THE "RED" CYTOPLASMIC VARIANT OF *ASPERGILLUS NIDULANS*

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1. INTRODUCTION

SPONTANEOUS cytoplasmic mutations are known in fungi (see Jinks, 1961 for review) but they are sufficiently rare to impose serious limitations on any systematic study of cytoplasmic heredity. Their frequency, however, may be raised by experimental treatments including the use of certain well-known mutagens. The present paper describes some of the properties of one such mutation in *Aspergillus nidulans* produced by ultra-violet irradiation (Arlett, 1957).

Unless otherwise stated the colonies were grown throughout on Czapek minimal agar at 25° C. Where a complete medium is used it is that given by Pontecorvo (1953).

2. THE NATURE OF THE RED VARIANT

The red variant of *Aspergillus nidulans* was produced by ultra-violet irradiation of asexual spores of a wild type strain. There was nothing about its mode of origin to distinguish it from the many nuclear mutations induced at the same time. Subsequently, however, two of its properties led to its classification as an extranuclear mutation. These were primarily (i) its persistent segregation and (ii) its behaviour in a heterokaryon with normal partners.

(i) Persistent segregation

Although the initial variant colony arose from a single irradiated asexual spore, the spores in this species being uninucleate and haploid, the colony itself was a mosaic. Sectors appeared with a reduced density of perithecia, which contained no viable sexual spores, an increased rate of growth and a marked red coloration some of which was secreted into the medium (plate Ia). The asexual progeny of this and similar colonies obtained by hyphal transfers invariably segregated into two major phenotypic classes, one apparently normal, which we shall call *r*-normal, the other like the abnormal sectors of the parent colonies, which we shall call *r*-red (plate Ib). Neither the *r*-normal nor the *r*-red colonies, however, were completely stable for when extensive vegetative growth of either occurred the *r*-normal colonies often gave *r*-red sectors and very occasionally *r*-red colonies gave *r*-normal sectors. Furthermore both *r*-normal and *r*-red colonies

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gave rise to the two phenotypes in their asexual progenies although with widely differing relative frequencies ranging from 0 to 95 per cent. *r*-red segregants. The frequencies of *r*-red segregants in the asexual progenies of 61 *r*-normal and 83 *r*-red colonies are given in text-fig. 1. From this it will be seen that a proportion of the *r*-normal colonies never give any *r*-red asexual progeny nor do these same colonies give *r*-red sectors, *i.e.* they are true breeding wild type colonies. In contrast, even though deliberately looked for, no true breeding *r*-red colony has ever been obtained.

TEXT-FIG. 1.—The distribution of percentage *r*-red segregants in the asexual progenies of 61 colonies classified as *r*-normals ■ and 83 classified as *r*-reds □.

While the *r*-normal and *r*-red segregants differ in a number of properties which make it possible to classify them unambiguously the segregation is in other respects continuous. Thus while the *r*-red colonies on average grow 27 per cent. faster than the *r*-normals and have some 49 per cent. less perithecia there is considerable variation among *r*-red and *r*-normal colonies for these two properties and also an overlap between one extreme of the *r*-red distribution and the opposite extreme of the *r*-normals. Furthermore, while no *r*-normal colony has ever given more than 20 per cent. *r*-red segregants in its asexual progeny and an *r*-red never less than 5 per cent. *r*-red progeny there is in fact a continuous distribution of proportions of *r*-red segregants among asexual progenies of *r*-normal and *r*-red parents (text-fig. 1). All three characters, therefore, agree in indicating a continuous variation and indeed rate of growth, perithecial density and per cent. *r*-red segregants are highly correlated in random samples of asexual progenies (*r* varies between 0.76 and 0.91 in all cases). The *r*-red, *r*-normal discontinuity in surface morphology must result

from a threshold effect in an underlying continuously varying system, the threshold coinciding with that part of the distribution where a colony gives approximately 10 to 15 per cent. *r*-red segregants in its asexual progeny.

We shall return to other properties of this segregation later. For the present it is sufficient to note that this segregation has been repeated in every asexual progeny of an *r*-red colony and in many progenies of *r*-normal colonies during a period of five years. Although this is the first and most fully documented example of persistent segregation it is no longer unique to the red variant of *A. nidulans* nor indeed to variants of this species (Jinks, 1960, 1961). Where, as in *A. nidulans*, persistent segregation coincides with a uninucleate haploid condition of the asexual spores it is presumptive evidence of an extra-nuclear origin of the segregation. That persistent segregation may coincide with other expressions of extra-nuclear behaviour is illustrated in the next section.

