NUCLEO-CYTOPLASMIC INTERACTIONS IN THE 'RED' CYTOPLASMIC VARIANT OF *ASPERGILLUS NIDULANS*

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1. INTRODUCTION

THERE is considerable evidence from studies of a wide range of both plant and animal materials which suggests an extrachromosomal, if not cytoplasmic, basis for the inheritance of certain characters (see Caspari, 1948; Jinks, 1963, 1964 for reviews). Few examples, however, have been analysed systematically to ascertain the extent to which cytoplasmic hereditary determinants are dependent on nuclear genes for their replication and expression. Recent studies of the 'red' variant of *A. nidulans* (Arlett, Grindle and Jinks, 1962) showed that its phenotype and properties could be altered substantially by associating the mutant cytoplasm of the variant with different nuclear genotypes. Consequently, it was suggested that it would be profitable to examine nuclear, cytoplasmic relationships in the variant. This paper is an extension of the previous work and describes the behaviour of the 'red' variant following the introduction of nuclear gene mutations.

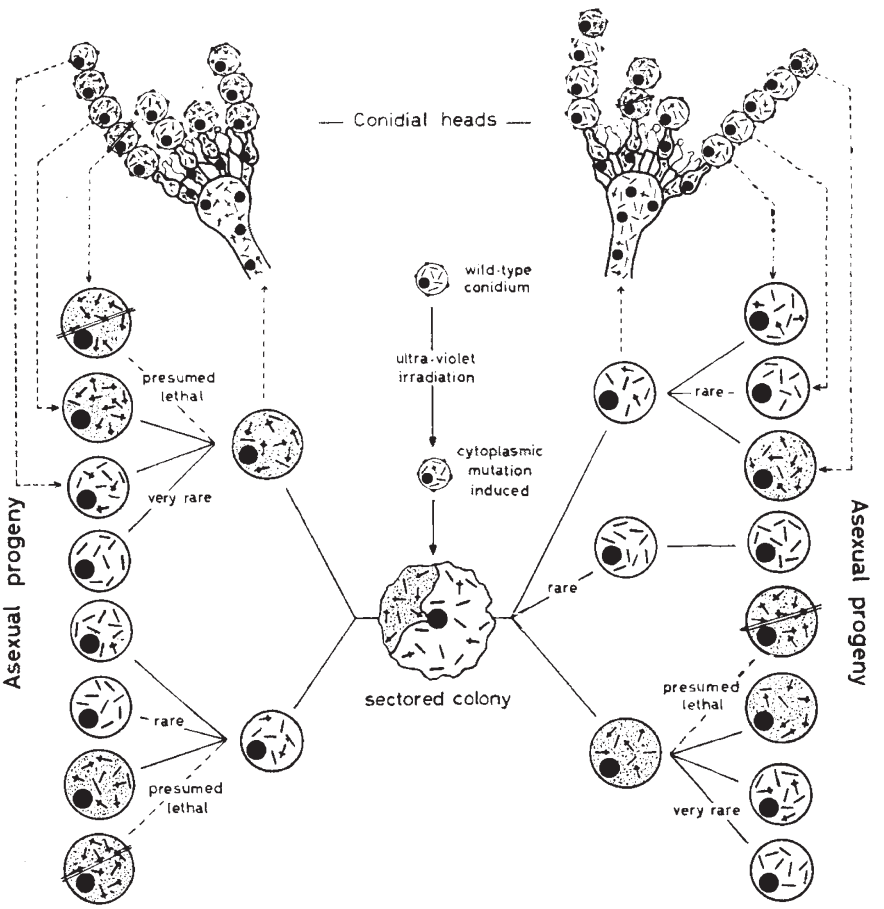
The materials and methods used are those given by Arlett *et al.* (1962).

2. BEHAVIOUR OF THE 'RED' VARIANT WITH WILDTYPE NUCLEI

It was proposed earlier (Arlett *et al.*, 1962) that the 'red' variant is a cytoplasmic variant produced by the action of ultra-violet irradiation on a wild isolate (Birmingham isolate 1) of *A. nidulans*. Furthermore, it was suggested that the variant is an heteroplasmon carrying normal and mutant forms of a cytoplasmic determinant. The data now to be presented confirm this suggestion and support the original interpretation of the properties of the 'red' variant. This interpretation is outlined schematically in text-fig. 1.

To account for the origin and subsequent behaviour of the 'red' variant it is proposed that a mutation '*rho*' was induced in a cytoplasmic component of an asexual spore of the wild isolate. The mutant site will be referred to as the '*rho*' homologue and the normal, wildtype site as '*Rho*'. Previous studies (Arlett *et al.*, 1962) showed that the mean number of '*rho*' homologues in asexual spores of the 'red' variant was at least 5·8. The total number of homologues ('*rho*' plus '*Rho*') in each spore, therefore, must be greater but for this discussion we shall assume that each asexual spore of the wild strain W_3'*Rho*' contains 6 '*Rho*' homologues and that the spore from which the 'red' variant arose contained 5 '*Rho*' homologues plus one that had mutated, that is 1 '*rho*' homologue. If the '*rho*' homologue was at an advantage

in replication and expression over '*Rho*' homologues, some of the hyphæ in the colony produced by the mutant spore would eventually contain a high proportion of '*rho*' homologues and these hyphæ would be phenotypically distinctive. This assumption would explain the



TEXT-FIG. 1.—A schematic representation of the origin of the 'red' variant from a wildtype conidium and its subsequent behaviour. The nuclei are shown as solid black circles; mutant '*rho*' and normal '*Rho*' cytoplasmic homologues are shown as crossed and uncrossed rods, respectively. The small rough-walled circles denote conidia (uninucleate asexual spores); the large smooth-walled circles denote colonies. Colonies with a red phenotype (*i.e.* '*rho*>*Rho*') and also conidia that give rise to red colonies are stippled. Broken arrows indicate that assortment of '*rho*' and '*Rho*' homologues in conidial heads of both red, W_3'*rho*>*Rho*' and green, W_3'*Rho*>*rho*' colonies leads to both red and green progeny. Conidia containing only '*rho*' homologues are presumed to be inviable; spores and colonies of this constitution are struck out.

origin of the variant as a red sector that grew faster than the rest of the colony. It is proposed, therefore, that the red sector consisted of hyphæ containing a sufficiently high proportion of '*rho*' homologues for the phenotypic expression of the '*rho*' homologues to be favoured ('*rho*>*Rho*') and that the green parts of the colony had so few '*rho*' homologues that the expression of the '*Rho*' homologues was favoured

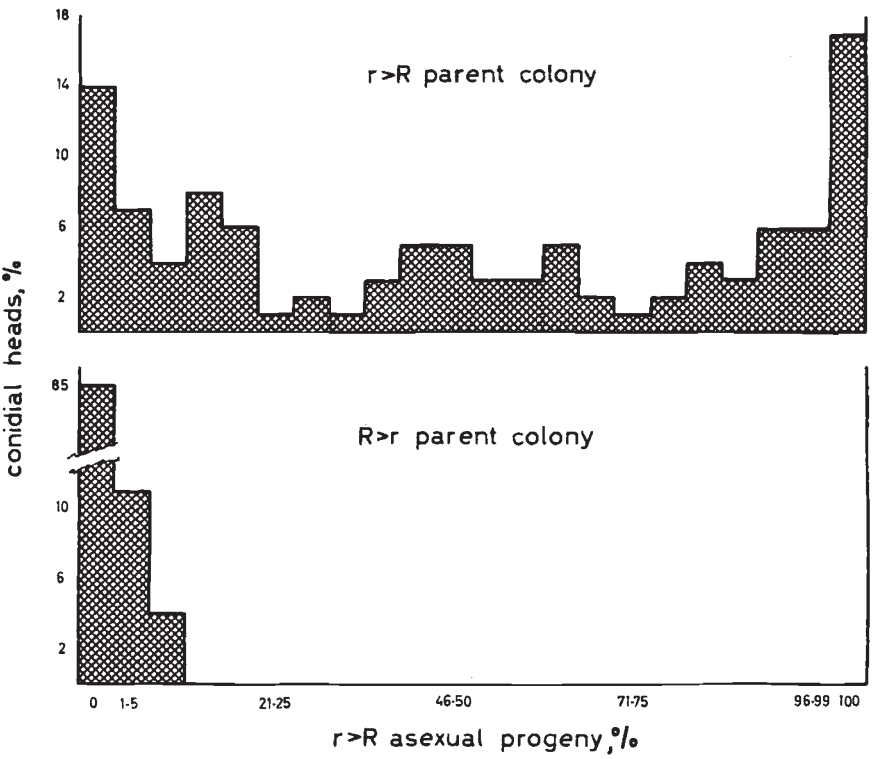
('*Rho*>*rho*'). Similarly, the asexual progeny obtained from these sectors were of two main kinds, red ('*rho*>*Rho*') and green ('*Rho*>*rho*').

