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Mitotic non-conformity in *Aspergillus nidulans*: the production of hypodiploid and hypohaploid nuclei

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1. INTRODUCTION

Strains of *Aspergillus nidulans* with a chromosome segment in excess of the haploid complement are unstable at mitosis (Bainbridge & Roper, 1966; Nga & Roper, 1968); they undergo frequent intrachromosomal changes involving, in all the variants so far analysed, one or other of the segments present in duplicate. Many variants arising by a chromosomal deletion, of variable size, have been analysed definitively. Variants of a second class have not yet been analysed completely; these are usually very highly unstable and probably carry new tandem duplications in addition to the initial duplication. Nga & Roper (1969) termed this instability 'mitotic non-conformity' and showed, by a study of balanced and unbalanced diploids, that it is provoked by the imbalance of chromosome segments.

Thus the basic genetic cause of this instability and an outline of at least some of its results are known. But more refined knowledge of the genetic consequences is needed in an attempt to explain how imbalance causes instability and for assessment of the possible occurrence of the processes in other species. Previous work had suggested that deletions at mitotic non-conformity were confined to either of the duplicate segments, but the use of haploid duplication parents favoured recovery of such types. This study was designed to investigate whether deletions might sometimes extend beyond the unbalanced segments to give hypohaploids from haploid duplication strains. It was expected that hypohaploids, if any, would be far more difficult to detect, maintain and analyse than would hypodiploids. Accordingly, to extend the scope of search for possible hypoploids, deletion variants from unbalanced diploids have also been analysed.

2. METHODS

Media. Minimal medium (MM) was Czapek–Dox 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.

Methods of genetic analysis and strain preparation. General techniques were those of Pontecorvo *et al.* (1953). Allocation of mutant alleles, duplications and deletions to their linkage groups via mitotic haploidization (Forbes, 1959) was facilitated by the use of *p*-fluorophenylalanine (PFA) (Morpurgo, 1961).

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Organisms. Strains of *A. nidulans*, all derived from Glasgow stocks, were kept on CM slopes at 5 °C. They were purified before use by single colony isolation. 'Master' strains (MSE and MSF), carrying markers on all eight linkage groups, were those of McCully & Forbes (1965). Mutant alleles are given in the legend to Fig. 1. The component haploids of diploid strains are separated by the symbol //.

Incubation was at 37 °C.

3. RESULTS

Figure 1 gives the genotypes of the three strains tested for the production of hypoploids. Haploid strains *A* and *B* were obtained as segregants from crosses of Pritchard's (1956, 1960) duplication strain to appropriately marked standard strains. Diploid *A*//MSF was obtained from *A* and MSF by Roper's (1952) method.