(ii) *Heterokaryon tests*

The heterokaryon test for distinguishing between nuclear and extranuclear control of a difference in phenotype was devised for use with stable differences in species in which stable heterokaryons can be made without recourse to complementary biochemical requirements between the components of the heterokaryon (Jinks, 1954, 1958). Both limitations, however, can be overcome, although in this particular instance the requirement of complementary biochemical differences for heterokaryotic stability leads, as we shall see, to considerable difficulties.

For this test we require a heterokaryon between a red variant with nucleus A and a non-red strain with a different nucleus B. If the difference between the red variant and normal is nuclear we should then recover in the asexual progeny of the heterokaryon both *r*-red and *r*-normal segregants with nucleus A but only normal colonies with nucleus B. On the other hand, if the difference is cytoplasmic our simplest expectation is to recover the *r*-red and *r*-normal phenotypes with both nucleus A and B. The distinction between nuclear and cytoplasmic control, therefore, rests solely on the recovery of *r*-red colonies with the B nucleus. The recovery of the reciprocal class, normals with the A nucleus, in this instance proves nothing since a small proportion would be present, in any case, in the asexual progeny of the red component of the heterokaryon.

This simple expectation could be complicated in two ways, both of which could invalidate the test. Firstly, the normal component of the heterokaryon could be suppressive (see section 3 (iii)) in which case the recovery of *r*-red colonies with the B nucleus could be extremely difficult or impossible even if the difference between red and normal were cytoplasmic. Secondly, there could be nuclear-cytoplasmic interaction whereby both the maintenance and phenotypic expression

of the red condition depended on genes which are present only in the A nucleus (see, for example, Roper (1958)). This again would negate the attempt to distinguish between nuclear and cytoplasmic control. We can attempt to overcome these difficulties in a number of ways. The effect of the first complication could be minimised by (1) using an *r*-red component which gives a high frequency of *r*-red segregants in its asexual progeny, *i.e.* increase its input into the heterokaryon, and (2) analysing the asexual progeny of the heterokaryon as soon after its formation as possible. Both of these steps have proved helpful under similar circumstances (Jinks, 1961). The second complication can also be minimised by ensuring that nuclei A and B are identical apart from the few major gene differences necessary for distinguishing between them and by using different major genes for this purpose in a series of parallel tests.

Since complementary biochemical requirements are needed to obtain a stable heterokaryon the first attempt to obtain one was made with an *r*-red *ad*⁻ and a normal *lys*⁻ strain; both nuclei being identical apart from these two auxotrophic mutations. The heterokaryon was stable but the *r*-red phenotype could not be maintained in the presence of the *ad*⁻ mutant nor were any *lys*⁻ *r*-red segregants obtained in the asexual progeny of this heterokaryon. This double failure, the reasons for which we shall return to later (section 4 (i)), led to a new approach which has proved to be of general applicability.

An *r*-red colony with a wild type nucleus was confronted by a normal colony carrying a white spored mutant (*w*₃), a compact variant of the latter (*w*₃*co*) and a diffuse mutant (*w*₃*df*). Although no stable heterokaryon was formed, hyphal anastomoses were observed at the interface with the occasional production of heterokaryotic conidiophores. The latter were recognised by their production of chains of both green (*W*₃) and white (*w*₃) asexual spores. Thus these spores are the product of a heterokaryotic association of the two component nuclei and of the mixing of their cytoplasm which was initiated at hyphal anastomosis and quickly resolved by the spore production of the conidiophore.

The asexual spores from single heterokaryotic conidiophores were analysed for each of the three combinations between normal and the red variant. The results are summarised in table 1. Every conidiophore sampled gave asexual progenies which segregated both for the known nuclear differences and for the red, non-red difference. Furthermore, in each of the three combinations tested the critical class, *i.e.* the red phenotype with the nucleus of the initially normal component, was obtained although with a somewhat lower frequency than expected. In fact there is a significant deviation from random association between the two alternative nuclear genotypes and the *r*-red, non-red difference and in each case a shortage of the class, *r*-red *w*₃, is primarily responsible. Furthermore, no colony in this class was initially a typical *r*-red, they all originated as mosaics with both red and normal sectors and the

former only gradually increased in frequency to give a uniform *r*-red growth. The relatively low frequency with which the class showing reassortment is recovered is shown by other presumed cytoplasmic variants when examined by this technique (Grindle, unpublished).

