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Altered instability due to genetic changes in a duplication strain of *Aspergillus nidulans*

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SUMMARY

Strains of *Aspergillus nidulans* with a duplicate segment are mitotically unstable; they produce phenotypically improved variants following deletions in either duplicate segment, and morphologically deteriorated types. The number of variants produced is characteristic of each duplication strain under the same conditions. After ultraviolet treatment two variants, one more stable and the other less stable than the original strain, were selected. Genetic analysis showed that the increased instability in the less stable variant was due to a translocation involving linkage groups V and VIII. The increased stability of the more stable variant was due to a recessive factor (*stf–1*) located in linkage group VIII. In the homozygous condition this factor also reduces the number of sectors in a diploid strain. The possible genetic mechanisms explaining the instability alterations are discussed.

1. INTRODUCTION

Strains of *Aspergillus nidulans* with a duplicate chromosome segment are mitotically unstable. Several strains, each with a different chromosome segment in excess of the standard haploid genome have so far been examined and all show similar patterns of instability at mitosis (Bainbridge & Roper, 1966; Ball, 1967; Nga & Roper, 1968; Clutterbuck, 1970*a*). These patterns of instability have never been observed in standard haploids and it seems likely that this type of instability is a feature of all duplication strains of *A. nidulans*. Duplication strains which have a characteristic 'crinkled' morphology and reduced growth rate produce sectors showing various degrees of phenotypic improvement. These arise from nuclei which have lost a variable part of one or other duplicate segment by an intrachromosomal process. Deletions are provoked by, and probably confined largely to, the segments carried in duplicate (Nga & Roper, 1969; Roper & Nga, 1969). Stability and a quantitatively haploid state may be reached either through several deletions or by a single deletion of the whole of a duplicate segment. Duplication strains also produce, infrequently but regularly, sectors with deteriorated morphology and some of them show modified instability (Azevedo & Roper, 1970). Duplication strains produce a regular number of sectors per colony which is characteristic for each strain maintained under the same conditions (Azevedo,

1971). Although it has been shown that alterations in the environment can produce modifications in the number of sectors in duplication strains (Cooke, Roper & Watmough, 1970; Roper, Palmer & Watmough, 1972) much less attention has been paid to the production of duplication variants with distinct degrees of instability due to genetic changes. The present work was carried out to isolate duplication variants which show modified instability in relation to the original duplication strain and to study the genetic changes responsible for the modifications of instability.

2. METHODS

(i) *Media*

Minimal medium (MM) was Czapek-Dox with 1% (w/v) glucose. Complete medium (CM) contained yeast extract, hydrolysed casein, hydrolysed nucleic acids, vitamins, etc. Solid media contained 1·5% agar.

(ii) *Methods of genetic analysis*

General techniques were those of Pontecorvo *et al.* (1953). Diploids were prepared by Roper's (1952) technique. Allocation of mutants alleles and chromosomal aberrations to their linkage groups by mitotic haploidization (Forbes, 1959) was facilitated by the use of *p*-fluorophenylalanine (PFA) (Lhoas, 1961; Morpurgo, 1961). Incubation was at 37 °C.

(iii) *Induction and detection of variants with modified instability*

Saline suspensions of conidia from a duplication strain (strain A) were ultraviolet irradiated with a mercury-vapour lamp, to give about 5% survival. Treated conidia were plated on CM in low densities and incubated 4–5 days. One hundred colonies which at this stage of growth showed no sectors and ten colonies with two or more sectors were isolated, purified and each was inoculated at the centre of 9 cm dishes of CM (ten dishes for each isolate) and the number of sectors was scored after 7 days incubation. The most stable (variant AA), that is, the one which produced fewer sectors, and the most unstable (variant AB) were chosen for genetic analysis in order to detect the causes of modified instability.

(iv) *Organisms*

The strains of *A. nidulans*, which were all derived from Glasgow stocks, were kept at 5 °C on CM slopes. Master Strain E (MSE), carrying markers on all eight linkage groups was that of McCully & Forbes (1965). The duplication strain was strain A (Nga & Roper, 1968) (Fig. 1). Mutant alleles were designated according to Clutterbuck's (1970*b*) suggestions as: *wA3*, *yA2*, white and yellow conidia respectively; *adE20*, *biA1*, *nicB8*, *pabaA6*, *proA1*, *pyroA4*, *riboB2* and *sB3*, requirement respectively, for adenine, biotin, nicotinic acid, *p*-aminobenzoic acid, proline, pyridoxin, riboflavine and thiosulphate; *galA1*, *facA303*, inability to grow on galactose and acetate respectively *suA1–adE20*, suppressor of *adE20*.