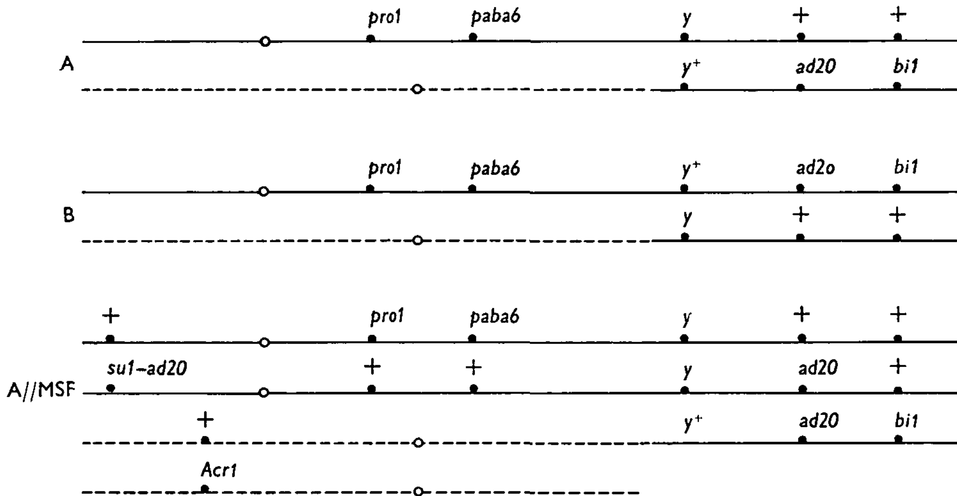

Fig. 1. Linkage groups I (unbroken line) and II (broken line) of the strains tested. Mutant alleles determined: *y*, yellow conidia (as opposed to wild-type green); *Acr1*, resistance to acriflavine; *ad20*, *bi1*, *paba6* and *pro1*, requirement, respectively, for adenine, biotin, *p*-aminobenzoic acid and proline; *su1-ad20*, suppressor of *ad20*. The diploid was heterozygous also for MSF 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. MSE, used in some analyses, differed from MSF only in having *w* (white conidia, epistatic to colour), instead of *Acr1*, as its linkage group II marker.

Strain A. This strain was known to give sectors bearing yellow conidia by deletion of the y^+ locus (Nga & Roper, 1968). Deletions beyond the duplicate segment would produce hypohaploids lacking the right, distal end of linkage group II. It was expected that hypohaploids, if they arose at all, would occur only as very small patches of yellow supported by surrounding parental growth. Low-density conidial platings on CM were made from about twenty-five yellow mitotic variants arising as tiny patches or single conidial heads. All gave yellow colonies, usually with morphology more-nearly wild type than that of parent *A*.

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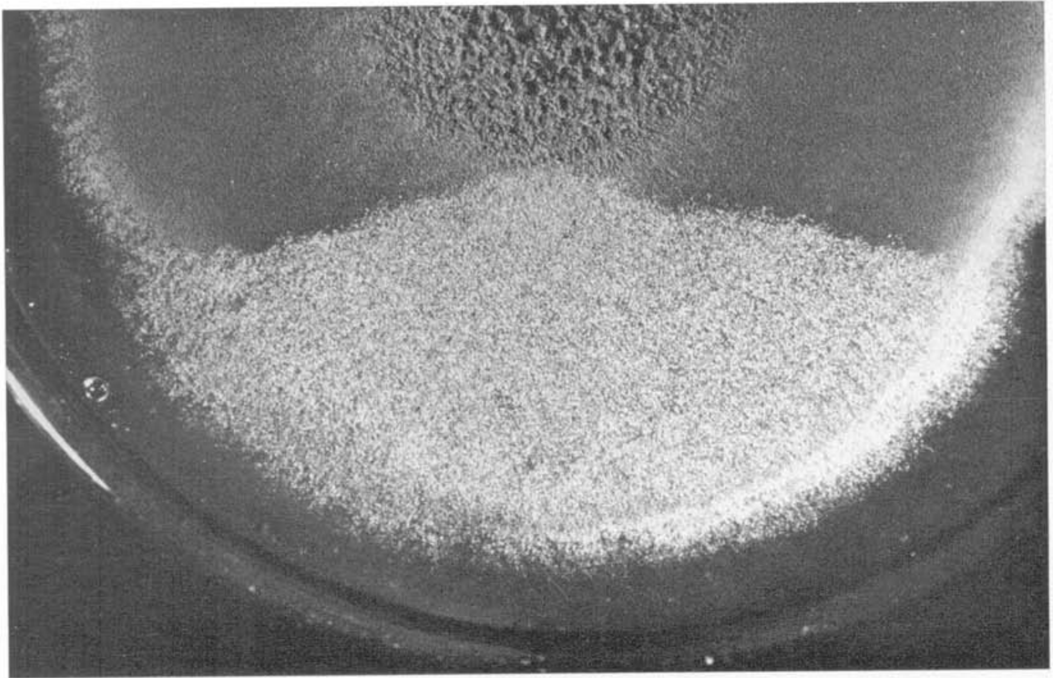
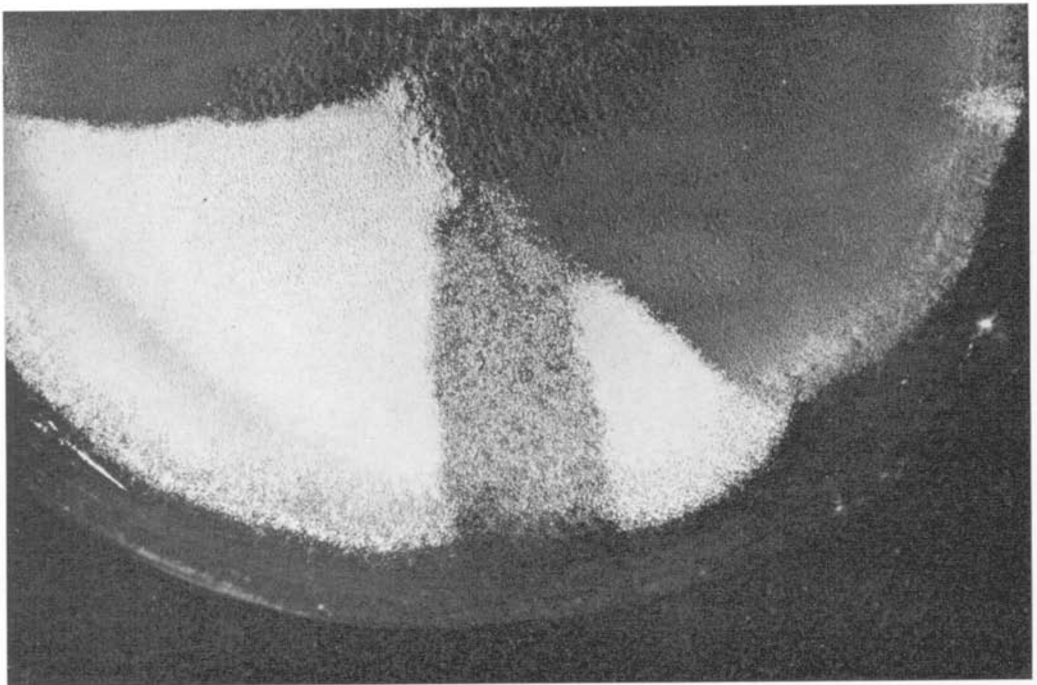