TABLE 1

Segregations in the asexual progenies of single heterokaryotic conidiophores between r-red and normal colonies differing by the nuclear genes indicated

1. Heterokaryon between W_3 *r*-red and w_3 non-red

	Asexual progeny from 5 conidiophores	
	W_3	w_3
<i>r</i> -red	211	51
non-red * . . .	43	762

2. Heterokaryon between W_3DF *r*-red and w_3df non-red

	Asexual progeny from 6 conidiophores	
	W_3DF	w_3df
<i>r</i> -red	10	13
non-red * . . .	17	123

3. Heterokaryon between W_3CO *r*-red and w_3co non-red

	Asexual progeny from 3 conidiophores	
	W_3CO	w_3co
<i>r</i> -red	24	13
non-red * . . .	48	65

* The non-red class includes all colonies with non-red phenotypes, *i.e.* both true breeding normals and *r*-normals whose asexual progenies would contain some *r*-red segregants.

It appears, therefore, to be a consequence of the brief opportunity for nuclear, cytoplasmic reassortment offered by the unstable, short lived heterokaryotic association. Nevertheless, our results suggest that the red variant is cytoplasmic in origin but at the same time they show that there is an association between the nucleus and the cytoplasmic determinants of the red variant which may survive the nuclear, cytoplasmic mixing which occurs during the heterokaryotic association.

3. THE HETEROPLASMIC SYSTEM OF THE RED VARIANT

The evidence presented in section 2 supports a cytoplasmic basis for the red variant. The difference between the red variant and wild type, however, must be complex. Since the red phenotype is transmissible to other strains by heterokaryosis its basis is not merely the absence of some cytoplasmic determinant but rather the presence of a functionally changed one. Furthermore, the ability of the red variant to produce wild type segregants suggests the presence of cytoplasmic determinants which are functionally normal. Either, therefore, the functionally normal determinants are present alongside the functionally abnormal, that is the red variant is a heteroplasmon or, alternatively, the functionally normal determinants arise by back mutation in a homoplasmic mutant cytoplasm. Since, however, the latter will also lead to a heteroplasmon the only difference between these two alternatives is the mode of origin and maintenance of the heteroplasmic condition. The ability of *r*-red colonies to segregate persists through successive asexual propagations; the basis of this segregation, namely the heteroplasmic condition, must therefore, also persist or be continually reinitiated following each propagation. Hence either asexual spores produced by the heteroplasmon are themselves heteroplasmic or, if they are homoplasmic, the heteroplasmic condition must be restored by back mutation. We shall now consider the evidence for these alternatives.

(1) *Parent-Offspring correlations*

We have seen that the large majority of asexual spores produced by a red variant give rise to colonies whose own asexual spores repeat the segregation into *r*-red and *r*-normal phenotypes. Furthermore, this segregation is observed irrespective of how soon after an asexual spore germinates its own asexual spores are sampled. Indeed, the spores produced in the first conidiophore following germination will show this segregation. It would seem, therefore, that the ability to segregate is inherent in the contents of the spores themselves. That is, the spores may be heteroplasmic and in the majority of cases appear to be so. In addition there are high correlations between the percentage of *r*-red segregants contained in the asexual progeny of a colony and the percentage of *r*-red segregants which the progeny produce in their own asexual progenies ($P < 0.001$). Thus not only does the heteroplasmon appear to persist through asexual spore propagations but the composition of the heteroplasmon may differ between colonies and these differences persist through asexual spore propagations. Irrespective, therefore, of how the heteroplasmic condition of the red variant originated the ability to produce heteroplasmic asexual spores allows its persistence through asexual propagations.

(ii) Segregation pattern

Given that the asexual spores carry more than one representative of the determinant whose mutation gave rise to the red variant and therefore have the potentiality to be heteroplasmic, it follows that the segregation pattern of the asexual progenies of *r*-red colonies must be relatable to the mean number of such determinants per spore and their distribution among spores. The situation is formally identical with that relating the proportion of nuclear types in the multinucleate asexual spores of a heterokaryon. Thus if there is random distribution of normal and mutant determinants at asexual spore formation and there is always the same total number of determinants per spore then the segregation is given by the binomial expression $(p+q)^n$,

where, p = the proportion of normal determinants

q = the proportion of mutant determinants

and n = the number of determinants per spore. From which it follows that we should expect

p^n spores which contain only normal determinants,

i.e. pure breeding homoplasmic wild types.

q^n spores which contain only mutant determinants,

i.e. pure breeding homoplasmic mutant types.

