Genet. Res., Camb. (1970), **16**, *pp.* 79–93
With 1 *plate and* 3 *text-figures*
Printed in Great Britain

Mitotic non-conformity in *Aspergillus*: successive and transposable genetic changes

By J. L. AZEVEDO and J. A. ROPER
Department of Genetics, The University, Sheffield S10 2TN

(*Received* 6 *April* 1970)

SUMMARY

Strains of *Aspergillus nidulans* with a duplicate chromosome segment are mitotically unstable; in addition to phenotypically improved variants, arising following deletions in either duplicate segment, they give morphologically deteriorated types, some with enhanced stability. In one isolate, deterioration and increased instability were determined by mutation in a duplicate segment; a more stable derivative no longer had this mutation but had one in another linkage group. Another variant, too unstable for analysis, gave derivatives whose single, new mutations were in different linkage groups. It is proposed that deterioration and increased instability result from tandem duplications on either duplicate segment; transposition of these to non-duplicated regions reduces instability. Another 17 variants had a single new mutation each; mutations, possibly clustered, occurred in all linkage groups. In these strains perhaps transposition preceded analysis. Deteriorated variants gave lineages of types with morphological changes caused by further, superimposed mutations. This continued instability is explained as interaction, in fidelity of replication, of non-homologous chromosome segments.

Instability in *A. nidulans* stems from chromosome imbalance. As imbalance is known or suspected in other cases of instability it may be possible to show common mechanisms for apparently diverse phenomena.

1. INTRODUCTION

Three strains of *Aspergillus nidulans*, each with a different chromosome segment in excess of the standard haploid genome, have been examined and all are unstable at mitosis (Bainbridge & Roper, 1966; Nga & Roper, 1968). The three strains have a similar pattern of instability, never observed in standard haploids, and it seems likely that this type of instability is a feature of all duplication strains of *Aspergillus*. Analysis of the consequences of this 'mitotic non-conformity' has been concentrated so far on those frequently observed events which result in stable types. 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, by an intrachromosomal process, a variable part of one or other duplicate segment. Frequent deletions are provoked by, and probably confined largely to, the segments carried in duplicate (Nga & Roper, 1969; Roper & Nga, 1969). Stability and a quanti-

tatively haploid or near-haploid genome are achieved either by a single, large deletion or a series of independent, smaller deletions.

Much less attention has been paid to the production of variants with increased or otherwise modified instability. Duplication strains produce, infrequently but regularly, sectors with deteriorated morphology. The most interesting feature of this class is that some of them show modified, sometimes greatly enhanced, instability. The present work was designed to probe the genetic changes responsible for modified instability and to analyse some of its consequences.

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 2 % agar.

