed were genetically related. The species *A. nidulans* is known to be genetically heterogeneous, with strains belonging to the Birmingham collection differing from each other, and from the Glasgow isolates, in several characters (Jinks, Caten, Simchen & Croft, 1966; Butcher, 1968, 1969). This communication extends the system to other wild-types and mutant isolates of the Birmingham collection, and shows that, irrespective of the residual genotype of the strain studied, aneuploidy resulting from the presence of an additional specific chromosome gives rise to a single specific recognizable phenotype.

2. METHODS

(i) *Media*

Minimal medium, supplemented with appropriate growth factors, was used throughout.

(ii) *Organisms*

Twenty wild-type isolates were selected from the Birmingham collection, together with any spore colour and auxotrophic mutant derivatives (obtained by

u.v. irradiation). For Birmingham isolates 1 and 29, the yellow and *paba* mutations have been located, via sexual crosses, on the homologue of chromosome 1 of the Glasgow stock (Upshall, unpublished). Before these investigations were undertaken the locations of the other mutations induced in the Birmingham isolates had not been determined. The auxotrophic strains employed are listed in Table 1.

(iii) *Methods of genetic analysis*

Prototrophic green and yellow Glasgow strains were derived from a cross between strains *j* and *k*. Heterokaryons and/or heterozygous diploids were formed between strains *a* and *b*, *c* and *d*, *c* and *f*, *e* and *g*, *h* and *i*, *l* and *m*, and *m* and *n*, in Table 1.

Table 1

(*a*) *lys; w*	Birmingham isolate 1 mutant strains
(*b*) *arg;*	
(*c*) *paba; w*	
(*d*) *y; ribo*	
(*e*) *bi*	
(*f*) *pyro*	
(*g*) *paba; w; pyroA5*	See note * below
(*h*) *arg*	Birmingham isolate 29 mutant strains
(*i*) *paba, y; meth; ribo; ad*	
(*j*) *pabaA6, yA2*	Glasgow mutant strains†
(*k*) *biA1*	
(*l*) *proA1, pabaA6, yA2; wA3*	
(*m*) *biA1; pyroA6*	
(*n*) *suadE20, yA2, adE20; wA3; galA1; pyroA4; facA303; sB3; nicB8; riboB2*	

Locus letters and allele numbers are given for mutants of Glasgow origin. In the present account no such designation is given for mutant alleles induced in the Birmingham (wild-type) strains.

For details of the locations of the markers of the Glasgow strains see Dorn (1967).

The nutritional requirements are as follows: *ad*, adenine; *arg*, arginine; *bi*, biotin; *lys*, lysine; *nic*, nicotinic acid; *paba*, p-aminobenzoic acid; *pro*, proline; *pyro*, pyridoxine; *s*, thiosulphate; *ribo*, riboflavine.

Other markers are *w* (white conidial colour), *y* (yellow conidial colour), *suad20* (suppressor of adenine requirement), *gal* and *fac* (inability to use galactose and acetate as a carbon source respectively).

* This strain is a recombinant from a sexual cross between (*c*) and a Glasgow mutant strain with the genotype *proA1, yA2; pyroA5*.

† The symbols used are those suggested by Clutterbuck (1970).

Unstable variants were obtained from the following known sources. (1) By treatment of both haploid and diploid strains with acridine dyes or para-fluorophenylalanine (Upshall & Croft, 1967). (2) Spontaneously from diploid strains (Käfer, 1961). (3) From conidial samples and ascospore samples of selfed cleistothecia of haploid strains (Upshall, 1966). (4) From ascospore samples of hybrid cleistothecia (Pritchard, 1954; Faulkner, 1967).

A further source of unstable variants was found to be among conidial samples of balanced heterokaryons. More detail as to their occurrence and formation will be given in a later paper (Upshall & Croft, in preparation).

To identify an unstable variant as aneuploid genetically one screens for the segregation of markers among the breakdown sectors by a technique known as sector analysis. For the results to be unequivocal the aneuploid must of necessity be heterozygous for one or more chromosomes. Such an analysis was carried out on all unstable variants recovered from the products of heterozygous combinations, and involved the following stages. The variant was first purified by single-colony isolation, i.e. one variant colony was subcultured so as to produce the next generation. The genotype of a sample of the variant progeny was then determined by testing their ability to grow on the range of differential media corresponding to the markers present in the parental strains. Fifty of the variant colonies were sampled by mass hyphae on to fully supplemented medium and allowed to produce breakdown sectors. One sector was then taken at random from each of these colonies and transferred to fresh fully supplemented medium, from which they were replicated on to the same range of differential media as the variant phenotype. Any chromosomal segregation can thus be easily observed by a comparison of the sector and variant genotypes.