Since red, W_3'*rho*>*Rho*' colonies have often given more green, W_3'*Rho*>*rho*' than red asexual offspring (Arlett *et al.*, 1962) it seems that some colonies with the red phenotype contain more '*Rho*' than '*rho*' homologues. Consequently, '*rho*' must be dominant in action to '*Rho*'. We do not know, however, precisely how frequent the '*Rho*' homologues must be before a phenotypically green colony is produced although breeding tests show that the '*Rho*' homologues must be substantially in excess of the '*rho*' homologues. For this discussion we shall simplify the situation and consider all those colonies having more '*rho*' than '*Rho*' homologues as being red ('*rho*>*Rho*') and all those with more '*Rho*' than '*rho*' homologues as being green ('*Rho*>*rho*').

During the formation of asexual spores by both the red and green sectors of the original mosaic colony, 6 cytoplasmic homologues and 1 nucleus would be cut off into each spore. If the '*rho*' and '*Rho*' homologues are distributed randomly, spores containing '*rho*' and '*Rho*' homologues in all ratios between 0:6 and 6:0 would be produced. Those spores with 5 '*rho*': 1 '*Rho*' or 4 '*rho*': 2 '*Rho*' homologues would give red colonies since the mutant homologues would be in excess of the normals. Because the '*rho*' homologues appear to be dominant to, and replicate faster than, the '*Rho*' homologues spores with 3 '*rho*' homologues would also form red colonies. On our simplified model, spores containing 1 or 2 '*rho*' homologues would form green colonies. To account for the recovery of a few true breeding, green wildtype colonies among the asexual progenies we must assume that spores were produced in which only '*Rho*' homologues (*i.e.* 6 '*Rho*': 0 '*rho*') were present. On the basis of this model, some spores must be formed that contain only '*rho*' homologues, but no true breeding red colony with the wildtype nucleus W_3 has ever been recovered from the 'red' variant since it arose in 1956. It seems, therefore, that homoplasmic mutant spores, W_3'*rho*', are inviable (for further arguments favouring this, see Arlett *et al.*, 1962). Alternatively, there may be continual backmutation of the '*rho*' homologues in which case, homoplasmic mutant spores would be viable but the colonies they produced would always contain '*Rho*' homologues. Although we cannot prove conclusively which of these explanations is the correct one, most of the circumstantial evidence favours the inviability hypothesis.

Our model shows that both red, W_3'*rho*>*Rho*' and green, W_3'*Rho*>*rho*' colonies can produce red (W_3'*rho*>*Rho*') and green (W_3'*Rho*>*rho*' and W_3'*Rho*') segregants in their asexual progenies; that is, there are qualitative and quantitative differences in the cytoplasmic constitution of the segregants. Since only 6 cytoplasmic homologues are present in each spore, the number of different proportions of '*rho*:*Rho*' is strictly limited. In the hyphæ of any colony produced by a spore, however, this limitation will not apply because the homologues will presumably multiply ('*rho*' faster than '*Rho*') and increase the total

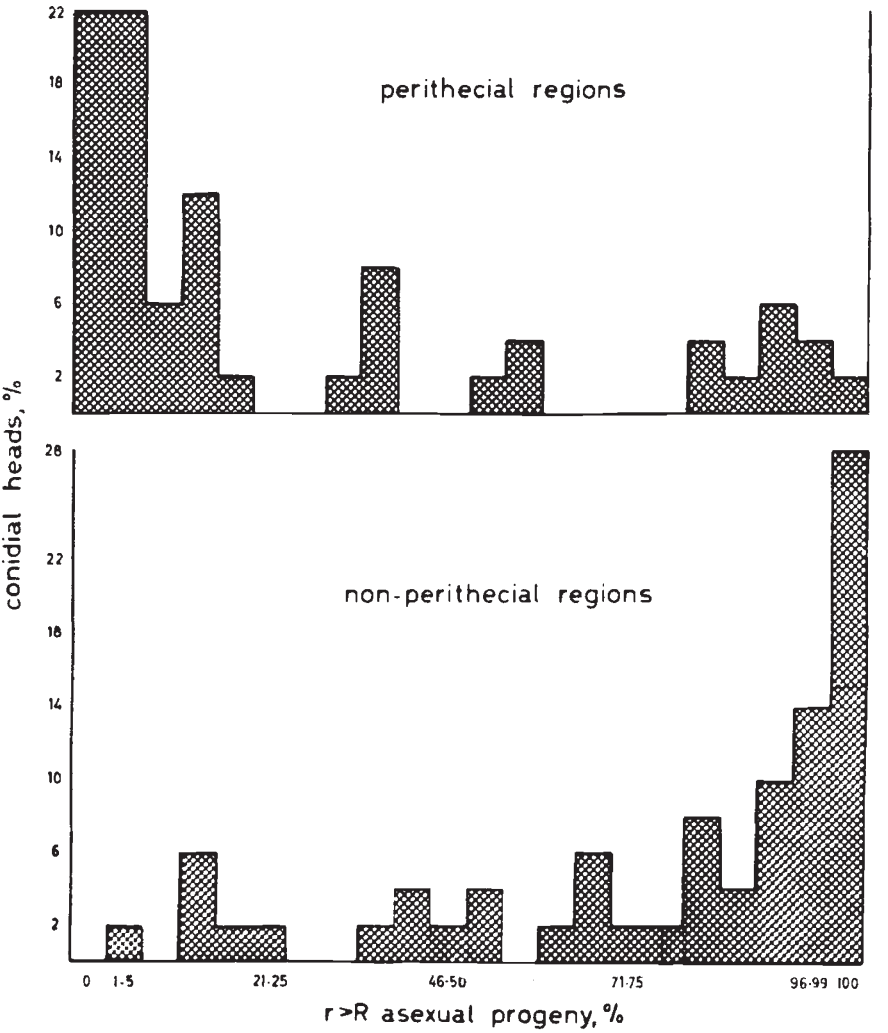
number considerably. Thus, it is reasonable to assume from our model that the proportion of '*rho*' :'*Rho*' homologues in the red and green heteroplasmic colonies varies considerably and that the properties of these colonies vary according to the dosage of '*rho*' homologues. In practice, the heteroplasmic colonies W_3'*rho*>*Rho*' and W_3'*Rho*>*rho*'



TEXT-FIG. 2.—The distribution of percentage red segregants in the asexual progenies of a red, W_3'*rho*>*Rho*' colony and a green, W_3'*Rho*>*rho*' colony. Conidial heads (aggregations of asexual spores)—200 from the red colony and 100 from the green colony—were sampled at random and each head was progeny tested individually. The conidial heads from the green colony never gave more than 10 per cent. red, W_3'*rho*>*Rho*' segregants and the vast majority (85 per cent.) gave no red segregants at all (*i.e.* 100 per cent. green segregants). Conversely, most (75 per cent.) of the conidial heads from the red colony gave more than 10 per cent. red segregants and only 14 per cent. of the heads gave no red segregants at all. Note, however, that when 100 per cent. green segregants was obtained from a conidial head, these segregants were very rarely homoplasmic, true breeding colonies; most of them gave both red and green colonies in their asexual progenies.

r = '*rho*'; *R* = '*Rho*'.

give two main classes of segregant, red and green, which are easily distinguishable (plate I, fig. 1) but neither of these classes is composed of colonies that are uniform in appearance or breeding behaviour. For example, the red segregants differ in the intensity of their red colouration, in rate of growth and in the percentage of red segregants they give in their asexual progenies. The green segregants also differ for these characters but principally in the rate at which they sector to form red colonies.



TEXT-FIG. 3.—The distribution of percentage red segregants in the asexual progenies of conidial heads taken from perithecial and non-perithecial areas of a red, *W*$_3$'*rho*>*Rho*' colony. Each histogram shows the segregations in 100 conidial heads that were progeny tested individually. Most (76 per cent.) of the conidial heads from perithecial regions gave less than 50 per cent. red, *W*$_3$'*rho*>*Rho*' segregants and 22 per cent. of the heads gave no red segregants at all (*i.e.* 100 per cent. green segregants). Conversely, only 20 per cent. of the conidial heads from non-perithecial regions gave less than 50 per cent. red segregants. Thus, conidial heads from perithecial regions give predominantly green offspring and those from non-perithecial regions give predominantly red offspring. (Compare with text-fig. 2 which shows the segregation in conidial heads taken *at random* from a *W*$_3$'*rho*>*Rho*' colony.)

r = '*rho*'; R = '*Rho*'.