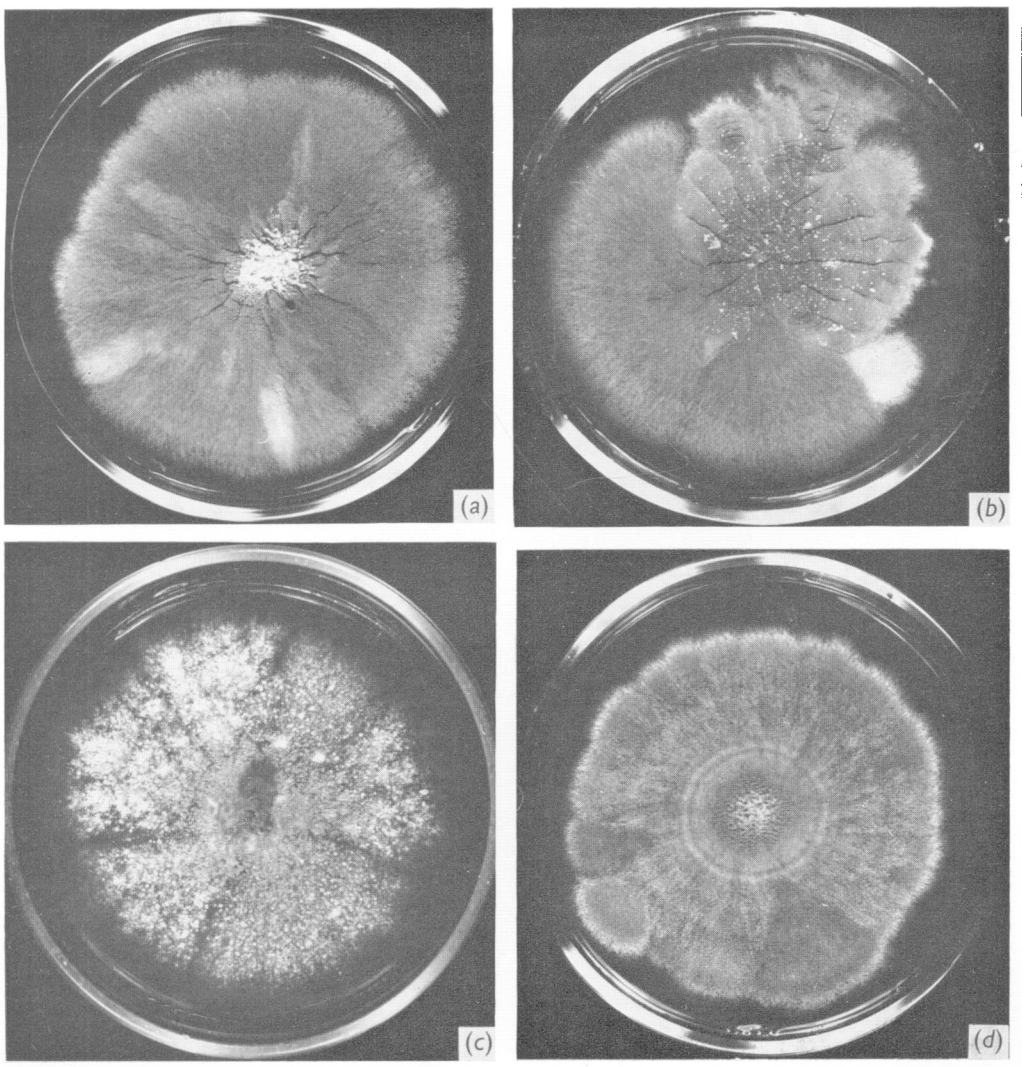
(ii) *Methods of genetic analysis*

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

In the case of highly unstable strains the technique for heterokaryon production was modified so as to reduce the number of mitoses needed before perithecia and diploid conidia could be isolated. Small wells were cut in MM agar plates and filled with liquid MM + 2 % CM; pairs of strains were inoculated into these wells and the usual first growth in liquid medium alone was eliminated. In view of the correlation between size of perithecia and proportion of hybrids the largest were taken from each cross (Baracho, Vencovsky & Azevedo, 1970).

(iii) *Organisms*

Strains of *A. nidulans*, 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). Mutant alleles of importance in this work were the following: *w* and *y*, white and yellow conidia respectively; *fl1*, fluffy; *Acr1*, resistance to acriflavine; *gal1* and *facA*, inability to grow on galactose and acetate respectively; *ad20*, *bi1*, *cho*, *lys5*, *meth2*, *nic2* and *nic8*, *paba6*, *pro1*, *pyro4*, *ribo2* and *ribo5*, *s1* and *s3*, requirement, respectively, for adenine, biotin, choline, lysine, methionine, nicotinic acid, *p*-aminobenzoic acid, proline, pyridoxin, riboflavine and thiosulphate; *su1 ad20*, suppressor of *ad20*; *co*, compact colony. Independently arising, deteriorated variants were isolated from sectors in colonies of strains A and B (Fig. 1). Some, kindly supplied by Dr B. H. Nga, had been stored for up to one year, without subculture, before analysis. Others were analysed within a few weeks of isolation.



Examples of deteriorated variants. (*a*) V2, (*b*) V3, (*c*) V8, (*d*) V4.

https://doi.org/10.1017/S0016672300002299 Published online by Cambridge University Press

(iv) *Symbols*

Deteriorated strains were labelled V1, V2, etc. Second- and later-order variants, arising during vegetative growth, were designated so as to show their lineage. This is made clear in Fig. 3. In almost every case the determinant of morphological change segregated as a single gene. Each original and subsequent determinant was designated as the variant but with a lower case *v*. Haploid components of diploids are separated by the symbol //.

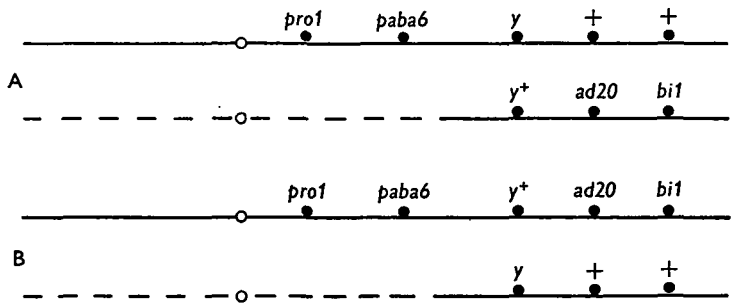


Fig. 1. Duplication strains from which deteriorated variants were obtained. Linkage groups I and II are shown by unbroken and broken lines respectively. Centromeres are designated by open circles.

3. RESULTS

(i) *The origin, incidence and phenotype of deteriorated variants*

It was important to determine whether deteriorated variants arose only from duplication strains and not from standard haploids. Inocula were made at the centre of 9 cm dishes of CM and incubated for 7 days, by which time the colonies covered the agar surface. The yield of deteriorated sectors was: duplication strains A and B, 14 and 11 sectors respectively from 150 colonies of each; 4 standard haploids *pro1 paba6 y; w3, pro1 y bi1, paba1 y; Acr1; co, and bi1*, 0 from 100 colonies of each.

Some of these colonies were produced from single conidia and others from point inoculation of a mass of conidia. In the former case, but not in the latter, there were instances of twin spots with adjacent improved and deteriorated sectors.

Freshly isolated and stored strains were very varied in their morphological characteristics and patterns of instability (Plate 1). Their main common features were a linear growth rate usually about that of the parent duplication strain, reduced conidiation, and differently increased degrees of pigmentation. There was no obvious correlation between morphology and degree of instability. Diploids formed between MSE and each original variant had near-normal morphology. In this the determinants of morphological change were recessive. However, some of these diploids showed greater instability than A//MSE, even when the deteriorated, haploid component was almost totally stable.