(*a*)



(*b*)

Heterokaryotic sectors, with inviable yellow and parental green conidia, produced by strain *A*. 1*b* also had homokaryotic yellow sectors.

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The first inviable yellow (IY) variant, potentially hypohaploid, was detected in a colony permitted to grow over the whole of a 9 cm dish of CM (Plate 1). The colony showed a heterokaryotic sector with an unusually intimate mixture of yellow and green conidial heads and a high proportion of individual heads with both yellow and green conidia. Conidia from this sector, plated on CM, gave only colonies with green conidia although about 400 yellow conidia had been plated. Yellow colonies were never obtained from similar platings on a variety of media at various temperatures; this showed that the mutation responsible for the IY was neither temperature sensitive nor readily compensated by change of medium. Further heterokaryotic sectors were sought and the results are shown in Table 1. Some of these heterokaryons with an IY component were maintained by transfer of blocks of hyphae to fresh dishes of CM. However, many were lost after a few subcultures, either by outgrowth of the green component or by the confusion resulting from new, viable, yellow variants produced by the green component.

Table 1. *Heterokaryotic yellow/green sectors from strains* A *and* B

Strain	No. of colonies examined	Total no. of heterokaryotic sectors	No. with inviable yellow component
A	435	23	22
B	824	16	1

All colonies were 9 cm diameter.

Initially it was thought possible that each IY was maintained in heterokaryotic condition with a green mitotic variant of *A*, rather than with *A* itself. Because of the labour involved, the green component of only one heterokaryon was analysed completely. From genetic analyses, morphology and study of instability it was concluded that this green component was identical with parent strain *A*.

Attempts to analyse the IY variants were made as follows. A block of solid CM with hyphae of the yellow and green heterokaryon was placed beside a similar block of the white-spored MSE on MM with 0·004 % (w/v) PFA. It was hoped to produce three-component heterokaryons comparable with those studied by Azevedo & Roper (1967). PFA was used to discourage growth of the *A* component, which is particularly sensitive to this inhibitor (Nga & Roper, 1968), and so encourage growth of a two-component heterokaryon of MSE and IY. In only one case, out of six attempted, was a balanced, two-component heterokaryon of an IY and MSE obtained. Hybrid perithecia from this heterokaryon had eight-spored asci but, in suspensions, it was noted that 50 % of the ascospores had a 'burst' appearance. Ascospores plated on CM gave 232 white and 214 yellow colonies, all of normal morphology. This confirmed deletion of the y^+ allele in the IY strain. All the segregants were biotin-independent, showing that the deletion included the *bi* allele. When the proximal part of the duplication remains in yellow variants of *A*, a cross to a standard strain gives, by recombination, a proportion of 'crinkled' (duplication morphology) segregants. Absence of such segregants showed that the