And, $1-p^n-q^n$ spores which contain both normal and mutant determinants *i.e.* the segregating heteroplasmic mutant types.

In applying this model to the observed segregations we are making one additional assumption, namely, the equal viability of all classes of spores. The validity of this assumption is critical in a number of ways for while we regularly recover pure breeding homoplasmic wild type segregants and heteroplasmic mutants in the asexual progenies of red variants we never recover the third class, the pure breeding homoplasmic mutant. This could, of course, mean that our interpretation of the cytoplasmic state of the red variant is incorrect. Too many properties of the red variant, however, are understandable on the basis of a heteroplasmic nature for us to consider seriously an alternative unless all attempts to explain the absence of this third class fail. Three possible explanations of the absence of pure breeding red segregants suggest themselves.

1. No pure breeding homoplasmic mutant spores are produced. Thus successful differentiation of a conidiophore and its asexual spores might only occur if more than a certain proportion of functionally normal determinants were present in the cell lineage. The low sporulation of the red variant and disorganised appearance of the heads with their short chains of spores all suggest difficulties in differentiating the asexual stage, thus lending some support to this explanation.

2. Pure breeding homoplasmic mutant spores are produced but they are inviable. This is clearly an indistinguishable extension of the previous explanation that merely requires delaying the inability of a homoplasmic mutant cytoplasm to function alone until spore germination.
3. There is continuous backmutation of the mutant determinants. This has already been suggested as one explanation of persistent segregation. If backmutation to functionally normal determinants occur then there would be no possibility of obtaining or retaining a pure breeding homoplasmic mutant clone even if spores with this constitution were produced.

The alternatives are, therefore, an explanation based on the inability of a pure breeding homoplasmic cytoplasm to function at certain stages in development with a resulting selection for heteroplasmons at these stages or continuous backmutation restoring the heteroplasmic state. Before pursuing this argument further we will examine another relevant property of the red variant, namely, suppression.

(iii) *Suppression*

In every mixture of mutant and normal cytoplasms so far investigated one component has been suppressive. Sometimes it is the normal component but more often it is the variant (Jinks, 1959, 1961). This high frequency of suppressive mutants can be related to the techniques whereby they were recognised and selected. Thus, if as we have suggested for the red and other variants (Jinks, 1958) the initial mutation arises in a spore or hypha containing many like determinants not all of which mutate then only a suppressive mutant would build up to the frequency and level of phenotypic expression necessary for it to be recognised and hence selected for further analysis. The red variant shows two aspects of suppression. Firstly, the percentage of *r*-red segregants in the asexual progeny of an *r*-red colony in general increases at a mean rate of 12.7 ± 4.1 per cent. per day during the log phase of growth up to a maximum of 74 per cent. although the percentage actually falls during staling. Thus during the period of most active growth the mutant determinants appear to have a superior rate of multiplication to their wild type counterparts. Secondly, colonies which give up to 90 per cent. *r*-normal segregants in their asexual progenies may nevertheless have an *r*-red phenotype. Thus the mutant determinants, which are clearly in the minority, have a superiority in action over their wild type counterparts. Why, we may therefore ask, does this suppressive mutant fail to replace completely its normal counterpart in a mixed cytoplasm? Again we have two possible explanations, inviability and backmutation. In the former case we would need to extend our earlier postulate that asexual spores containing only mutant cytoplasm either fail to be

formed or fail to germinate to homoplasmic mutant hyphæ. On this view the cytoplasmic system of the red variant would, therefore, be a dynamic equilibrium between a suppressive mutant component and a normal component which is essential, albeit in low concentrations, for growth to occur. The alternative explanation would assume that homoplasmic mutant hyphæ like spores of similar constitution could not give rise to homoplasmic mutant colonies because of a continual backmutation of the mutant determinants. Thus the dynamic equilibrium on this view would be between a suppressive mutant component which is continually replacing its normal counterpart but which in turn is continually replaced by backmutation of the mutant component. Either explanation would account for the remarkable stability of the red variant through prolonged vegetative propagation.