(ii) *Analysis of the original variants*

Each variant was analysed by a cross to MSE to investigate the segregation of the determinants of deterioration and to try to separate the possible elements of modified instability. Mitotic haploidization was used to locate each determinant to its linkage group. In some cases further crosses were made to locate determinants more precisely, to look for possible segregation anomalies and to test allelism among determinants on the same linkage group. Some of the results, summarized in Table 1,

Table 1. *Meiotic and mitotic segregation of deteriorated phenotype*

Variant	Strain of origin	Meiotic segregation, deteriorated:non-deteriorated	Linkage group implicated by haploidization*
V6	A	42:110	Deletion on I
V12	A	—	Deletion on I
V22	A	92:86	I
V2	B	366:403	II–I
V7	B	166:167	II–I
V3	B	304:324	II–I
V4	B	73:75	II–I
V19	A	31:45	III
V9	A	119:136	III
V18	A	99:139	IV
V8	A	117:137	IV
V15	B	125:208	IV
V11	A	224:253	V
V21	A	—	V
V1	B	191:221	V
V16	B	61:105	VI
V17	B	88:104	VII
V10	A	104:112	VII
V5	A	52:75	VIII
V13	A	172:192	VIII
V20	A	46:62	VIII
V14, V23, V24	A	Too unstable for analysis	

Variants 1–12 inclusive were kindly supplied by Dr B. H. Nga.

* All variants had a segment of linkage group I attached to II. This complex segregated as a unit in mitotic haploidization.

showed the genetic diversity among the variants and suggested a provisional grouping as follows: types with deletions from linkage group I; those with mutations in linkage group I or the II–I complex; those with mutations elsewhere in the genome. Most of the variants were analysed in some detail but the total results are too extensive for full presentation and only representative examples will be given.

V6 and V12 were deletion types and each differed from A by a single genetic change. V6 required biotin, presumably as a result of a deletion which included the *bi*$^+$ locus. The cross V6 × MSE showed a recessive lethal 15 units distal to *paba6*; segregation of morphological types was 42 deteriorated, 40 non-deteriorated

crinkled and 70 normal. These results showed that deterioration resulted from the deletion in linkage group I. Meiotic analysis, and later analyses of derivatives of V6, did not indicate any change in the I segment of the II–I complex. Haploids from V6//MSE supported the meiotic results. Linkage groups III to VIII of V6 were obtained in normal as well as deteriorated haploids. Haploids of pro paba type, all deteriorated, were recovered only in combination with the II–I complex; the I segment of this complex was needed because of the linkage group I deletion. V6 deterioration stemmed solely from a deletion with breaks determining modified morphology.