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IY strain lacked this region. The IY strain resulted from a deletion extending at least from the *bi* locus to the point of attachment to linkage group II. Assuming, as was likely, that this variant arose by a single event, it was probable that the deletion extended into the distal right end of linkage group II. On CM with PFA, diploid IY//MSE gave only white haploids; classification of a sample of these is shown in Table 2. With the exception of the member carrying w^+, both members of all linkage groups were recovered.

Table 2. *Haploids from an inviable yellow variant of* A//*MSE*

Linkage group	Phenotype	
I	pro$^+$ paba$^+$	11
	pro paba	12
II	w$^+$	0
	w	23
III	gal$^+$	12
	gal	11
IV	pyro$^+$	8
	pyro	15
V	fac$^+$	8
	fac	15
VI	s$^+$	13
	s	10
VII	nic$^+$	6
	nic	17
VIII	ribo$^+$	9
	ribo	14

The diploid gave only white haploid segregants. For any two linkage groups, all four combinations of markers were recovered among the above haploids; this showed that there was no translocation.

The recessive lethal of the IY strain was linked in coupling with w^+ on linkage group II. Unfortunately, the chromosomes of *A. nidulans* are too small to permit detection of a deletion (Pontecorvo *et al.* 1953; Elliott, 1960) but the combined results of meiotic and mitotic analyses left little doubt that IY was a hypohaploid.

Strain B. This was studied in the hope of finding yellow variants with linkage group I deletions extending beyond the region duplicated in the translocated segment. It would have been possible then to locate the proximal point of break, by determining the recombination frequency between the recessive lethal and *pro1* and *paba6*, as had been done by Nga & Roper (1968) for deletions within the duplicated region.

The viability of twenty-one yellow variants occurring as small patches was examined; all gave yellow colonies. Results of the search for heterokaryotic, possibly inviable, yellows is given in Table 1. Heterokaryotic sectors were far less frequent than in *A* and only one gave an IY. Unfortunately, this was lost on sub-culture. Most of the remaining yellows from these heterokaryotic sectors had

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extremely abnormal morphology and high instability. They had undoubtedly undergone a deletion but their high instability and abnormal morphology showed that they differed in some respect from previously analysed deletion variants, all of which had improved morphology (Nga & Roper, 1968).

Table 3. *Haploids from a yellow variant of* A//*MSF and from a yellow, hypodiploid variant of* A//*MSF*

Linkage group	Phenotype	From yellow variant of *A*//MSF	From yellow hypodiploid variant of *A*//MSF
I	pro^+ $paba^+$	11	19
	pro paba	10	17
II	Acr^+	7	0
	Acr	14	36
III	gal^+	14	20
	gal	7	16
IV	$pyro^+$	9	22
	pyro	12	14
V	fac^+	10	9
	fac	11	27
VI	s^+	11	24
	s	10	12
VII	nic^+	10	21
	nic	11	15
VIII	$ribo^+$	8	11
	ribo	13	25

The diploid produced only yellow haploid segregants. For any two linkage groups, all four combinations of markers were recovered among the above haploids; this showed that there was no translocation.

Strain A//*MSF*. Diploid strains of this constitution produced very frequent yellow sectors following deletion from the translocated segment carrying y^+ (Nga & Roper, 1969). Mitotic segregation for the MSF and some *A* markers showed that almost all of these sectors were diploid, hyperdiploid or, possibly, hypodiploid. The last class was open to detection by direct haploidization of a sample of yellow sectors.