Any temperature of incubation between 25 and 35 °C was found to make no difference to the recovery of unstable variants or to their behaviour. General techniques were those of Pontecorvo *et al.* 1953.

3. RESULTS AND DISCUSSION

Heterozygous aneuploid variants, i.e. those showing a segregation of markers on sector analysis derived from the Glasgow strains, confirmed the findings of Käfer and her associates, since independently isolated variants which segregated for a particular marked chromosome had identical phenotypes. Variants segregating for different chromosomes differed in phenotypes. Hyperdiploid trisomic phenotypes deviated only slightly in appearance from the corresponding hyperhaploid disomic phenotype, the former generally being larger in size and bearing more conidia.

Hyperhaploid and hyperdiploid variants heterozygous for chromosome I were recovered from the combinations between the Birmingham isolate 1 derivatives (*c* and *d*, Table 1), and from between the Birmingham isolate 29 derivatives (*h* and *i*, Table 1). These segregated for green/yellow conidiospore colour and for the known linked *paba* mutation thus confirming the results of the sexual crosses. These variants had identical gross phenotypes to the corresponding variants from the Glasgow strains and to each other (Plate 1 *a*, *b*).

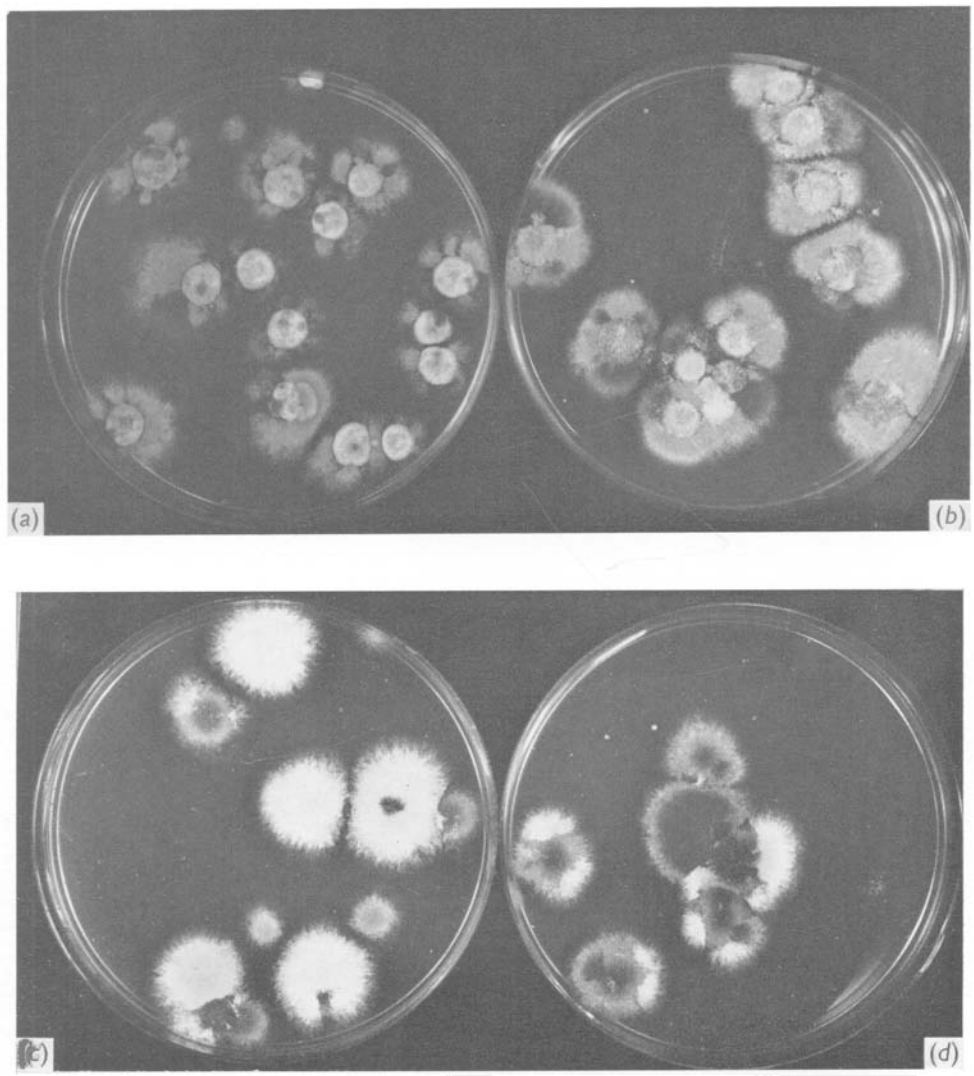
Also from Birmingham isolate 1 combinations (*c* and *d*, Table 1) variants were recovered which segregated for green/white conidiospore colour. These had phenotypes virtually identical to green/white conidiospore colour-segregating disomic aneuploids derived from a Birmingham isolate 1/Glasgow progeny hybrid cleistothecium (*e* and *g*, Table 1), and from fully Glasgow combinations segregating for yellow/white or green/white conidiospore colours (*l* and *m*, *m* and *n*, Table 1) (Plate 1 *c*, *d*). Since it is known that in the Glasgow strain the wA3 locus is situated on chromosome II, then it is highly probable that the white locus in Birmingham