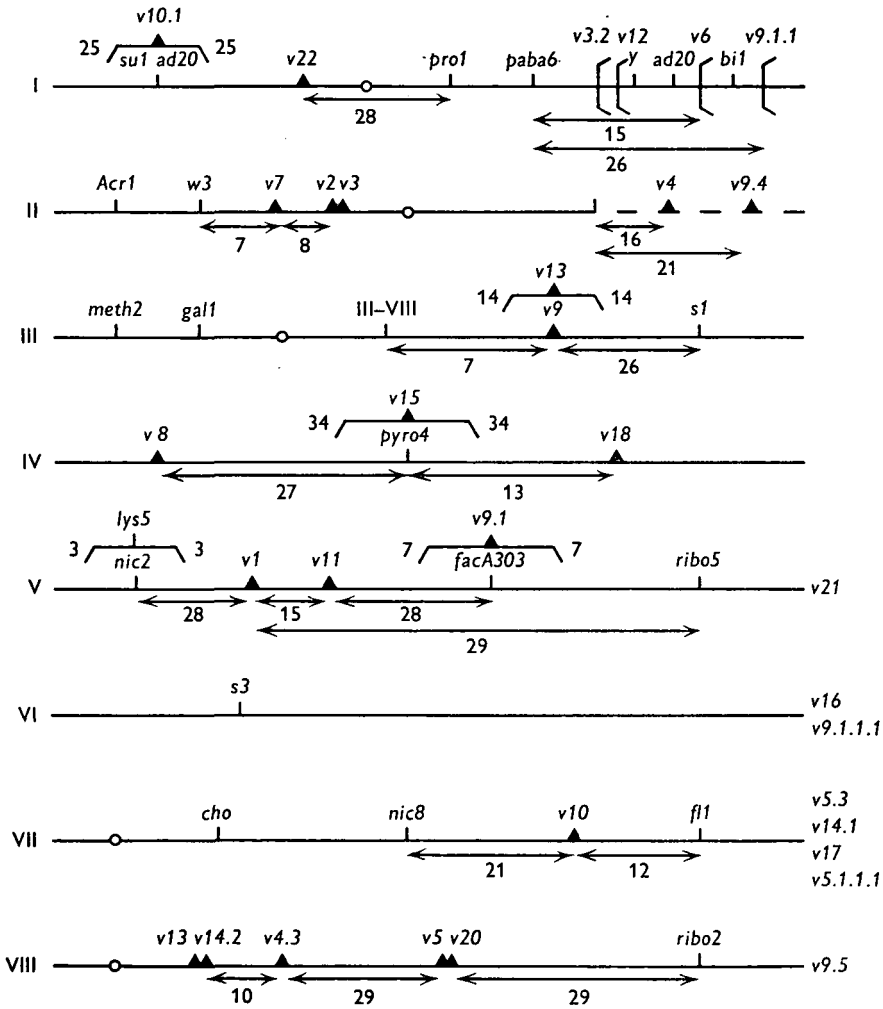


Fig. 2. Locations of the mutations in original variants and strains produced mitotically by them. Linkage maps are not drawn to scale. The symbol ⊂ designates a deletion. The broken line represent the duplicate segment of linkage group I terminally attached to linkage group II. Mutations allocated only to their linkage group are shown at the right. The symbol III–VIII represents the point of break in a particular translocation strain used to locate *v9*. Mutations not yet tested for allelism are shown by joined triangles.

https://doi.org/10.1017/S0016672300002299 Published online by Cambridge University Press

Mitotic and meiotic analyses of V12 and its derivatives showed the similarity of V12 and V6. V12 had a different, probably larger, deletion from its linkage group I and required both adenine and biotin,

V22 showed a single genetic change linked to *pro1* in linkage group I. Haploidization of V2//MSE, V7//MSE and V3//MSE implicated only the II–I complex in deterioration. In a wide range of crosses to normal strains, deteriorated versus non-deteriorated segregated as a single gene difference, in each case located in the II component of the II–I linkage group complex. More refined locations and the results of tests of allelism are shown in Fig. 2.

Table 2. *Haploids from V4//MSE and V4.3//MSE*

		V4//MSE		V4.3//MSE	
Linkage group	Marker	Deteriorated	Non-deteriorated	Deteriorated	Non-deteriorated
I	*pro*$^+$*paba*$^+$	3	15	6	27
	pro paba	0	8	5	3
II	*w*$^+$	3	0	0	2*
	w	0	23	11	28
III	*gal*$^+$	0	14	5	11
	gal	3	9	6	19
IV	*pyro*$^+$	1	12	6	10
	pyro	2	11	5	20
V	*fac*$^+$	0	9	2	14
	fac	3	14	9	16
VI	*s*$^+$	1	14	6	12
	s	2	9	5	18
VII	*nic*$^+$	3	13	3	9
	nic	0	10	8	21
VIII	*ribo*$^+$	3	15	11	0
	ribo	0	8	0	30

* These two haploids carried the II–I linkage group complex of V4.3 and linkage group I of MSE. They were typical duplication types and showed no deterioration. V4 was derived from duplication strain B (Fig. 1) and pro paba haploids which did not have the translocated I segment required also adenine and biotin.

Among the original variants V4 was outstanding in its high instability and in the location of its determinant. It was difficult to obtain deteriorated haploids from V4//MSE but the results unequivocally implicated the II–I complex (Table 2). In crosses to normal, deterioration segregated as a single gene difference with *v4* located 16 units from the point of attachment of the I segment. This did not permit distinction between location in the II and I components of the complex. Later analysis of a derivative, V4.1, showed that *v4* was located in the I segment.

Variants 1, 5, 8–11, 13, 15 and 16–21 constituted another group. In crosses to normal most showed a segregation of deteriorated to non-deteriorated consistent with a 1:1 ratio. In some cases there was a statistically significant deviation from

1:1; this was due to differential viability as the many subsequent crosses gave no indication, in any instance, of more than one genetic difference between a variant and its duplication parent. Analysis of V9 is given as an example. Segregation from the cross V9 × MSE was 77 white, 28 yellow, 31 green non-deteriorated, and 62 white, 25 yellow, 22 green deteriorated. Each marker of V9 and MSE showed 1:1 segregation with the expected linkage relationships. Crinkled types, determined by the translocated duplicate segment, also segregated as expected. Haploidization of V9//MSE is shown in Table 3. As is often the case, there was a deficiency of pro paba types and this was not regarded as significant. There was the expected

Table 3. *Haploids from V9//MSE*

Linkage group	Marker	Deteriorated	Non-deteriorated
I	*pro*$^+$*paba*$^+$	15	19
	pro paba	1	6
II	*w*$^+$	3	5
	w	13	20
III	*gal*$^+$	16	0
	gal	0	25
IV	*pyro*$^+$	8	14
	pyro	8	11
V	*fac*$^+$	9	12
	fac	7	13
VI	*s*$^+$	9	16
	s	7	9
VII	*nic*$^+$	7	11
	nic	9	14
VIII	*ribo*$^+$	10	12
	ribo	6	13

Except for the known translocated I segment, there was no evidence of any segregating chromosome aberration.

shortage of types carrying the translocated I segment; they are at a disadvantage on the PFA medium used in haploidization. Mitotic analysis unequivocally located *v9* in linkage group III. More precise locations of *v9* and other determinants of this group are shown in Fig. 2. A full examination has not yet been made of segregants which are deteriorated but which do not carry the I duplicate segment. A first inspection of a few such segregants suggested that they are not unstable.