The green, *W*$_3$'*Rho*>*rho*' colonies always produce more perithecia and bigger conidiophores than are produced by red, *W*$_3$'*rho*>*Rho*' colonies and these colonies give predominantly green asexual progeny (text-figs. 2 and 6). Red colonies, however, can give predominantly red or green offspring (text-figs. 2 and 6). Most of the conidiophores

on red colonies are small and abnormal but a few normal, large conidiophores occur also and these are found close to the perithecia. When progeny tested, the small conidiophores give predominantly red offspring whereas the large ones give mainly green offspring (text-fig. 3). This result is further evidence that colonies with a red phenotype are mosaics consisting of hyphæ in which either the '*rho*' or the '*Rho*' homologues (*i.e.* W_3'*rho*>*Rho*' or W_3'*Rho*>*rho*' hyphæ) predominate; in these mosaic colonies, the perithecia and large conidiophores are formed in those regions where W_3'*Rho*>*rho*' hyphæ are present (colonies of the " minute " variant of *A. nidulans* may also be mosaics composed of hyphæ carrying different dosages of extrachromosomal elements—see Faulkner and Arlett, 1964). Clones of the heteroplasmic green segregants produce phenotypically red colonies (plate I, fig. 2) and during the growth of these colonies the percentage of red offspring increases rapidly (text-fig. 4). This suggests that the '*rho*' homologues replicate faster than the '*Rho*' homologues so that no matter how few '*rho*' homologues are present in the hyphæ of an inoculum, they will soon outnumber the '*Rho*' homologues during hyphal growth. Because of their superior rate of growth, W_3'*rho*>*Rho*' hyphæ will outpace the W_3'*Rho*>*rho*' hyphæ in the same colony. It is presumed that this process stops short of producing pure breeding mutant colonies because homoplasmic mutant hyphæ, like homoplasmic mutant spores, fail to grow. But, as we shall see in the next section, even the introduction of single nuclear major gene mutations can effect the behaviour of the ' red ' variant by promoting the replication and expression of either the '*rho*' or '*Rho*' homologues and thereby leading to the recovery of homoplasmic mutant, as well as homoplasmic normal, colonies.

3. MEANS OF INTRODUCING NUCLEAR GENE MUTATIONS INTO THE ' RED ' VARIANT

In *A. nidulans*, different combinations of nucleus and cytoplasm can be achieved in two ways:—(i) by exposing spores to mutagenic agents, and (ii) by heterokaryotic exchange between two different parental strains. Both of these methods have been used to associate the mutant cytoplasm of the ' red ' variant with various nuclear genotypes (Arlett *et al.*, 1962) but it was clear from these early studies that the success of either method depended on the nature of the nuclear gene mutations with which the mutant cytoplasm was to be associated. For instance, by using the technique of heterokaryotic exchange, nuclei from the morphological mutant strains w_3'*Rho*', w_3co'*Rho*' and w_3df'*Rho*' were introduced successfully into the ' red ' variant. Yet all attempts to introduce similar mutations affecting morphology into the ' red ' variant directly by using ultra-violet irradiation failed. On the other hand, only the irradiation experiments were successful in producing auxotrophic forms of the ' red ' variant. Of these two techniques, heterokaryotic exchange has the considerable advantage

of enabling the effect of mutant nuclei on the phenotypic expression of the '*Rho*' homologues to be evaluated prior to the association of the same nuclei with the heteroplasmon '*rho,Rho*'.

(i) *Ultra-violet irradiation studies*

Asexual spores (conidia) of the 'red' variant were exposed to U.V. irradiation for $\frac{1}{2}$-8 min. and plated on Czapek minimal medium (MM) and on complete medium (CM) at a density not exceeding 50 viable spores per Petri dish. The colonies obtained were cloned on to MM and CM and examined after 5 days growth. No auxotrophic mutants were recovered from more than 2000 viable U.V. irradiated spores plated on CM and only 5 prototrophic mutants were obtained from nearly 7000 spores plated on MM. In later studies, however, abnormal colonies produced on MM or CM by viable U.V. irradiated spores were progeny tested immediately as well as being cloned on to fresh media before they were tested. In this way, an additional 4 morphological mutants (mutations m_1, m_2, m_3 and m_5) were obtained. These were recovered initially as extremely small colonies ('minute reds') which, apart from their size, were typical red, W_3'*rho*>*Rho*' colonies in appearance (plate III, fig. 1). Conidia from each of these 'minute reds' gave two abnormal types of colony but when clones of these same 'minute reds' were progeny tested, the abnormal types of colony were not recovered (Grindle, 1963).

(ii) *Heterokaryotic exchange*

The application of the heterokaryon test (Jinks, 1954) as a means of examining the effects of different nuclear genes on the 'red' variant has been discussed in previous reports (Arlett *et al.*, 1962; Grindle, 1963). Heterokaryons were obtained in which the cytoplasm of the 'red' variant was associated with its own wildtype nucleus (W_3) and the nuclei from (1) a normal colony with white spores, w_3'*Rho*' (2) a compact nuclear mutant of the white-spored strain, w_3co'*Rho*' and (3) a diffuse mutant, w_3df'*Rho*'. Heterokaryotic conidial heads from each of the three different heterokaryons were single-spored on to MM and the young colonies obtained were cloned on to fresh MM. Colonies were recovered (Arlett *et al.*, 1962) which had the phenotype of the 'red' variant but the nuclei of the initially normal components, that is (1) white-spored colonies with red colouration and abnormal conidiophores, w_3'*rho*>*Rho*', (2) compact, white-spored colonies with red colouration, w_3co'*rho*>*Rho*', and (3) diffuse, red, white-spored colonies, w_3df'*rho*>*Rho*'. In each case, the new red phenotypes arose in mosaic colonies consisting of both red and normal sectors.

Similarly, the '*rho*' homologues from colonies of the 'red' variant were associated with nuclei from a white-spored, riboflavineless strain, w_3rb^-'*Rho*', of isolate 1 and a white-spored mutant, *w*, of Birmingham wild isolate 26 of *A. nidulans* (*Grindle*, 1963).

4. PROPERTIES OF THE MODIFIED FORMS OF THE ' RED ' VARIANT

Ten different nuclear mutants of the ' red ' variant have been analysed. The data obtained are listed in table 1. In this table the system of nomenclature discussed in section 2 is used throughout to distinguish between the two classes of asexual progeny obtained from colonies of the ' red ' variant carrying mutant genes. The morphological nuclear mutations induced in the ' red ' variant by U.V. irradiation are designated as mutations m_1—m_5.

The properties of these variants have been assessed as follows:—

(*a*) The '*rho*>*Rho*', '*Rho*>*rho*' distinction is based on colonial appearance. That is, where two different classes of segregant are recovered among the progeny of the same parent colony, those with the more intense red colouration and more abnormal conidiophores are designated as the '*rho*>*Rho*' segregants.

(*b*) The range of percentages of '*rho*>*Rho*' and '*Rho*>*rho*' segregants obtained from the various strains was determined, where possible, from numerous asexual progenies examined over a period of several months.

(*c*) The rates of growth indicate the mean daily increase in colony diameter as measured from the 3rd-8th days after inoculation. Four colonies of each strain were grown, two per Petri dish, at 25° C. on 20 ml. MM.

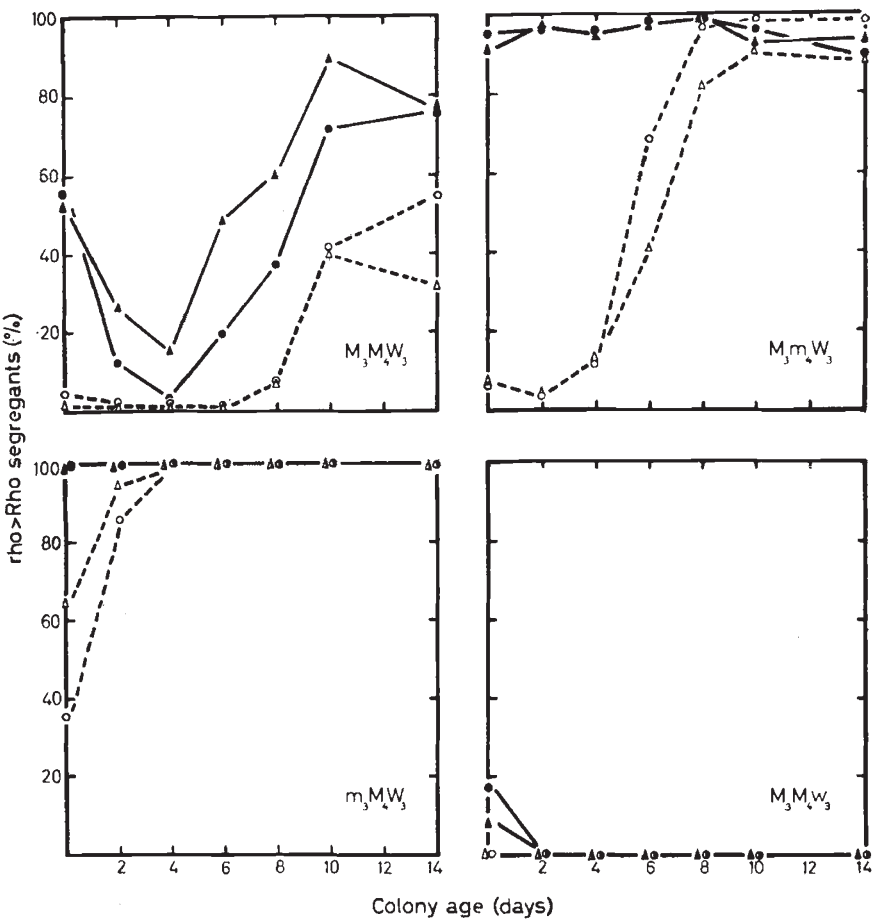
(*d*) Since either the '*rho*>*Rho*' or the '*Rho*>*rho*' colonies from most of the modified forms of the ' red ' variant are very unstable vegetatively they break down, when cloned, to give the phenotype of the corresponding stable form. Consequently, growth-rate measurements of these clones just indicate how rapidly the unstable phenotype breaks down. To compare more accurately the growth rates of '*rho*>*Rho*' and '*Rho*>*rho*' colonies, therefore, the relative sizes of the two types of uncloned colony were determined. This comparison was made for each ' Red ' strain by selecting 6- or 7-day-old asexual progenies and measuring the diameters of all the '*rho*>*Rho*' and '*Rho*>*rho*' colonies that had neither sectored nor grown into neighbouring colonies. The difference between the mean diameters of the '*rho*>*Rho*' and '*Rho*>*rho*' colonies is expressed in the table as a proportion. Whenever possible, the asexual progenies of 8 different parent colonies of each ' red ' strain were analysed independently. Thus, each range of proportions given in the table shows the least and the greatest differences between the mean diameters of the appropriate '*rho*>*Rho*' and '*Rho*>*rho*' colonies, as determined from 8 independent tests.