3. RESULTS

(i) *Patterns of instability of strain A and variants AA and AB*

The number of improved green and yellow sectors and deteriorated sectors was scored in the original duplication strain (A) and on both, more stable (AA) and more unstable (AB) variants. The duplication strain A, AA and AB had the same phenotypic appearances and growth rates and were distinguished only by the number of sectors produced (Table 1; Plate 1). There were more yellow than green sectors in the three strains analysed. This is consistent with previous experience of this system and showed that deletions which included the yA^+ allele on the translocated segment exceeded the sum of all other deletions (Cooke *et al.* 1970). Also, deteriorated sectors are much less frequent than improved sectors. The number of sectors per colony showed a Poisson distribution in the control strain A as well as in the variants. Statistical significance of comparisons (Duncan's test) regarding the number of sectors produced by A, AA and AB are shown in Table 1.

Table 1. *Sectors produced by strains A, AA and AB*†

Strains	No. of dishes	Mean number of sectors per dish			
		Yellow	Green	Deteriorated	Total
A	24	2·417	0·625	0·083	3·125
AA	74	0·676**	0·108 N.S.	0·081 N.S.	0·865**
AB	34	3·235 N.S.	1·088 N.S.	0·441*	4·765*

† Comparisons between number of sectors produced by AA and AB were significantly different from each other at 1 % level in all cases. N.S., Not significant. *, 5–1 % significance. **, 1 % significance.

(ii) *Genetic analysis*

Diploids constructed from A, AA or AB plus MSE produced the same pattern of instability (Plate 1); the mean number of sectors produced by A//MSE (14 sectors), AA//MSE (15·2 sectors) and AB//MSE (15 sectors) did not differ significantly indicating recessivity of the factor(s) for altered instability. Mitotic haploidization was used to determine the possible causes of modified instability. The results showed that the original translocation–duplication was still present in all three strains. The evidence for this was a rarity of wA^+ sectors which would carry the duplication and were seleted against by the Lhoas (1961) technique. Five exceptional wA^+ sectors (2 out of 23 from the diploid AB//MSE and 3 out of 38 from diploid AA//MSE) were yellow sectors that had probably lost the translocated segment prior to haploidization. However, an additional translocation in strain AB between linkage groups V and VIII was indicated by the total absence of recombinants between *facA* (linkage group V) and *riboB* (linkage group VIII) among the 23 sectors from AB//MSE. Duplication green crinkled sectors are known to be inhibited by PFA (Nga & Roper, 1968; Azevedo & Roper, 1970). However, the use of Morpurgo's (1961) technique, duplication green crinkled sectors (Table 2) did show that all green crinkled haplid sectors fac^+ $ribo^+$ from the diploid AB//MSE

were as unstable as AB; sectors *fac ribo* from this diploid had the same pattern of instability as the original A strain. This suggests that it is the V–VIII translocation presented in AB that causes increased instability. Green haploid crinkled sectors from AA//MSE could be divided into two classes: the first (*ribo*$^+$ sectors) had the AA pattern of instability; the second class (*ribo* sectors) were as unstable as the original A strain. These results suggest the presence of a determinant of stability in linkage group VIII in the AA strain, which causes decreased instability.

Table 2. *Duplication green haploids from AB//MSE AA//MSE and A//MSE*

		AB//MSE		AA//MSE		A//MSE (control)	
Linkage group	Marker	Stability, type AB	Stability, type A	Stability, type AA	Stability, type A	Stability, type A	Stability Type AA or AB
I	pro$^+$paba$^+$	3	2	6	6	17	0
	pro paba	1	2	4	4	5	0
II	w$^+$	4	4	10	10	22	0
	w	0	0	0	0	0	0
III	gal$^+$	2	2	6	4	16	0
	gal	2	2	4	6	6	0
IV	pyro$^+$	2	2	2	3	10	0
	pyro	2	2	8	7	12	0
V	fac$^+$	4	0	6	5	12	0
	fac	0	4	4	5	10	0
VI	s$^+$	1	2	5	7	18	0
	s	3	2	5	3	4	0
VII	nic$^+$	3	1	6	6	18	0
	nic	1	3	4	4	4	0
VIII	ribo$^+$	4	0	10	0	16	0
	ribo	0	4	0	10	6	0