isolate 1 is situated on the corresponding homologue since segregators for that mutation had the same variant phenotype. This is made even more likely when one considers the observations on the other unstable variants recovered. Thus from all heterozygous combinations, other non-segregating variants were recovered which had identical gross phenotypes to the heterozygous aneuploid variants. These must be aneuploid states in which the disomic chromosome pair is homozygous in the hyperhaploid $n+1$ state and where in the hyperdiploid $2n+1$ state, two of the chromosomes carry the wild-type alleles.

In considering the wild-type isolates, any aneuploid variants, which might arise by non-disjunction, from ascospores of selfed cleistothecia and conidial samples, must be homozygous and therefore non-segregating. From such samples, a relatively high frequency of unstable variants is recovered, some of which can be classified according to phenotype (Upshall, 1966). The phenotypes of some of these classified variants were found to be identical to those of the segregating and non-segregating chromosome I and II aneuploid phenotypes arising from the heterozygous combinations of the same and different strains, and must therefore represent disomy for those chromosomes respectively. Other phenotypes were recovered which were virtually identical to segregating phenotypes from the Glasgow strains (Plate 2*a–d*) and to some non-segregating phenotypes arising from heterozygous combinations of the Birmingham strains. These spontaneous unstable variants, with recognizable phenotypes, arising from haploid conidia or from ascospores derived from selfed cleistothecia must be aneuploid disomic for different chromosomes, the homologue in excess being identifiable by the specific phenotype. (Frequency data for the occurrence of these spontaneous variants will be presented in a later paper.) It is clear therefore that phenotypic specificity exists for the different aneuploid states of *Aspergillus nidulans* irrespective of genetic background differences.

In other fungi, disomic aneuploids are generally isolated as intermediate stages in the breakdown of heterozygous diploid nuclei. In *Neurospora crassa* they are recovered as pseudo-wild-type ascospores from sexual crosses (Pittenger, 1954). In all known examples, the aneuploid condition can only be confirmed by segregation of markers and they cannot be recognized when they arise from haploid mitoses. In *A. nidulans* the specific phenotype produced by the particular extra chromosome permits one to visually recognize and identify the aneuploid genotypes irrespective of the source from which they were isolated. A further advantage of the specific phenotype is that it allows one to assign an unmapped marker in a Birmingham isolate to the homologue of the known Glasgow chromosome without recourse to sexual crossing. In this context, it has so far proved impossible to obtain stable diploid strains between Birmingham and Glasgow isolates (Croft, unpublished) and further the occurrence of hybrid cleistothecia between such isolates is very infrequent, especially if multiply marked auxotrophic strains are involved.