Nine diploid yellow sectors were taken; all had normal or near-normal morphology. Haploids of each variant were selected on CM + PFA. In eight cases both members of all eight linkage groups were recovered among these haploids. An example is given in Table 3. This showed that the deletions responsible for these eight variants did not extend into linkage group II since this would have resulted in failure to recover the Acr^+ allele. For the remaining yellow variant, no Acr^+ types were found in thirty-six haploid segregants, although both members of the other seven linkage groups were recovered (Table 3). This was strong indication of a recessive lethal on the linkage group II member which had carried the duplicate

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segment. Further analysis was not possible but, by analogy with the hypohaploid from *A*, it seemed highly likely that the yellow variant from *A*//MSF was a hypodiploid.

4. DISCUSSION

Previous work had suggested that the deletions at mitotic non-conformity were confined to the unbalanced segments; the possibility of frequent deletions throughout the whole genome had been excluded (Nga & Roper, 1969). Now, with the discovery of hypoploids, susceptibility to deletion is known to extend beyond the unbalanced segments into the contiguous chromosome regions. With the present experimental design we do not know whether the contiguous regions may undergo deletion alone, or whether they may be lost only as part of a larger deletion which includes part of an unbalanced segment. The relative immunity of the balanced part of the genome suggests that the latter is the more likely.

The results give no measure of the frequency of hypohaploids from haploid duplication parents. Furthermore, hypohaploids survive only as heterokaryons and many may go undetected; for this reason there can be no useful comparison even with the observed frequency of viable deletion variants. Strain *A* yielded far more inviable variants than did *B*. The two classes of presumed hypohaploids involved different deficiencies and the result could indicate a difference either in frequency of occurrence or of recovery. The genotype of diploid *A*//MSF favoured detection of yellow variants which had deletions from a translocated segment and, possibly, from the distal end of the chromosome to which it was attached. This gave a direct ratio of diploids and hyperdiploids (8) to hypodiploids (1). Despite the small numbers it is clear that, among deletion variants from an unbalanced diploid, hypodiploids are not rare.

Strains *A*, *B* and *A*//MSF showed frequent mutant sectors on a wild-type background. For example, *A*//MSF gave an average of five yellow sectors per colony of 5·4 cm mean diameter. Previously we have drawn a parallel between the mosaicism resulting from mitotic non-conformity and that ascribed either to genetic instability or to gene inactivation. Without analyses of the sectors—equivalent in higher organisms to the analysis of somatic cells—we might have interpreted our findings in terms of position-effect variegation (P-EV) or, perhaps less likely, of mutable genes. P-EV may arise following transposition of a euchromatic segment to a region of heterochromatin. The effect has been studied most thoroughly in *Drosophila* and is presumed to arise from suppression, in some cell lineages, of certain of the genes in the translocated euchromatin. Mutation was earlier proposed and extensively discussed as an explanation (for summary and references see Schultz, 1936); but evidence to the contrary, summarized by Lewis (1950) and Baker (1963, 1968), has brought general acceptance of the gene inactivation (or suppression) hypothesis. P-EV and mitotic non-conformity (MN-C) can be seen now to have points of similarity which may justify a reopening of this issue.

In P-EV the mutant sectors are clonal (Baker, 1967), and this is clearly so in MN-C. Both MN-C (Lieber & Roper, unpublished) and P-EV (Gowen & Gay,