Meiotic green erinkled segregants from the cross AA × MSE were also analysed for the number of sectors. Again two classes of segregants could be distinguished. From 48 segregants, 29 showed the AA type of instability and 19 showed the A type of instability. The factor responsible for decreased instability (stability factor = *stf–1*) behaved therefore as a single gene (χ^2 = 2·08 to fit a 1:1 ratio). The factor *stf–1* was not meiotically linked to *riboB2* in linkage group VIII. A diploid between AA and an AA//MSE haploid mitotic segregant *riboB*$^+$ showed that in a homozygous condition *stf–1* also reduces the instability of the diploid (Plate 1). Such diploids produced a mean of 6·2 sectors per colony.

4. DISCUSSION

It has already been shown that in duplication strains it is the chromosome imbalance which provokes frequent deletions and production of phenotypically improved variants. Nga & Roper (1969) suggested that this is due to errors arising from competition for sites initiating replication of chromosome segments. As a

formal explanation of deletions Nga & Roper (1968) proposed unequal sister chromatid exchange or crossing-over within a intrachromosomal loop. Either of these could give tandem duplications as well as deletions. The origin of morphological deterioration and enhanced instability was tentatively explained by new duplication arising within one or other duplication segment and greater stability is achieved by transposition of all or only part of this extra genetic material to another site in the non-duplicated part of the genome (Azevedo & Roper, 1970).

In the present paper it has been shown that instability can be altered through genetic changes. A point mutation was responsible for decreased instability. A similar situation was recently described by Lee & Nga (1974), where a strain with the I duplication and a VI–VIII translocation possibly with a small segment of chromosome VI in duplicate was extremely stable. It could be thought that, since linkage group VIII was involved in both cases of increased stability, the same point in this linkage group was responsible for the altered instability. However, diploids constructed between the strain described by Lee & Nga (1974) and the master strain were relatively stable, showing the semi-dominant nature of the stability factor. In our case, the *stf-1* factor leading to decreased instability was recessive in diploids. Crosses between two more stable strains might indicate whether the same point in linkage group VIII was involved. On the other hand, the involvement of this linkage group might have been only coincidental, mainly due to the fact that linkage group VIII, and consequently the corresponding chromosome, seems to be the largest one in *A. nidulans* (Pollard, Käfer & Johnston, 1968). A third case of extreme stability was described by Azevedo & Roper (1970) where a deteriorated variant (V 8) derived from a strain with the I duplication presented a determinant of deterioration in linkage group IV; in this case also the stability character was recessive in diploids.

Cases of increased instability, besides the one presented here, have already been described by Burr Palmer & Roper (1971), incorporating a mutation (*uvs*B), in unstable duplication strains. This probably affects excision repair and instability was greatly enhanced. Lieber (1975) obtained a slight enhancement in the frequency of deletions from the I duplication when the III duplication (Bainbridge & Roper, 1966) was incorporated into the system; a partial deletion in this last duplication lead to a great enhancement of the instability of the I duplication.

All these cases show that altered instability can probably be achieved in different ways, which makes any unifying hypothesis premature. In one case (Burr *et al.* 1971) it was suggested that in the duplicate segments of duplication strains there are frequent spontaneous lesions which, failing repair due to introduction of *uvs*B, give deletions. Both variants AA and AB used in the present research, however, did not differ in sensitivity to ultraviolet light when compared to the original A strain. Even when germinating conidia were irradiated, which is known to increase ultraviolet sensitivity (Jansen, 1970), no differences were detected. So no effects due to excision repair seem to be involved here. Factors leading to decrease or increase in recombination might also affect instability, and it would be useful therefore to test the recombination behaviour of strains with modified

instability. So far, crosses between AA × AA showed that frequencies of meiotic crossing-over are not affected by *stf-1*. Meiotic recombination frequencies in crosses AB × AB were not tested since all were infertile. It is known that certain agents, such as trypan blue, produce increased instability, and this was explained in terms of greater liability to replication errors (Cooke *et al.* 1970). Caffeine also increased the frequency of deletions from the duplicated segments of the duplication strain, perhaps by stimulating the mechanism which in unbalanced strains produces replication errors leading to deletions, or by exposing the intrinsic instability of duplication by preventing the repair of spontaneous replication errors (Roper *et al.* 1972). An endogenous substance with similar effects might also be present in duplication strains. In this case, a decrease in the formation of such product would cause a decrease in instability, as found in the AA variant. In the case of the AB variant it is more reasonable to suppose that it is the genetic imbalance due to a further aberration that increases instability. In this connexion it is interesting that chromosome translocation and duplications can affect other duplications, although a duplication does not affect the general stability of diploid regions of a diploid to the same extent. However, some deteriorated strains which probably originated by tandem duplications in the duplicated region transposed to other regions of the genome, giving a large number of deletions in diploids (Azevedo & Roper, 1970). Deletions not in duplicated regions in strains presenting a further chromosomal aberration would cause lethality, but studies could be carried out to see if recessive lethals are produced frequently in diploids with more than one chromosomal aberration.