No direct evidence is available for distinguishing between these two explanations of the failure to obtain pure breeding red variants. We have, however, observations which indirectly favour the explanation based on the lethality of the homoplasmic mutant state. We have already seen that asexual spore production is disorganised in the red variant and this is associated with a reduced viability of the spores. For example, in a random sample of *r*-red colonies giving from 22 to 67 per cent. *r*-red segregants in their asexual progenies there was a negative correlation ($r = -0.66$) between viability and percentage *r*-red segregants. Furthermore, on ageing, the spores which would give rise to *r*-red colonies become inviable five times as fast as those which would give rise to *r*-normal colonies. Therefore, asexual spores containing sufficient mutant determinants to give rise to an *r*-red colony are sub-vital, they have a reduced viability on formation and degenerate faster on ageing. While the higher the proportion of *r*-red segregants produced by a parent colony and hence, presumably, the higher the frequency of spores containing a high proportion of mutant determinants the greater is this inviability. We have, therefore, sufficient correlation between the presence and probable dosage of the mutant determinant and inviability to accommodate the explanation of the absence of a pure breeding mutant cytoplasm based on its lethality. While this does not constitute a proof it does allow us to accept this explanation on fewer assumptions than its alternative.

Irrespective of which explanation is correct the differential viability of spores containing different proportions of mutant and normal determinants is undeniable. Hence an important assumption underlying any method of estimating the mean number of determinants per spore is not fulfilled. We can still, however, obtain a minimal estimate based solely on the frequency of pure breeding wild type segregants which will of course be inflated by the viability disturbances. Since the frequency of this class is inversely related to the mean number of mutant determinants per spore our estimate of the latter will be correspondingly deflated. Our estimate obviously depends on what

we assume about the distribution of mutant determinants at spore formation. If we assume that it is random we may equate the frequency of pure breeding wild type segregants to the first term of a Poisson series. In a typical experiment the asexual progeny of a *r*-red colony giving 60 per cent. *r*-red segregants, gave 5 out of 1667, *i.e.* 0.3 per cent. colonies which were pure breeding wild types. This is equivalent to a mean number of mutant particles per spore of 5.8.

4. NUCLEAR-CYTOPLASMIC INTERACTIONS

We have previously mentioned the possibility of nuclear cytoplasmic interactions in the red variant and how this determined our choice of nuclear markers for the heterokaryon test. We shall now examine the evidence for such interactions. This evidence is of two types.

- (i) The behaviour of the red variant following the introduction of certain nuclear gene mutations.
- (ii) The spectrum of mutations which have been induced in the red variant compared with that obtained in wild type controls.

(i) *Auxotrophic red-variants*

As a preliminary to the heterokaryon test asexual spores of *r*-red colonies were extensively screened for mutations induced by ultra-violet irradiation. Only one auxotrophic mutation with a simple biochemical requirement, namely for adenine, was obtained. This adenine requiring colony even on an adenine supplemented medium had an unusual phenotype and produced few conidiophores and no perithecia. But like a typical red colony its asexual progeny segregated into two clear classes one of which was similar to the adenine requiring parent colony (plate 1c). The second class consisted of smaller colonies with raised centres and these frequently sectoried to give the other type of growth. Only the original unsectoried parts of the colonies in this class showed persistent segregation, the parental like colonies and the sectors were pure breeding although in the small progenies produced by these virtually sterile colonies a low frequency of segregation could not be ruled out. Nevertheless, as a result of a rapid decrease in the frequency with which the smaller segregants appeared, even in their own asexual progenies, and their continual sectoring this class was finally lost in spite of heavy selection in its favour. In interpreting this behaviour the parental colonies which either did not segregate or did so with a very low frequency would be equatable to our *r*-normals and the non-parental segregants which segregated again to our *r*-reds. However, this interpretation suggests that the *r*-red colonies regularly give *r*-normal sectors and that the *r*-normals become the predominant and finally the sole surviving phenotype. This is a complete reversal of the behaviour of the original *r*-red and *r*-normal phenotypes. And yet this interpretation appears to be correct for

Plate

Plate Ia

A colony of the original red variant showing sectoring into *r*-red.

Plate Ib

The asexual progeny of an *r*-red colony showing the segregation into two classes *r*-normal and *r*-red.

Plate Ic

The asexual progeny of an adenine requiring nuclear mutant of the red variant. The parent colony was believed to be equivalent to the *r*-red class and had the phenotype of the smaller of the two segregants shown in the photograph. Thus the adenine requiring mutant still shows segregation into two classes but with markedly modified phenotypes (compare plate *Ib*).

when a backmutation to adenine independence occurred in an *ad*⁻ parental colony, which we have suggested is equivalent to *r*-normal, it did indeed prove to have this phenotype and the breeding behaviour associated with it.