(iii) *Segregation anomalies*

In attempts to locate *v1* more accurately, V1 was crossed to strains carrying markers which, in normal crosses, are ordered as follows: *lys5*–3 units–*nic2*–> 50–*facA*–35–*ribo5*. A three-point cross located *v1* between *nic2* and *ribo5*. Recombination frequencies between *v1* and each marker were: *lys5*, 26·9 ± 4·3 %; *nic2*, 28·5 ± 2·8 %; *facA*, 27·6 ± 2·6 %; and *ribo5*, 29·2 ± 2·9 %. There was a strong suggestion of map reduction which might be explained by association of *v1* with an

inversion. In that case some recombination frequencies would be expected to return to normal in homozygous *v1* crosses. Several such crosses were attempted repeatedly but all were infertile.

Mitotic analysis of diploids containing V3 and V5 showed cases of a situation encountered frequently in this work. Haploids selected on PFA show a deficiency of certain alleles, but, apart from these known cases, the markers of a chromosome pair are generally recovered in approximately 1:1 proportions. Total absence of a class is good first evidence of a lethal linked in coupling with the missing marker (Azevedo & Roper, 1967). Two independent isolates of V5//MSE gave only the fac$^-$ types, as if there was a lethal in linkage group V of V5. However, the absence of fac$^+$ types could be ascribed to other causes such as unknown interactions affecting selective advantage on PFA, or to chromosome rearrangement. V3//MSE was unequivocal. The first isolate gave only pyro$^+$ types in 20 haploids, suggesting a lethal in coupling with *pyro4* on linkage group IV of MSE. A second, independent isolate gave both pyro and pyro$^+$ haploids. The lethal in the first V3//MSE isolate must have been generated in the diploid or in the heterokaryon from which it was obtained. Since spontaneous, recessive lethals have a frequency of about 1 in 250 (Azevedo & Roper, 1967; Azevedo, 1970) the origin of this and other lethals in this work could not be ascribed to mutation at normal frequencies.

(iv) *Mitotic variants from deteriorated strains*

The original deteriorated strains produced morphologically distinct types and each such variant was presumed to arise from its parent by a single genetic change. As judged by morphological criteria, each first- and later-order variant arose repeatedly. However, it is possible that there was genetic diversity among morphologically indistinguishable types. Variants from most deteriorated strains were analysed but attention was given mainly to those shown in Fig. 3.

V5. The phenotypic sequence to V5.1.1.1 was: V5.1, an improved green sector; V5.1.1, a deteriorated sector; V5.1.1.1, a sector with further deterioration. Each step involved a genetic change, which segregated as a single gene, superimposed on the existing changes. The various determinants showed interactions and segregation of morphological types from the cross V5.1.1.1 × MSE was very complex. Three points of interest stood out. Diploids formed with V5 line variants carried apparent recessive lethals though definitive evidence of lethals, as opposed to other interactions, was not sought. These possible lethals were in addition to the mutations determining morphology. V5//MSE showed a recessive lethal on linkage group V of V5; V5.1//MSE, a lethal on III of V5.1; V5.1.1//MSE, lethals on III and V of V5.1.1; V5.1.1.1//MSE, a lethal on III on V5.1.1.1. The final step in deterioration arose by a change on linkage group VII. Segregants with this mutation grew only on medium supplemented with yeast extract or peptone. The precise nutritional requirement is not yet known but seems likely to be complicated. Finally the cross V5.1 × MSE gave, among deteriorated segregants, a proportion with compact colonies. Possible explanations of these and other compact types are considered later.

V8. This was apparently very stable and, over a long period of study, gave no sectors. However, the diploid V8//MSE was far more unstable than diploids such as A//MSE. Plated conidia of V8 gave V8-type colonies and a new, compact variant, V8.1. The latter was too unstable for mitotic analysis but segregated in ascospores of 1 out of 15 perithecia tested from the cross V8.1 × MSE. There was a 1:1:1:1 segregation of non-deteriorated non-compact: non-deteriorated compact: deteriorated non-compact: deteriorated compact. All compact segregants, whether or not they carried *v8* and/or the duplicate I segment, showed extreme instability. Like V8.1, the compact segregants grew as small colonies completely surrounded by a faster growing, more stable, breakdown product. Compact segre-