Regardless of the causes, it has been shown that different patterns of instability can be achieved through genetic mutations in a duplication strains of *A. nidulans* It would be interesting to know if the same mechanisms can be found in other duplication strains or if the factors which modify instability can also alter the instability of other duplication strains. Finally, reduction of instability by genetic methods as presented in this paper can be useful for yield preservation in commercial strains. Certain commercially useful strains may show an instability pattern due to low-yielding derivatives in the population of stored spores of the strain. Reduction of instability is in part achieved by environmental control or through a balanced lethal system (Ball & Azevedo, 1974). The use of point mutations as described here can also be useful for this purpose.

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REFERENCES

AZEVEDO, J. L. (1971). Mitotic non-conformity in *Aspergillus nidulans*. Ph.D. thesis, University of Sheffield.

AZEVEDO, J. L. & ROPER, J. A. (1970). Mitotic non-conformity in *Aspergillus*: successive and transposable genetic changes. *Genetical Research* **16**, 79–93.

BALL, C. (1967). Chromosome instability related to gene suppression in *Aspergillus nidulans*. *Genetical Research* **10**, 173–183.

Ball, C. & Azevedo, J. L. (1974). The applied significance of genetic instability in parasexual fungi. *Proceedings of the 2nd International Symposium in Genetics of Industrial Microorganisms*. New York: Academic Press. (In the Press.)

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

Burr, K. W., Palmer, H. M. & Roper, J. A. (1971). Mitotic non-conformity in *Aspergillus nidulans*: The effect of reduced DNA repair. *Heredity* **27**, 487.

Clutterbuck, A. J. (1970*a*). A variegated position effect in *Aspergillus nidulans*. *Genetical Research* **16**, 303–316.

Clutterbuck, A. J. (1970*b*). *Aspergillus* symbols, locus letters and allele numbers. *Aspergillus Newsletter* **11**, 25–33.

Cooke, P., Roper, J. A. & Watmough, W. A. (1970). Trypan blue induced deletions in duplications strains of *Aspergillus nidulans*. *Nature* **226**, 276–277.

Forbes, E. (1959). Use of mitotic segregation for assigning genes to linkage groups in *Aspergillus nidulans*. *Heredity* **13**, 67–80.

Jansen, G. J. O. (1970). Survival of *uvs* B and *uvs* C mutants of *Aspergillus nidulans* after U.V.-irradiation. *Mutation Research* **10**, 21–32.

Lee, Y. T. & Nga, B. H. (1974). Mitotic stability of a duplication in *Aspergillus nidulans*. *Genetics* **77**, 38–39.

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

Lieber, M. M. (1975). Environmental and genetic factors affecting instability at mitosis in *Aspergillus nidulans*. *Aspergillus Newsletter* **12**, 26–27.

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

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

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

Nga, B. H. & Roper, J. A. (1969). A system generating spontaneous intrachromosomal changes at mitosis in *Aspergillus nidulans*. *Genetical Research* **14**, 63–70.

Pollard, D. R., Käfer, E. & Johnston, M. T. (1968). Influence of chromosomal aberrations on meiotic and mitotic non-disjunction in *Aspergillus nidulans*. *Genetics* **60**, 743–757.

Pontecorvo, G., Roper, J. A., Hemmons, L. M., MacDonald, K. D. & Bufton, A. W. J. (1953). The genetics or *Aspergillus nidulans*. *Advances in Genetics* **5**, 141–238.

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

Roper, J. A. & Nga, B. H. (1969). Mitotic non-conformity in *Aspergillus nidulans*: the production of hypodiploid and hypohaploid nuclei. *Genetical Research* **14**, 127–136.

Roper, J. A., Palmer, H. M. & Watmough, W. A. (1972). Mitotic nonconformity in *Aspergillus nidulans*: the effects of caffeine. *Molecular and General Genetics* **118**, 125–133.