We have already mentioned the anomalous results obtained in a heterokaryon test between this *ad*⁻ red and a *lys*⁻ normal strain (section 2 (ii)). From this heterokaryon the spores produced by single conidiophores were sampled. Eight such samples totalling 1478 colonies contained both *ad*⁻ and *lys*⁻ phenotypes and hence came from heterokaryotic conidiophores. But only two of these samples segregated for the red, normal difference and this segregation was confined to the *ad*⁻ colonies. Since we know from much more extensive tests that the red, normal difference reassorts with the nuclear components in a heterokaryotic conidiophore we can hardly accept this evidence as proof to the contrary. Nevertheless it requires an explanation. The first point to bear in mind is that the *ad*⁻, *lys*⁻ heterokaryon produced less than 3 per cent. of the segregants which we have equated with *r*-red, hence the frequency of mutant determinants is presumably extremely low. Secondly where we observed reassortment in the earlier heterokaryon tests the mutant determinants were suppressive; in the present heterokaryon tests where we have failed to observe reassortment the mutant determinants are being suppressed and rapidly lost altogether. We might reasonably conclude, therefore, that the opportunities for reassortment were considerably more restricted in the latter case and hence the failure to observe it, in the size of progeny examined so far, inconclusive. On the other hand, there is the alternative possibility that the *lys*⁻ nucleus is producing an even more extreme form of nuclear-cytoplasmic interaction with the mutant determinants than we have already observed with the *ad*⁻ nucleus; so extreme in fact, that the mutant determinants cannot coexist with the *lys*⁻ nucleus. Only further investigations can tell us whether this is indeed the case.

(ii) *Induced mutation rates*

The induction of mutations by ultra-violet irradiation has been attempted both in the red variant and in the wild type strain from which it arose. Various techniques have been used including total isolation of the irradiated asexual spores onto complete medium (Pontecorvo, 1953) and selective enrichment followed by isolation onto any preferred medium (Catcheside, 1954) but always on parallel samples of the red variant and wild type. The cumulative results given in table 2 are therefore strictly comparable. These results show a consistent deficiency of induced mutations in the red variant which is common to all types of mutant. Either, therefore, the red variant is less susceptible to the mutagenic action of ultra-violet irradiation or we are not recovering the results of nuclear mutations in the red variant. The latter possibility is the more interesting since its basis

would presumably reside in some form of incompatibility between certain nuclear changes and the mutant cytoplasm. Indeed we would need to postulate that go per cent. of those mutations which occur in the wild type strain are lethal and hence never recovered when they occur in the red variant, that is, these nuclear mutations cannot survive in the mutant cytoplasm. This interaction while basically similar is resolved in a different manner from that which followed the introduction of the *ad*⁻ mutation into the red variant for in this case it was the mutant cytoplasm that could not survive following the nuclear change. This explanation of the deficiency of nuclear mutations in the red variant is, however, suspect for a different reason. Pigmentation and morphological mutations which have been recovered in the wild type strain but not so far in the red variant

TABLE 2

The types and frequencies of mutations induced in the red variant and in the wild type strain from which it arose by U.V. irradiation

	Wild type	Red variant
Colonies tested . .	1368	3241
Types of mutant		
pigmentation . .	2	0
morphological . .	15	2
biochemical . .	7	2

have nevertheless been associated with the mutant cytoplasm by heterokaryotic reassortment. These reassortments are perfectly viable. On the other hand preliminary attempts to reassort the red variant with biochemical requiring mutants using exactly the same technique which was successful with the morphological and pigmentation mutants have so far failed. Thus while the alternative explanation appears at the moment to be favoured a final decision must await more critical evidence than is yet available.

5. SUMMARY

The red variant is a vegetatively stable mutant of *Aspergillus nidulans* induced by ultra-violet irradiation. The persistent segregation in its asexual progenies which include some wild type segregants and its behaviour in heterokaryotic association with non-red partners suggest that the red variant is a heteroplasmon between mutant cytoplasmic determinants and their wild type counterparts. The failure to recover a pure breeding red variant further suggests that the mutant component of this heteroplasmon is lethal in the homoplasmic state. The vegetative stability of the variant is then understandable as a dynamic equilibrium between the mutant component

which suppresses its normal partners and the ultimate reliance of the mutant component on the presence of the normal for its survival.

If we assume that the distribution of mutant determinants during asexual spore production is random then we require at least 5.8 determinants per spore on average to account for the observed segregations in the asexual progenies.

The failure of the mutant determinant to maintain itself following the introduction of an adenine requiring nuclear mutation into the red variant suggests that it may be profitable to examine nuclear-cytoplasmic relationships in this variant.

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