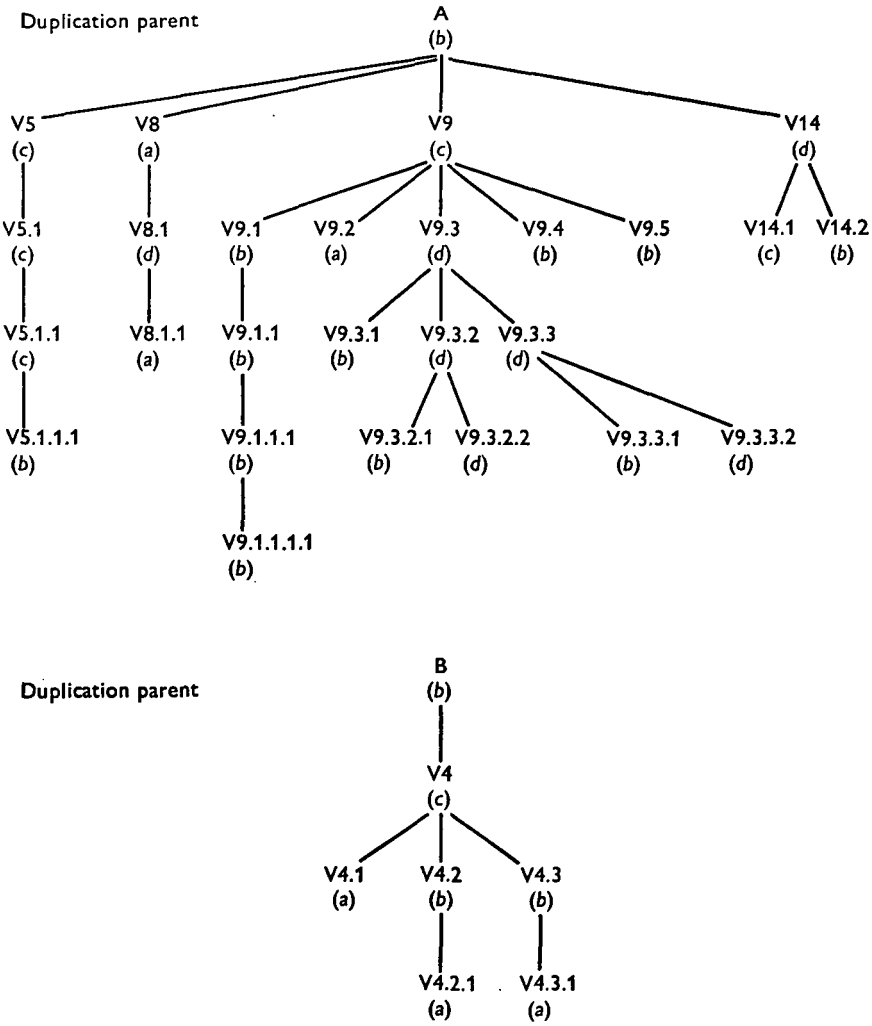


Fig. 3. Lineages of some of the mitotic derivatives from deteriorated strains. Instability was assessed subjectively on sector frequency and graded: (*a*) approaching that of standard haploids; (*b*) stability like duplication parents A and B: (*c*) and (*d*) increasing degrees of instability.

https://doi.org/10.1017/S0016672300002299 Published online by Cambridge University Press

gants from the cross V8.1 × MSE were tested to see whether they showed mitotic segregation for any of the V8.1 or MSE markers. This was found for the gal marker. Some individual, compact, gal$^+$ segregants gave gal$^+$ and gal$^-$ breakdwon products. This type of behaviour is shown by disomics, through the loss of one or other member of the chromosome pair (Käfer, 1961); it is also shown by strains with a duplicate chromosome segment, through loss of all or part of either segment. Although disomy is a possible explanation of V8.1, two pieces of evidence favour interpretation involving a duplicate segment of linkage group III attached to another linkage group. Disomics arise at meiosis, and with increased frequency when one parent has a translocation (Pollard, Käfer & Johnston, 1968), but they have not been detected among the vegetative progeny of chromosomally standard or non-standard haploids. In addition, the 1:1 meiotic segregation of compact:non-compact was more regular than segregation of the extra chromosome in a disomic × normal cross (Ball & Roper, 1966). Mitotic analysis, if it proves possible, would offer definitive distinction between disomy and an attached duplicate segment.

V9. This was moderately unstable and gave repeated origin to a variety of types. All of the analysed derivatives retained the *v9* mutation. V9.2 was an improved sector, of normal or near-normal stability, which had lost most or all of the translocated duplicate segment. V9.3, and derivatives which included a compact type, have not so far been analysed because of their extreme instability. V9.4 arose as a deteriorated sector; it carried a new mutation located in the translocated segment of linkage group I, 9 units from y^+. V9.5 also arose as a deteriorated sector, morphologically distinct from V9.4; it had a further mutation in linkage group VIII. The sequence of phenotypic and genetic changes leading to V9.1.1.1.1 is summarized in Table 4. The final variant in this lineage was severely deteriorated and still unstable.

V4. Mitotic and meiotic analyses had located *v4* on the II–I complex, 16 units from the point of attachment. Definitive location of *v4* was provided by V4.1. This green, stable improved variant required adenine and biotin, presumably following deletion of most or all of the translocated I segment. No deteriorated or crinkled segregants were recovered from the cross V4.1 × MSE or from haploidization of V4.1//MSE; this showed that most or all of the translocated I segment had been deleted together with the *v4* locus which must have been carried on it. V4.2, like V4.1, arose by deletion of part of the translocated I segment. It was still deteriorated as the deletion did not include *v4*. A derivative, V4.2.1, was a stable, green normal. It had undergone a further, probably total, deletion from the translocated segment and in analysis it behaved like V4.1.