The variants described in table 1 can be distinguished into 3 main categories of interaction, (i) " neutral " mutations, (ii) mutations favouring the normal cytoplasmic homologues, and (iii) mutations favouring the mutant cytoplasmic homologues.

(i) *Neutral mutations*

Two of the mutant nuclei, m_2W_3 and m_4W_3 had only a minor effect on the ' red ' variant. Asexual progenies of the red-coloured colonies

(m_2W_3'*rho*>*Rho*' and m_4W_3'*rho*>*Rho*') were similar in appearance to those of the ' red ' variant itself (W_3'*rho*>*Rho*') differing only slightly in colouration and rate of growth (plate I, figs. 3 and 5). Although the heteroplasmic non-red segregants of the two mutant strains were more stable phenotypically than those of the original ' red ' variant, they always broke down to give colonies having the phenotype and properties of the corresponding heteroplasmic red colonies (plate I, figs. 4 and 6; text-fig. 4). But, no true breeding homoplasmic red colonies, m_2W_3'*rho*' or m_4W_3'*rho*', were ever recovered. Homoplasmic



TEXT-FIG. 4.—Changes in the percentage of red segregants obtained during the growth of single colonies of various forms of the ' red ' variant. At time 0, individual colonies among 14-day old progeny were each single-spored; immediately afterwards, these same colonies were cloned on to fresh MM and conidia were sampled at 2-day intervals from just behind the growing edges of the colonies so formed. The four sets of graphs refer to the behaviour of the ' red ' variant associated with four different nuclear backgrounds. The first set (upper left) refers to the ' red ' variant with wildtype nucleus and illustrates the gradual increase in the proportions of red, '*rho*>*Rho*' segregants from each of two different red, $M_3M_4W_3$'*rho*>*Rho*' parents (solid lines) and also from each of two different green, $M_3M_4W_3$'*Rho*>*rho*' parents (dotted lines). Note that no true breeding red colonies, $M_3M_4W_3$'*rho*', are produced. The remaining three sets of graphs show how the situation is modified by introducing the nuclear gene mutations indicated.

TABLE 1

Properties of the 'red' variant with the wildtype nucleus W_3 and with the nuclear gene mutations indicated

Parent colony			How obtained	Asexual progenies		Sexual progenies	Pure-breeding cytoplasmic mutant obtained	Mass-hyphal subcultures	
Genotype		Phenotype		Segregation	Colony size differences '$r>R$': $R>r$'			Phenotype after 8 days growth	Growth-rate mm/day
Nucleus	Cytoplasm								
W_3 (wild type)	'R' (wild type)	dark green (green conidia)	from atmosphere	100 per cent. W_3'R'	...	ascopores inviable	...	green	5·1
w_3	'R'	white (white conidia)	u.v.-irrad. of W_3'R' conidia	100 per cent. w_3'R'	...	100 per cent. w_3'R'	...	white	4·8
w_3co	'R'	white, compact growth	u.v.-irrad. of w_3'R' conidia	100 per cent. w_3co'R'	...	non-sexual	...	white, compact	1·9
w_3df	'R'	white, diffuse growth	u.v.-irrad. of w_3'R' conidia	100 per cent. w_3df'R'	...	non-sexual	...	white, diffuse	5·4
W_3	'$r>R$'	red	u.v.-irrad. of W_3'R' conidia	16-95 per cent. W_3'$r>R$'	1·1:1-1·3:1	inviable	No	red	4·1
W_3	'$R>r$'	green	ditto	1-20 per cent. W_3'$r>R$'	...	inviable	No	red/green mosaic	3·8
m_4W_3	'$r>R$'	red-brown	u.v.-irrad. of W_3'$r>R$' conidia	78-92 per cent. m_4W_3'$r>R$'	1·4:1-1·6:1	non-sexual	No	red-brown	4·0
m_4W_3	'$R>r$'	orange	ditto	2-22 per cent. m_4W_3'$r>R$'	...	non-sexual	No	orange red-brown mosaic	3·7

m_2W_3	'$r>R$'	red	ditto	23-90 per cent. m_2W_3'$r>R$'	1·4:1-2·0:1	inviable	No	red	4·2
m_2W_3	'$R>r$'	green	ditto	14-44 per cent. m_2W_3'$r>R$'	...	inviable	No	red/green mosaic	3·7
m_3W_3	'$r>R$' (='r')*	red-brown	ditto	100 per cent. m_3W_3'r'	4·0:1-4·6:1	inviable	Yes	red-brown	4·4
m_3W_3	'$R>r$'	pale brown	ditto	15-89 per cent. m_3W_3'$r>R$'	...	inviable	Yes	red-brown	4·4
m_5W_3	'$r>R$' (='r')*	pale red	ditto	100 per cent. m_5W_3'r'	2·4:1-2·9:1	inviable	Yes	pale red	?
m_5W_3	'$R>r$'	green	ditto	0-90 per cent. m_5W_3'r'	...	inviable	Yes	pale red	?
w_3co	'$r>R$' (='r')*	red	from heterokaryon of w_3co'R' and W_3'$r>R$'	100 per cent. w_3co'r'	...	non-sexual	Yes	red	2·3
w_3co	'$R>r$' (='R')*	white	ditto	100 per cent. w_3co'R'	...	non-sexual	No	white	?
w_3df	'$r>R$' (='r')*	red	from heterokaryon of w_3df'R' and W_3'$r>R$'	100 per cent. w_3df'r'	...	non-sexual	Yes	red	5·9
w_3df	'$R>r$' (='R')*	white	ditto	100 per cent. w_3df'R'	...	non-sexual	No	white	?
m_1W_3	'$r>R$'	pale red	u.v.-irrad. of W_3'$r>R$' conidia	0-4 per cent. m_1W_3'$r>R$'	1:1·6-1:3·8	inviable	No	green	?
m_1W_3	'$R>r$' (='R')*	green	ditto	100 per cent. m_1W_3'R'	...	inviable	No	green	?

TABLE 1 (*continued*)

Parent colony			How obtained	Asexual progenies		Sexual progenies	Pure-breeding cytoplasmic mutant obtained	Mass-hyphal subcultures	
Genotype		Phenotype		Segregation	Colony size differences '*r*>*R*':'*R*>*r*'			Phenotype after 8 days growth	Growth-rate mm/day
Nucleus	Cytoplasm								
w_3	'*r*>*R*'	red	from heterokaryon of w_3'*R*' and W_3'*r*>*R*'	0-55 per cent. w_3'*r*>*R*'	1:2·0-1:3·7	100 per cent. w_3'*R*'	No	pale pink	4·8
w_3	'*R*>*r*'	pale pink	ditto	0-1 per cent. w_3'*r*>*R*'	...	100 per cent. w_3'*R*'	No	pale pink	5·0
W_3ad^-	'*r*>*R*'	pink	u.v.-irrad. of W_3'*r*>*R*' conidia	0-15 per cent. W_3ad^-'*r*>*R*'	? ('*r*>*R*' smaller than '*R*>*r*' colonies)	?	No	green	?
W_3ad^-	'*R*>*r*' (='*R*')*	green	ditto	100 per cent. W_3ad^-'*R*'	...	?	No	green	?
w_3rb^-	'*r*>*R*'	brown	from heterokaryon of w_3rb^-'*R*' and W_3'*r*>*R*'	100 per cent. w_3rb^-'*R*'	? ('*r*>*R*' smaller than '*R*>*r*' colonies)	?	No	orange	?
w_3rb^-	'*R*>*r*' (='*R*')*	orange	ditto	100 per cent. w_3rb^-'*R*'	...	?	No	orange	?

r = '*rho*', *R* = '*Rho*'

(Note: the wildtype nucleus is given simply as W_3; no other wildtype alleles are given and only the gene mutations, indicated by small letters, are listed.)

* As these colonies always breed true in their asexual progenies, the '*Rho*' or '*rho*' homologues are presumably lost from the original unstable heteroplasmic parents so that homoplasmic mutant, '*rho*', or normal, '*Rho*', colonies result—see text.

mutant spores carrying the m_2W_3 or m_4W_3 nuclei, as well as those with the wildtype nucleus, therefore, are either not produced or they are inviable.