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1933*a*) have a negative temperature coefficient. In P-EV the expression of untranslocated genes is rarely affected and the phenomenon thus shows a type of *cis–trans* effect. In haploid duplication strains, such as *A* and *B*, either duplicate segment may suffer deletion, and in this there is no pronounced *cis–trans* effect. But, subject to the reservation made previously, MN-C does show a form of *cis–trans* effect in the production of hypoploids. Nga & Roper (1968) have pointed out that the duplicate segments in our strains share, with heterochromatin, the property of mitotic instability. For the present comparison with P-EV the duplicate segments, especially the translocated segment, might be regarded as the equivalent of heterochromatin; the balanced complement would be the equivalent of euchromatin. The unstable state of the translocated segment shows clearly in unbalanced diploids, in which deletions occur almost exclusively in that segment rather than in the untranslocated segments (Nga & Roper, 1969). Within the duplicate segments deletions as big as thirteen map units have been characterized (Nga & Roper, 1968) and Ball's (1967) study may indicate deletions of subgenic size. If deletions of variable size were involved in *Aspergillus* hypoploids, we could use these as a model to explain two aspects of P-EV. In *Drosophila* a single individual may show complex variegation involving the effects of one and more than one gene. This spreading effect (Muller, 1930; Demerec & Slizynska, 1937) is interpreted as polarized suppression extending from the heterochromatin to a variable number of euchromatic genes. Polarization is not found if the euchromatin is sandwiched between heterochromatin. Deletions of variable size originating in one heterochromatic region or, in the latter case, two regions would account both for polarization and non-polarization. Deletions would also explain the apparent irreversibility of suppression in a cell lineage with mutant phenotype. Here the work of Burns & Gerstel (1967), which they do not interpret as P-EV, is relevant. In a *Nicotiana* hybrid, a block of heterochromatin showed mitotic instability and suffered deletions of variable size; variegation resulted when the deletion included a flower colour locus in the contiguous euchromatin. In cases described as P-EV, and in other, possibly related situations, there is only equivocal evidence that the inactivated genes are still present in the phenotypically mutant cells. Demonstration of phenotypic reversion, or compelling molecular evidence, is needed for definitive proof. In *Drosophila*, analysis of polytene chromosomes offers the finest cytological resolution and Schultz (1965) has observed a case in which a band was still recognizable but failed to show its normal puff. However, definitive conclusions are usually precluded by heterochromatization of a variable region distal to the original heterochromatin (Caspersson & Schultz, 1938; Prokofieva-Belgovskaya, 1941).

Baker (1963, 1968) has summarized clearly the grounds on which mutation provides an unsatisfactory explanation of P-EV and we accept that the known details of MN-C cannot answer all of these; but some grounds for objection to a mutation hypothesis are less firm. Genes susceptible to variegation usually show germinal stability. If variegation stems from instability of heterochromatin, then the germ line may be protected, since a chromosome region need not be hetero-

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chromatic in all tissues (Lima-de-Faria & Jaworska, 1968; Priest, 1968). Such protection operates in, for example, *Cyclops*, in which there is loss of heterochromatic segments during embryogenesis (Beermann, 1966). Addition of heterochromatin, such as an extra *Y* chromosome, usually reduces the amount of mutant tissue in a case of P-EV (Gowen & Gay, 1933*b*) and this appears to favour the suppression rather than the mutation hypothesis. But even the former hypothesis faces difficulties; polarization and the *cis–trans* effect indicate a highly localized, intrachromosomal effect while, relatively speaking, the extra heterochromatin acts at a distance. The extra heterochromatin might change the balance or disposition of heterochromatin and so alter the susceptibility to deletion of a particular region. There is another possibility. Nga & Roper (1969) have sought an explanation for MN-C in terms of replication initiated at the nuclear membrane by segment-specific elements which, it is proposed, are limited in number. In these terms translocations, especially of euchromatin to late-replicating heterochromatin, might be expected to provoke errors of replication. Perhaps extra heterochromatin increases the nuclear volume and reduces replication errors through increased accessibility of chromosome segments and the proposed initiating elements.

Mitotic non-conformity produces effects like those observed in yet other situations. Most notable are the cases of mammalian mosaicism for sex-linked genes; these are usually explained either on the basis of an inactive *X* chromosome (Lyon, 1961) or as threshold effects (Grüneberg, 1966). Cases involving autosome-*X* translocations in the mouse (Russell & Bangham, 1959; Cattanach, 1961; Lyon, 1961) are of particular interest as some of these have a close analogy with P-EV. It would be premature to make a detailed comparison with more situations. Our main purpose in this exploration has been to ask whether, for some instances of phenotypic variegation, a re-examination of accepted interpretations is called for.

SUMMARY

Strains of *Aspergillus nidulans* with a chromosome segment additional to the normal complement are vegetatively unstable. Previous work suggested that the deletions occurring at mitosis were confined to the unbalanced segments. It has been shown now that deletions, while probably always involving a duplicate segment, may extend beyond it to produce hypohaploids and hypodiploids, respectively, from unbalanced haploid and unbalanced diploid parents.

Hypoploids have been proposed tentatively as an explanation for some cases of phenotypic variegation; on this basis it is possible to account for some of the diverse phenomena shown by, for example, position-effect variegation.

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