V4.3 was a slightly deteriorated sector from V4. The cross V4.3 × MSE gave 138 crinkled non-deteriorated, 147 non-crinkled deteriorated, 115 crinkled deteriorated and 207 fully normal segregants. In view of the higher viability of normal segregants this was acceptable as free recombination of the translocated I segment (determining crinkled) and the V4.3 determinant of deterioration. Haploids from V4.3//MSE located this determinant on linkage group VIII (Table 2). The change, V4 to V4.3, could be explained as a double event involving reversion

of *v4* and generation of a new deterioration determinant. This seemed unlikely as no other case of reversion had been observed. The alternative explanation, more economical in events but more speculative in nature, would be a transposition of *v4* from the translocated I segment to linkage group VIII. If this latter explanation held, then no substantial part of the I segment had been transposed with *v4*; such a transposition would have been revealed in the segregation of crinkled types from V4.3//MSE and from the cross V4.3 × MSE. V4.3.1 was a stable yellow variant which was still deteriorated. It had a deletion from linkage group I and this was confirmed by haploidization of V4.3.1//MSE. The determinant of deterioration remained on linkage group VIII. The cross, V4.3.1 × MSE, gave segregant duplication types in the frequency and of the phenotype expected for an essentially intact translocated I segment.

Table 4. *Genetic and phenotypic changes in one mitotic lineage*

Variant	Phenotypic change	Genetic change
V9	Deterioration	*v9* on linkage group III
V9.1	Improvement	*v9.1* on V, partially suppresses *v9*
V9.1.1	Improvement	Deletion from I.
V9.1.1.1	Deterioration	*v9.1.1.1* on VI. Not suppressed by *v9.1*
V9.1.1.1.1	Deterioration	Unlocated, dominant mutation

V14. This was too unstable for analysis but two derivatives, still deteriorated but morphologically distinct from each other and their parent, were fairly stable. In each of these, deterioration segregated as a single gene difference located on V14.1 on linkage group VII and in V14.2 on linkage group VIII. No firm conclusions could be reached about the genotype of V14. It seemed unlikely that it had two mutations and had produced V14.1 and V14.2 by reversions. Perhaps V14, like V4, had a transposable mutation in one of the duplicate segments. Other derivatives of V14 are being analysed and attempts are still being made to analyse V14 itself.

4. DISCUSSION

The frequency of deteriorated variants from duplication strains, compared with that from standard haploids, indicates that the duplication either increases the frequency of these variants or is totally responsible for them. In duplication strains it is chromosome imbalance which provokes frequent deletions and production of phenotypically improved variants. An explanation for this was offered in terms of errors arising from competition for sites initiating replication of chromosome segments (Nga & Roper, 1969) and it would be economical to suggest a single mechanism for the origin of both classes of variant. In fact, a small proportion of deteriorated types do arise by deletions, presumably with break points which produce modified morphology. But these are a minority and another type of genetic change must be involved in the majority. As a formal explanation of deletions

Nga & Roper (1968) proposed unequal sister chromatid exchange or crossing over within an intrachromosomal loop. Either of these could give tandem duplications as well as deletions and the latter mechanism could also generate circular fragments. From a preliminary analysis of two deteriorated variants Nga and Roper (1968) suggested that phenotypic deterioration and enhanced instability were caused by new tandem duplications on one or other duplicate segment. The present studies give some support to this idea but the evidence is still not conclusive. When colonies of strains A and B were grown from single conidia there was a probably significant frequency of twin spots with adjacent improved and deteriorated sectors. Perhaps each twin spot represented the complementary products of a single chromosomal event. Further evidence of this is being sought through the frequency of twin spots and analysis of their component sectors.

Evidence from the highly unstable variants V4 and V14 may be central to an understanding both of the initial genetic change in most deteriorated variants and of the events leading to increased stability. In V4 the determinant of morphological change and enhanced instability was located on the translocated duplicate segment and there was a strong indication that a derivative, V4.3, arose by transposition of this mutation to linkage group VIII. The proposed transposition, which involves loss of the mutation from its original site, resulted in some morphological change but, more important, in substantially increased stability. Only derivates of V14 were analysed as the parent was too unstable to survive unchanged through the mitoses preceding meiotic and mitotic analysis. The two V14 derivatives had a single mutation each, but in different linkage groups; perhaps these arose by transposition to different locations of one initial mutation.

Most of the variants analysed as presumed originals had mutations in the non-duplicated parts of the genome. However, we cannot be certain that these were original variants and not more stable derivatives which survived storage and purification. It is significant that among the freshly isolated variants there was a greater proportion of very unstable strains than among the older, stored variants. If they were all original, then perhaps tandem duplication within the vulnerable duplicate segments and transposition elsewhere can occur at a single mitotic division or as quickly successive events. There was an important further difference between this group on the one hand and V4 (and perhaps V14) on the other. The former retained their supposed original mutations during subsequent genetic changes while the latter lost theirs in favour of a differently located mutation. Although all linkage groups were involved in the possible original and known secondary mutations, there are indications of a non-random distribution (Fig. 2). Some individual mutants and some clusters are located towards the centromeres. Furthermore, mutations *v3*, *v2* and *v7* show a positive correlation, possibly fortuitous, between the proximity of a mutation to the centromere and the severity of its effect on morphology.