(ii) *Nuclear mutations favouring the normal cytoplasmic homologues,* ' Rho '

Each of the mutant nuclei m_1W_3, w_3, W_3ad^- and w_3rb^- had a distinctly adverse effect on the replication and expression of the '*rho*' homologues such that the '*rho*' homologues were maintained, if at all, with great difficulty. For example, the heteroplasmic red segregants of the four mutants m_1W_3'*rho*>*Rho*', w_3'*rho*>*Rho*', W_3ad^-'*rho*>*Rho*' and w_3rb^-'*rho*>*Rho*' were much smaller in size than the corresponding '*Rho*>*rho*' segregants whereas those of the ' red ' variant with the wildtype nucleus, W_3, were bigger than the '*Rho*>*rho*' segregants. Also, the red colonies carrying any of these mutant nuclei broke down rapidly to give the phenotype of the corresponding '*Rho*>*rho*' colonies. Of the latter only those carrying the w_3 nucleus ever produced any red segregants among their asexual progenies. The non-red colonies carrying the m_1W_3, W_3ad^- or w_3rb^- nuclei were true breeding and apparently normal; presumably, therefore, they contained only normal '*Rho*' homologues and should be designated more correctly as m_1W_3'*Rho*', W_3ad^-'*Rho*' and w_3rb^-'*Rho*'. In contrast, the red offspring of the original ' red ' variant, W_3'*rho*>*Rho*' do not break down to form non-red colonies, and true breeding, homoplasmic progeny, W_3'*Rho*' are rarely recovered.

The heteroplasmic red colonies sectored and broke down so rapidly that red segregants carrying the m_1W_3 nuclei were lost, despite repeated selection in their favour, after only two propagations by asexual spores. With the W_3ad^- nuclei, they were lost after five asexual generations (Arlett, 1960) and with the w_3rb^- nuclei they were lost after a single asexual generation.

By using the technique of heterokaryotic exchange, the '*rho*' homologues were associated with nuclei carrying a mutation for lysine requirement but not a single colony which carried both the W_3lys^- nucleus and the '*rho*' homologues, that is W_3lys^-'*rho*>*Rho*' or W_3lys^-'*Rho*>*rho*', was recovered (Arlett, 1960; Arlett *et al.*, 1962). It seems, therefore, that the '*rho*' homologues cannot survive in the presence of the W_3lys^- nucleus. Indeed our evidence suggests that the '*rho*' homologues may be almost or completely incompatible with the nuclei of most auxotrophic strains.

The '*rho*' homologues were also difficult to maintain in conjunction with the w_3 nucleus. The red, w_3'*rho*>*Rho*', segregants (plate II, fig. 5) produced very few red offspring and they broke down to form colonies that were identical in phenotype and properties with the W_3'*Rho*>*rho*' segregants (plate II, fig. 6; text-fig. 4). Consequently, the w_3'*rho*>*Rho*' phenotype was perpetuated only by sampling large numbers of asexual spores. This was surprising since the same nucleus carrying additional mutations affecting colonial morphology (w_3co,

w_3df) favoured the '*rho*' homologues to the extent that, ultimately, only pure breeding red colonies were obtained (section 4(iii)). Furthermore, a mutant sector that arose spontaneously from a *w_3*'*Rho*>*rho*' colony had an increased rate of growth, a very intense red colouration and it gave only pure breeding red offspring (*sw_3*'*rho*'). Thus, spontaneous and induced nuclear gene mutations negate the adverse effect which the *w_3* mutation has on the replication and expression of the '*rho*' homologues.

(iii) *Nuclear mutations favouring the mutant cytoplasmic homologues,* 'rho'

When associated with wildtype nuclei carrying the mutations *m_3*, *m_5*, *w_3co* or *w_3df* the '*rho*' homologues were at a considerable advantage in replication and expression over their normal, '*Rho*' partners. Homoplasmic mutant spores, *m_3*'*rho*', *m_5*'*rho*', *w_3co*'*rho*' and *w_3df*'*rho*', unlike those of the original 'red' variant (*W_3*'*rho*') were viable and produced pure breeding red colonies.

Compared with the 'red' variant *W_3*'*rho*>*Rho*', colonies carrying the *m_3W_3* nuclei had a slightly different red colouration, the '*Rho*>*rho*' segregants were much smaller than their '*rho*>*Rho*' partners (plate II, fig. 1) and clones of the small segregants broke down to give red sectors much more rapidly and completely (plate II, fig. 2; text-fig. 4). All of the red progeny were true breeding and should, therefore, be designated more correctly as *m_3W_3*'*rho*'.

Although the *m_5W_3* nucleus had little effect on the colour of the 'red' variant, it altered its behaviour in the same way as did the *m_3W_3* nucleus by making the homoplasmic mutant condition the more stable form. In spite of repeated selection in favour of the *m_5W_3*'*Rho*>*rho*' segregants, they were lost after three propagations by asexual spores.

The first visible sign that the '*rho*' homologues had been associated successfully with the *w_3co* or *w_3df* nuclei during heterokaryosis was that some spores from heterokaryotic conidiophores produced white-spored colonies consisting of both red and normal sectors (plate II, fig. 3). Both types of sector bred true in their asexual progenies, that is, they gave only homoplasmic red or normal offspring. Red colonies carrying either of these mutant nuclei had a rate of growth superior to that of the corresponding normal colonies (plate II, fig. 4). It appears, therefore, that the '*rho*' and '*Rho*' homologues cannot coexist in the presence of the *w_3co* or *w_3df* nuclei so that whenever the two types of homologue occur together the normals are soon lost and the mutants replace them.

The '*rho*' homologues can also be maintained in preference to their '*Rho*' partners in the presence of the kinds of nuclear gene differences that occur in nature. This was shown by introducing the mutant cytoplasm from a *W_3*'*rho*>*Rho*' colony into a white-spored mutant, *w*, of Birmingham wild isolate 26 of *A. nidulans* (plate II, fig. 2). Only

Plate I

FIG. 1.—Asexual progeny of the ' red ' variant with wildtype nucleus, showing the segregation into red, W_3'*rho*>*Rho*' and green, W_3'*Rho*>*rho*' colonies. Note the red sectors arising from some of the green progeny. Colonies 7 days old.

FIG. 2.—Clones of a red, W_3'*rho*>*Rho*' colony (left) and a green W_3'*Rho*>*rho*' colony (right). The latter has produced a red colony which is identical in phenotype and behaviour to the red colony. Colonies 10 days old.

FIG. 3.—Asexual progeny of the modified ' red ' variant, m_4W_3'*rho*>*Rho*' showing the segregation into red-brown, m_4W_3'*rho*>*Rho*' and orange, m_4W_3'*Rho*>*rho*' colonies. Note the red-brown sectors arising from most of the orange colonies. Colonies 8 days old.

FIG. 4.—Clones of a m_4W_3'*rho*>*Rho*' colony (left) and a m_4W_3'*Rho*>*rho*' colony (right). The latter has formed a colony having the phenotype and properties of a m_4W_3'*rho*>*Rho*' colony. Colonies 12 days old.

FIG. 5.—Asexual progeny of the modified ' red ' variant, m_2W_3'*rho*>*Rho*' showing the segregation into red, m_2W_3'*rho*>*Rho*' and green, m_2W_3'*Rho*>*rho*' colonies. Note the red sectors arising from the green colonies. Colonies 8 days old.

FIG. 6.—Clones of a m_2W_3'*rho*>*Rho*' colony (left) and a m_2W_3'*Rho*>*rho*' colony (right). The latter has broken down and formed a colony with the phenotype and properties of a m_2W_3'*rho*>*Rho*' colony. Colonies 12 days old.

All colonies growing on Czapek minimal medium.