As well as hyperhaploid $n+1$ variants, variants are occasionally recovered disomic for two different chromosomes and it has been noted and substantiated



(*a*), (*b*). Heterozygous hyperhaploid disomic chromosome I variants derived from Glasgow and Birmingham isolate 29 strains respectively. Disomic genotype:

$$\frac{paba \qquad\qquad y}{+ \qquad\qquad +}$$

I

(*c*), (*d*). Heterozygous hyperhaploid disomic chromosome II variants derived from Glasgow and Birmingham isolate 1 strains respectively. Disomic genotype:

(*c*) $\dfrac{y}{\quad}$ $\dfrac{w}{+}$ I II (*d*) $\dfrac{w}{+}$ II

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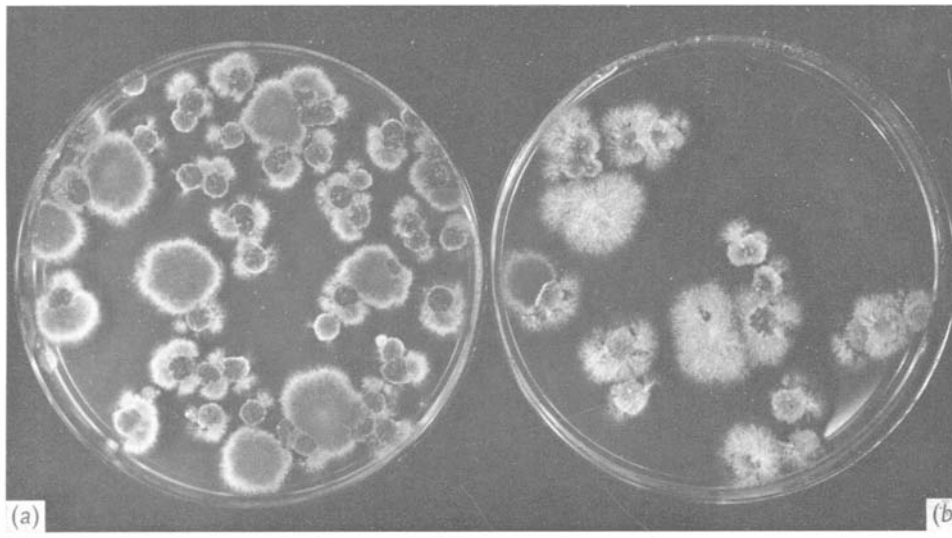

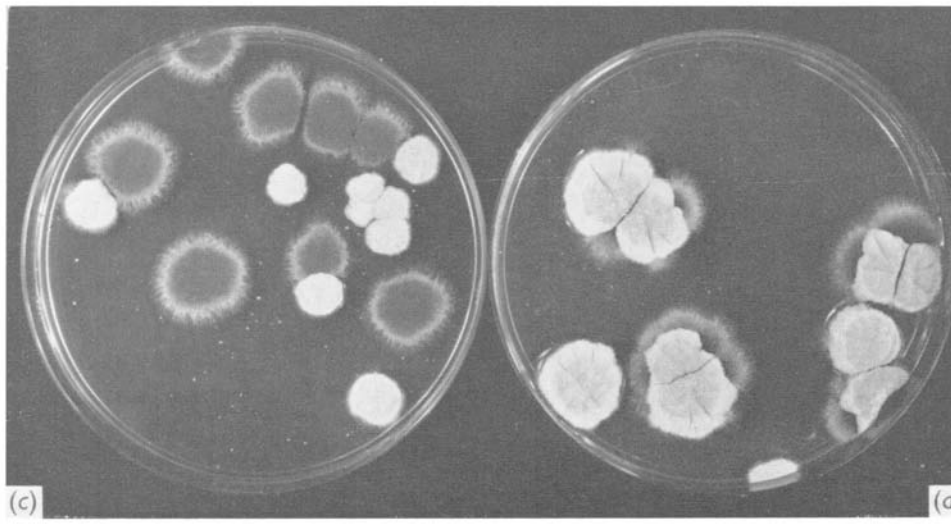

(*a*) Heterozygous hyperhaploid disomic chromosome V variant derived from Glasgow strains. Disomic genotype: $\frac{fac}{+}$

(*b*) Homozygous hyperhaploid disomic chromosome V variant derived from Birmingham isolate 1. (*c*) Heterozygous hyperhaploid disomic chromosome VI variant derived from Glasgow strains. Disomic genotype: $\frac{s}{+}$

(*d*) Homozygous hyperhaploid disomic chromosome VI variant derived from Birmingham isolate 1.

A. UPSHALL

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(E. Käfer, personal communication) that these also have specific phenotypes depending upon which two chromosomes are involved. A preliminary investigation upon isolates of *Aspergillus niger* has shown that the system of phenotypic specificity of aneuploid states does not appear to be operative.

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