The origin of morphological deterioration and enhanced instability may be explained tentatively, then, by new duplications arising within one or other duplicate segment. Greater stability is achieved by transposition of all or part of

this extra genetic material to another site in the non-duplicated parts of the genome. A scheme with points of similarity has been elaborated by Fincham (1967) to explain features of mutable genes; Fincham's scheme differed from the present one in being based on the master-slave hypothesis of Callan (1967). Duplication of a chromosome segment is proposed as the essential and single cause of mitotic instability; this provokes deletions and production of a second class of elements, tandem duplications. These latter duplications, once separated from the original duplicate segments which provoked them, do not appear to cause substantial instability. But these tentative ideas do not explain persistent instability in a number of lineages, some now analysed to fourth order variants. It is possible that these lines have, simply, a generalized increase in mutation rates and the occurrence of a high frequency of recessive lethals in the analysed diploids might be taken to support this. We are now measuring reversion rates of single genes in a variety of genetic backgrounds derived from duplication strains and the preliminary results do not support the idea of a general increase in mutation rates. The non-random location of *v* mutations also argues against an overall increase in mutation rates. If mutation rates are not increased generally, then the analysed lineages show that non-homologous chromosomes are not entirely autonomous in their fidelity of replication. Mutation in one chromosome may lead to mutation in one or other susceptible region of another. A structure concerned with replication of at least some segments of all chromosomes appears to be required, both to produce the inter-homologue mutations and, perhaps, to facilitate the proposed transpositions. We cannot specify yet even the formal, let alone molecular, properties of such a structure, but there is strong support for the basic idea from the work of Rosenberger & Kessel (1968). From a cytological study of chromatid segregation at mitosis in *A. nidulans* these authors proposed segregation units, attached to membrane sites, carrying DNA strands of the same age.

Within the formal genetic analysis of deteriorated variants there remain incompletely explored areas. First, we need to investigate further the behaviour of *v* mutations, without the initial duplicate segments, to see whether they provoke instability of meiosis or mitosis. In this connexion compact variants, obtained in several lines, may eventually provide important evidence about some of the secondary events of instability. At present these compact types can be interpreted either as disomics or, rather more likely, as strains with a new duplicate segment attached to another linkage group. Secondly, we need to analyse more independent derivatives of the very unstable variants to see whether the secondary mutations are to some extent programmed by the first. Thirdly, it may prove useful to study the apparent release of instability which is observed when a non-sectoring variant, such as V8, gives an extremely unstable diploid. In all of this the aim would be to probe the nature of the genetic changes involved and of their interactions with each other.

Similarities have been pointed out already at the level of observation, and perhaps of mechanism, between this instability in *Aspergillus* and cases ascribed either to genetic or phenotypic instability in bacteria, maize, *Drosophila*, mouse

and man (Nga & Roper, 1968; Roper & Nga, 1969). In revealing probable transposable elements, and inter-chromosomal effects, the present work has extended these similarities, particularly with those found in maize (McClintock, 1951, 1965), bacteria (Dawson & Smith-Keary, 1963) and *Drosophila* (Green, 1969). One feature common to a number of cases of instability, in various species, is the known or possible presence of duplicate genetic material. Further study of the effects of duplications in generating 'errors' of replication may bring some apparently diverse phenomena of genetic instability closer and offer hope of a common explanation. It may then be possible to see whether these cases of instability represent only disordered systems or whether they are abnormal manifestations of processes which, in orderly, programmed sequence, could account for aspects of development.

The authors are indebted to Mrs W. Watmough for skilled assistance and to the Foundation of Assistance to Research of São Paulo State for financial support for one of us (J. L. A.).

REFERENCES

AZEVEDO, J. L. (1970). Recessive lethals induced by nitrous acid in *Aspergillus nidulans*. *Mutation Research* (in the Press).

AZEVEDO, J. L. & ROPER, J. A. (1967). Lethal mutations and balanced lethal systems in *Aspergillus nidulans*. *Journal of General Microbiology* **49**, 149–155.

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.

BALL, C. & ROPER, J. A. (1966). Studies on the inhibition and mutation of *Aspergillus nidulans* by acridines. *Genetical Research* **7**, 207–221.

BARACHO, I. R., VENCOVSKY, R. & AZEVEDO, J. L. (1970). Correlation between size and hybrid or selfed state of the cleistothecia in *Aspergillus nidulans*. *Transactions of the British Mycological Society* **54**, 109–116.

CALLAN, H. G. (1967). The organisation of genetic units in chromosomes. *Journal of Cell Science* **2**, 1–7.

DAWSON, G. W. P. & SMITH-KEARY, P. F. (1963). Episomic control of mutation in *Salmonella typhimurium*. *Heredity* **18**, 1–20.

FINCHAM, J. R. S. (1967). Mutable genes in the light of Callan's hypothesis of serially repeated gene copies. *Nature* **215**, 864–866.

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

GREEN, M. M. (1969). Controlling element mediated transpositions of the white gene in *Drosophila melanogaster*. *Genetics* **61**, 429–441.

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

MCCLINTOCK, B. (1951). Chromosome organization and genetic expression. *Cold Spring Harbor Symposia on Quantitative Biology* **16**, 13–47.

MCCLINTOCK, B. (1965). The control of gene action in maize. *Brookhaven Symposia on Biology* **18**, 162–184.

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 changes 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 nondisjunction 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 of *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.

Rosenberger, R. F. & Kessel, M. (1968). Nonrandom sister chromatid segregation and nuclear migration in hyphae of *Aspergillus nidulans*. *Journal of Bacteriology* **96**, 1208–1213.