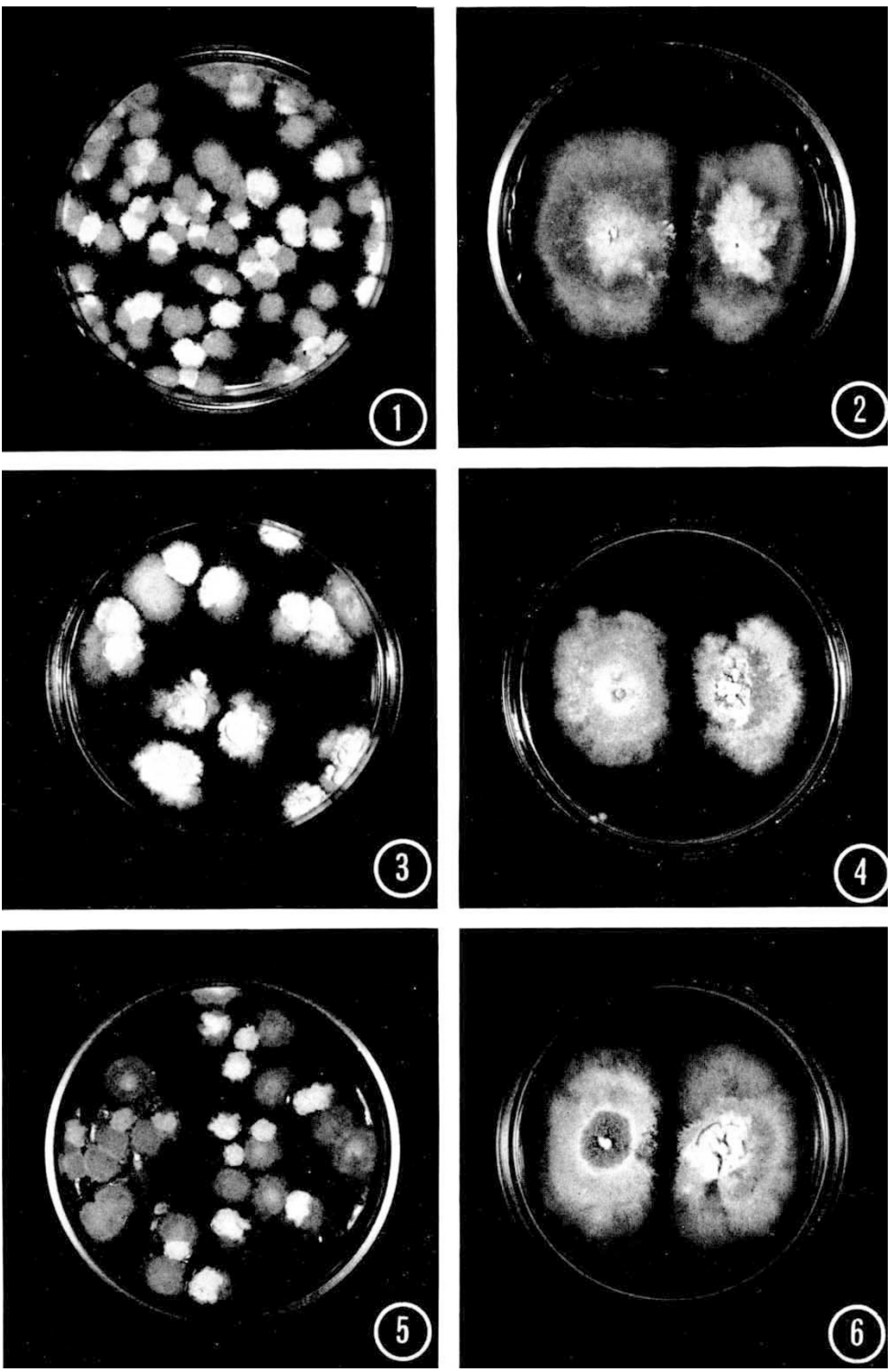

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Plate II

FIG. 1.—Asexual progeny of the modified ' red ' variant, m_3W_3'*Rho*>*rho*' showing the segregation into red-brown, m_3W_3'*rho*' and pale brown, m_3W_3'*Rho*>*rho*' colonies. Note the red-brown sectors arising from the small, pale brown colonies. Colonies 8 days old.

FIG. 2.—Clones of a m_3W_3'*rho*' colony (left) and a m_3W_3'*Rho*>*rho*' colony (right). The latter has broken down completely and produced a red-brown colony which is identical in phenotype and properties to a m_3W_3'*rho*' colony. Colonies 8 days old.

FIG. 3.—The result of associating '*rho*' cytoplasmic homologues with mutant nuclei by means of heterokaryotic exchange. The colonies shown are clones of four of the progeny from a heterokaryotic conidiophore taken from a mixed culture consisting of the ' red ' variant, W_3'*rho*>*Rho*' and a normal diffuse mutant, w_3df '*Rho*'. The red sector (arrowed) gives true breeding, red asexual progeny, w_3df '*rho*'; the rest of the colony gives only normal w_3df '*Rho*' offspring. Colonies 10 days old. (From a Kodachrome transparency.)

FIG. 4.—Colonies of the compact, white-spored strain, w_3co'*Rho*' (left and right) and the same strain carrying '*rho*' cytoplasmic homologues, w_3co'*rho*'. Colonies 14 days old.

FIG. 5.—Asexual progeny of the white-spored ' red ' variant, w_3'*rho*>*Rho*' showing the segregation into small, red-pigmented colonies, w_3'*rho*>*Rho*' and large, phenotypically normal colonies, w_3'*Rho*>*rho*'. Colonies 7 days old.

FIG. 6.—Clones of a w_3'*rho*>*Rho*' colony (left) and a w_3'*Rho*>*rho*' colony (right). The w_3'*rho*>*Rho*' clone has broken down and produced a colony whose phenotype and properties are identical to those of a w_3'*Rho*>*rho*' colony. Colonies 12 days old.

All colonies growing on Czapek minimal medium.

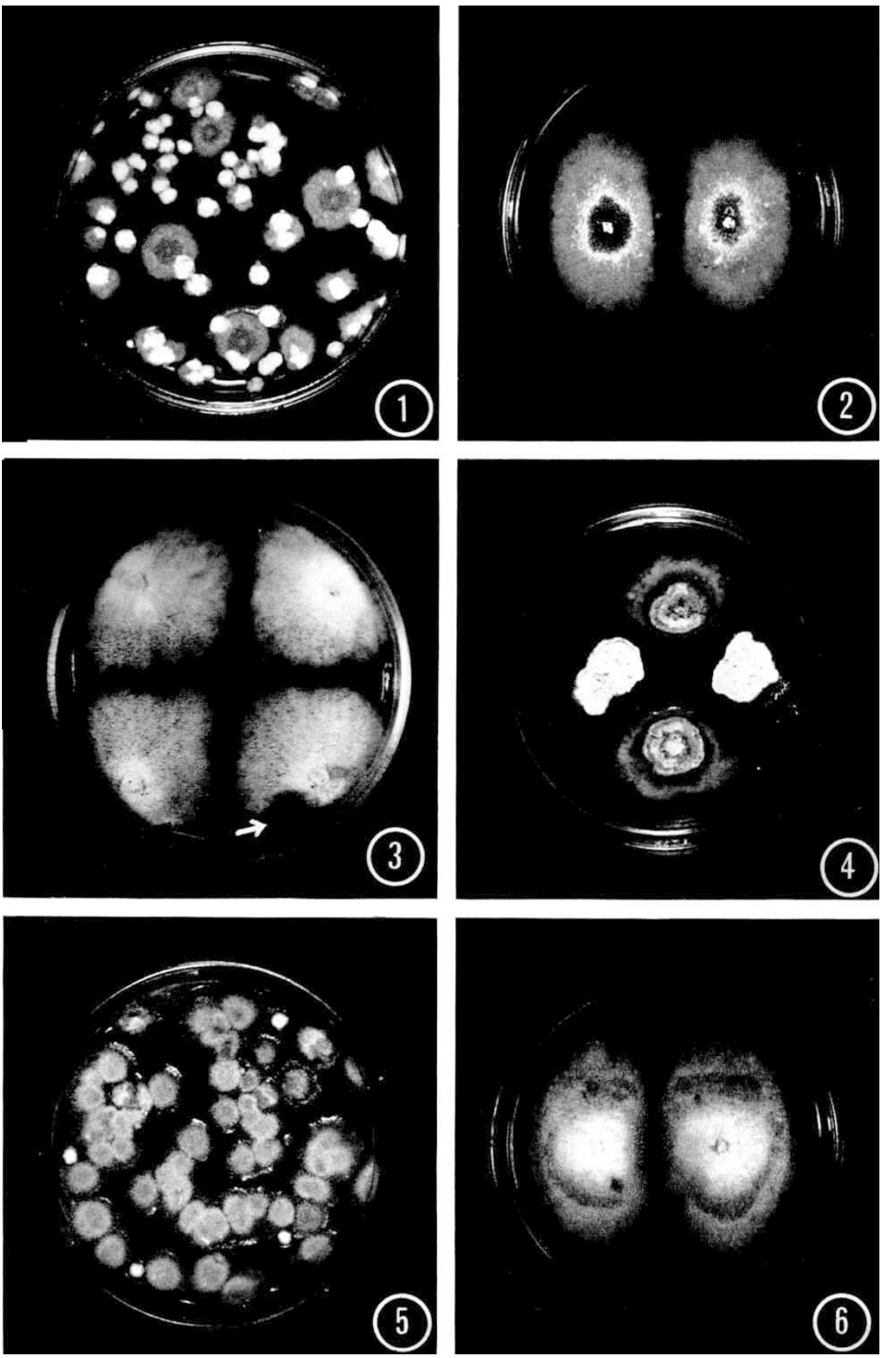
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Plate III

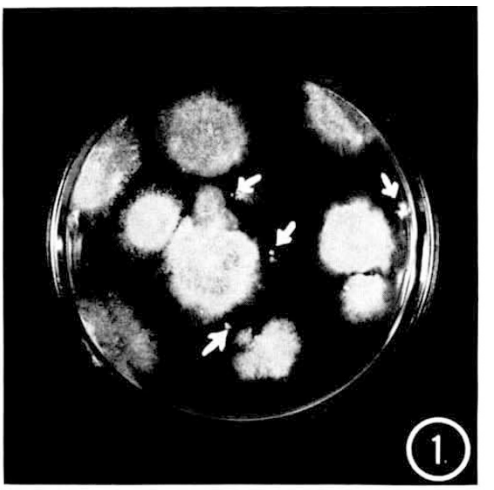
FIG. 1.—Colonies formed by U.V.-irradiated asexual spores of the 'red' variant, W_3'*rho*>*Rho*'. Arrows indicate 'minute red' colonies. Colonies 10 days old.

FIG. 2.—The effect of introducing '*rho*' cytoplasmic homologues from the 'red' variant into a white-spored mutant of Birmingham wild isolate No. 26 of *A. nidulans* by heterokaryotic exchange. The colonies shown (under-surface view of right-hand plate) are clones of two of the progeny from a heterokaryotic conidiophore. The white and red sectors both breed true in their asexual progenies, giving white and pink colonies, respectively. Colonies 10 days old.

FIG. 3.—Asexual progeny of the 'purple' variant with wildtype nucleus showing the segregation into purple, *WY*'*pi*>*Pi*' colonies and green, *WY*'*Pi*>*pi*' colonies (arrowed). The *WY*'*pi*>*Pi*' colonies have few conidiophores, many perithecia, raised centres and their hyphæ change from yellow to purple in colour with age. Colonies 12 days old.

FIG. 4.—Clones of a green, *WY*'*Pi*>*pi*' colony (left) and a purple, *WY*'*pi*>*Pi*' colony (right). The *Wy*'*pi*>*Pi*' clone has broken down and formed a colony which is identical in phenotype and properties to a *WY*'*Pi*>*pi*' colony. Colonies 12 days old.

All colonies growing on Czapek minimal medium.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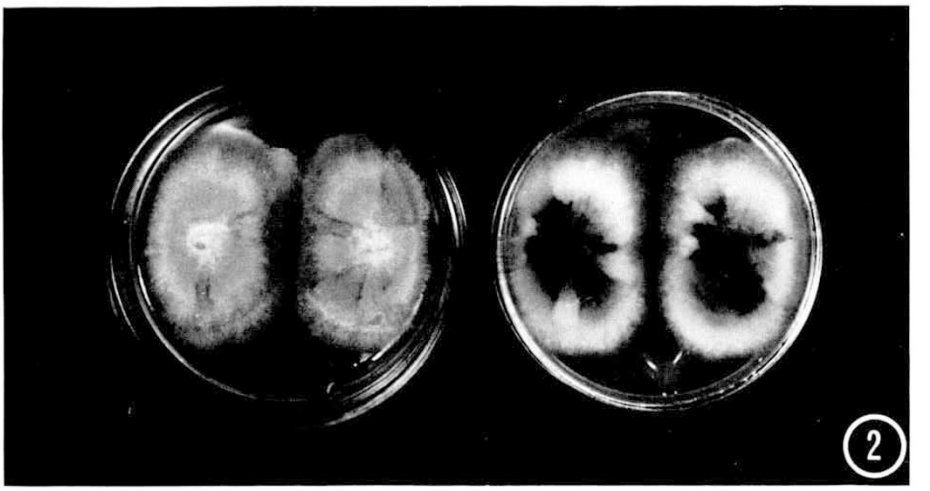
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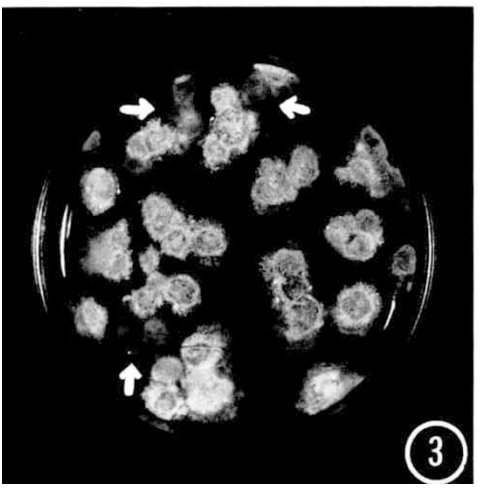
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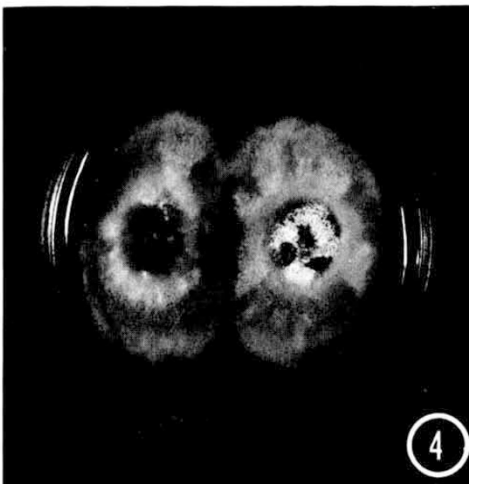
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pure breeding pink offspring, *w'rho'* were recovered from the red sectors.

Properties of these modified forms of the ' red ' variant are summarised in section 5 and compared graphically in text-fig. 6.

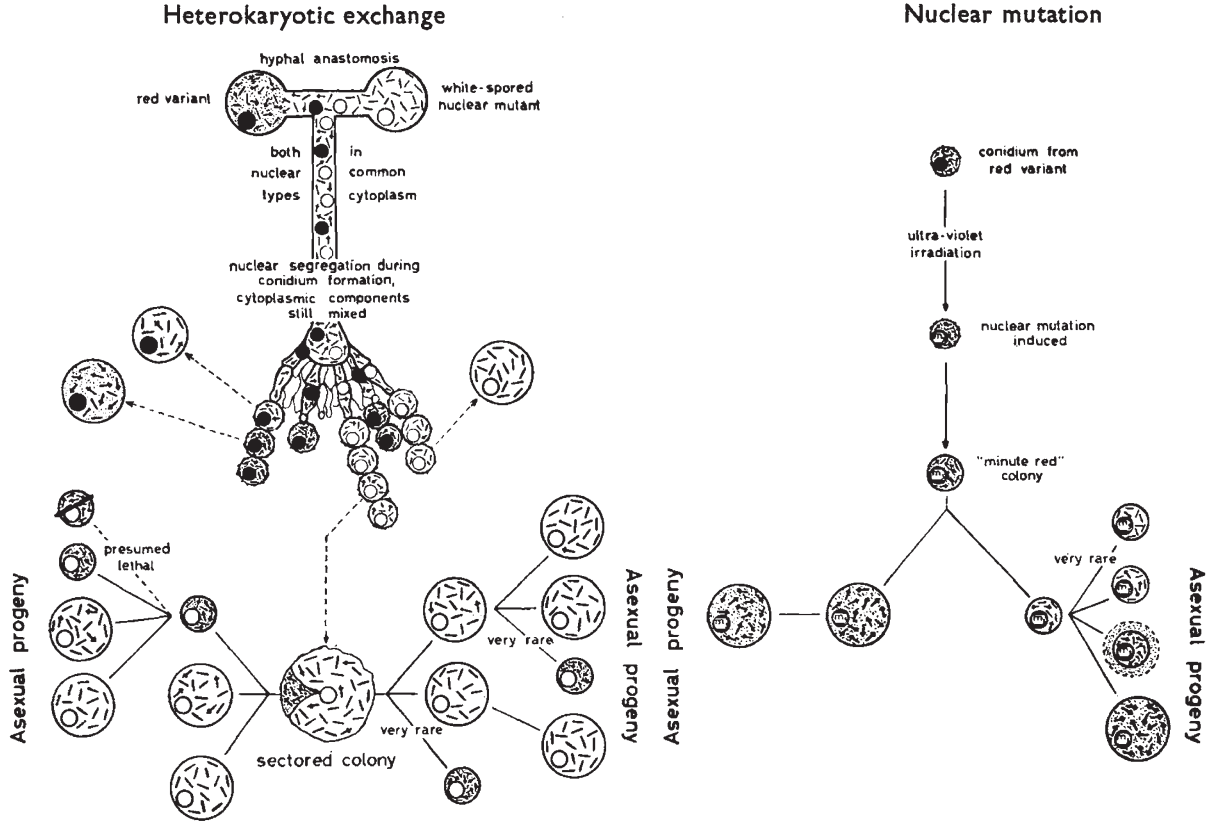
5. BEHAVIOUR OF THE ' RED ' VARIANT WITH MUTANT NUCLEI

We have shown that the equilibrium between the mutant '*rho*' and normal '*Rho*' cytoplasmic homologues can be altered, in favour of either type of homologue, by associating the heteroplasmon '*rho*,*Rho*' with various mutant nuclei. This was accomplished either by introducing nuclear gene mutations directly into the ' red ' variant by U.V. irradiation, or indirectly by heterokaryotic exchange.

The process of heterokaryotic exchange between prototrophic parents is illustrated schematically in text-fig. 5. Following the fusion of hyphæ of the ' red ' variant, W_3'*rho*>*Rho*' and a normal strain carrying nuclear gene mutations, one of which affects spore colour (*e.g.* w_3), cytoplasms and nuclei of the two parental strains become mixed in the heterokaryotic hypha so formed. If we assume that the '*rho*' and '*Rho*' homologues and the two parental nuclear types are distributed at random throughout the mycelium that develops from the heterokaryotic hypha, some of the conidiophores produced by the heterokaryon must contain both types of cytoplasmic homologue and both types of nucleus; that is, '*rho*', '*Rho*', W_3 and w_3. During the formation of asexual spores a single nucleus and 6 cytoplasmic homologues will be cut off into each spore. Therefore, while the two nuclear types segregate out the cytoplasmic homologues can be reassorted so that in the spores produced by a heterokaryon either type of nucleus can be associated with '*rho*' homologues. The new combinations of nucleus and cytoplasm can be recovered by sampling conidial heads in which both parental nuclear types are known to be present; such conidial heads can be distinguished easily since they have chains of both green and white spores.

The presence of '*rho*' homologues in spores carrying the mutant nuclei can be recognised only if those spores are viable and produce colonies that are (1) similar in phenotype and behaviour to the ' red ' variant, W_3'*rho*>*Rho*', or (2) quite different from the parental strain carrying the mutant nuclei. Only one of the heterokaryons tested, however, involving the ' red ' strain W_3ad^-'*rho*>*Rho*' and a normal lysine-requiring strain, W_3lys^-'*Rho*', consistently gave only the two parental strains in its asexual progenies (Arlett, 1960). In the other examples of heterokaryotic exchange, the '*rho*' homologues were associated with nuclei carrying the mutations w_3, w_3co, w_3df and w_3rb^- and progeny were recovered that carried the mutant nuclei but were different from the parental strains.

When nuclear gene mutations are introduced directly into conidia



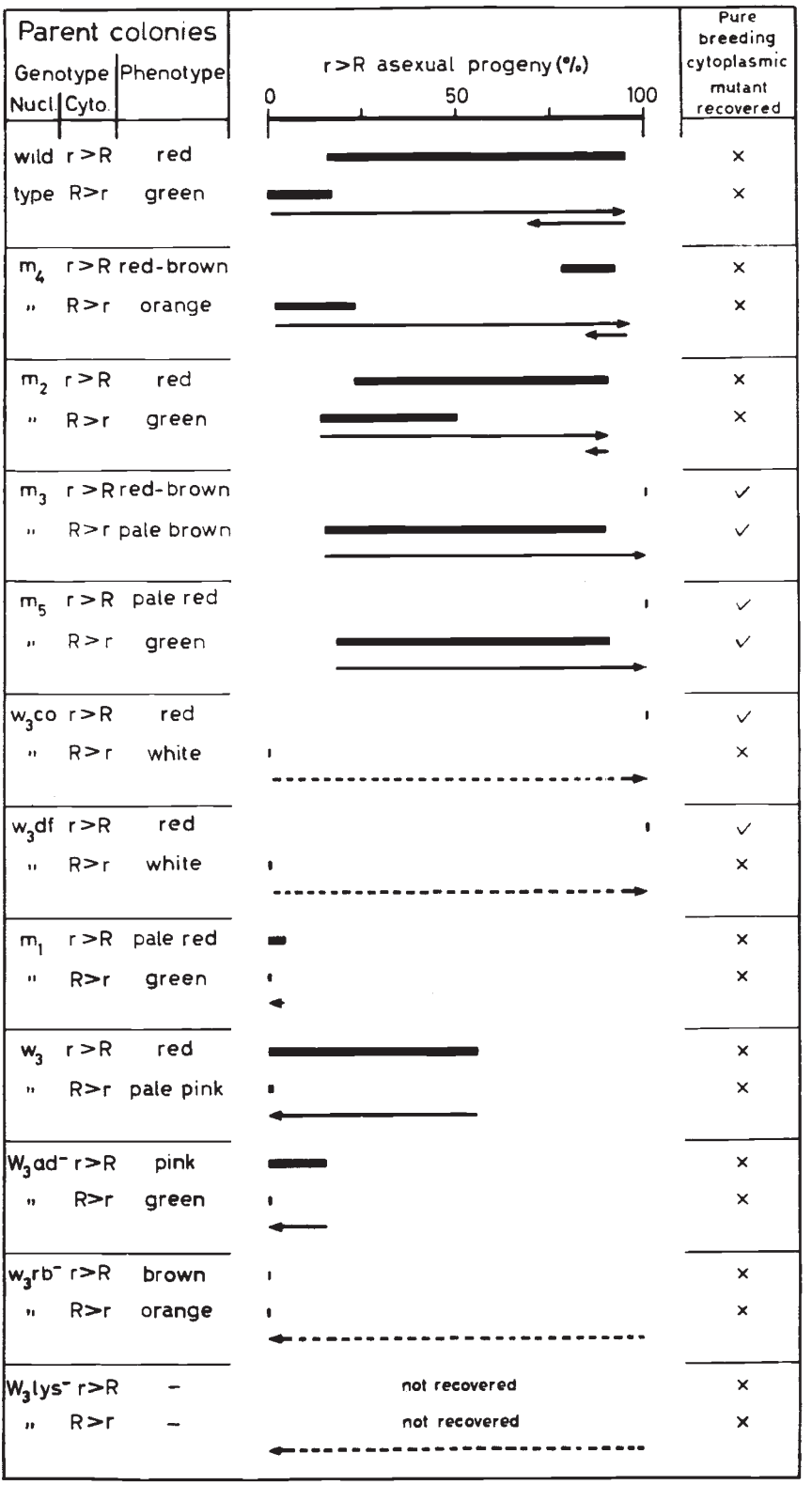
TEXT-FIG. 5.—A schematic representation of the effects of associating the mutant cytoplasm of the 'red' variant with mutant nuclei by heterokaryotic exchange and by direct mutation. Wildtype nuclei, W_3, are shown as solid black circles and w_3 nuclei as open circles. The other mutant nucleus shown carries the mutation m_3. Mutant '*rho*' and normal '*Rho*' cytoplasmic homologues are shown as crossed and uncrossed rods, respectively. The small rough-walled circles denote conidia. The smooth-walled circles denote colonies; relative sizes of the different colonies are shown but they are not to scale. Colonies with a red phenotype ('*rho*>*Rho*' or '*rho*') and also those conidia that give rise to red colonies are stippled. Broken arrows point to the type of colony expected to be formed by the appropriate conidium. Homoplasmic mutant colonies, w_3'*rho*', are not recovered and these are struck out. The m_3W_3'*rho*>*Rho*' colony, only half of which is stippled, is presumed to break down so quickly that it would have the phenotype and properties of a homoplasmic mutant colony, m_3W_3'*rho*'. The two nuclei depicted here are only examples; other nuclei have different effects on the 'red' variant. The type of behaviour of the U.V. induced mutant can also be obtained with other nuclei.

of the ' red ' variant, the heteroplasmon '*rho*,*Rho*' is associated immediately with a single mutant nucleus (text-fig. 5). These new combinations of mutant cytoplasm and mutant nucleus will be recovered only if the spores in which they occur are viable and produce colonies that differ from the ' red ' variant in phenotype or behaviour. By exposing conidia of the ' red ' variant to U.V. irradiation, '*rho*' homologues were associated with the nuclei m_1W_3, m_2W_3, m_3W_3, m_4W_3, m_5W_3 and W_3ad^-.

Our results show that the replication and expression of either the '*rho*' or '*Rho*' form of the cytoplasmic determinant may be favoured depending on the nuclear genes with which it is associated (text-fig. 6). With the wildtype nucleus, W_3, the '*rho*' and '*Rho*' homologues are in equilibrium and although the mutant '*rho*' has the superior rate of multiplication and is dominant in action to '*Rho*', it is reliant on its normal, '*Rho*' partners for its survival. Consequently, homoplasmic mutant colonies are not recovered and the most stable form of colony gives about 70 per cent. red, W_3'*rho*>*Rho*' segregants in its asexual progenies. By introducing the m_2W_3 or m_4W_3 nuclei, the '*rho*' : '*Rho*' equilibrium is altered slightly in favour of the '*rho*' homologues thereby increasing the rate at which the '*Rho*>*rho*' colonies break down to produce red, '*rho*>*Rho*' colonies. The homoplasmic mutant condition is still lethal, however, and the most stable form of colony gives about 90 per cent. red segregants.

Each of the mutant nuclei m_3W_3, m_5W_3, w_3co and w_3df favours the '*rho*' homologues to the exclusion of their normal, '*Rho*' partners. Heteroplasmic '*rho*>*Rho*' and '*Rho*>*rho*' colonies are very unstable vegetatively and they break down very rapidly to form pure breeding homoplasmic mutant colonies, m_3W_3'*rho*', m_5W_3'*rho*', w_3co'*rho*' and w_3df'*rho*'. The most stable form of colony gives 100 per cent. red asexual offspring (*cf.* colonies carrying the nuclei W_3, m_2W_3, or m_4W_3). We suggested earlier that the absence of true breeding red colonies among the offspring of the ' red ' variant W_3'*rho*>*Rho*' was due to the lethality of homoplasmic '*rho*' spores or, alternatively, to the continual backmutation of '*rho*' homologues in the hyphæ. Since heteroplasmic colonies carrying the m_3W_3, m_5W_3, w_3co or w_3df nuclei give rise to true breeding red progeny, the introduction of these mutant nuclei into the ' red ' variant must have (1) made the homoplasmic '*rho*' spores viable, or (2) stopped the backmutation of the '*rho*' homologues, or (3) put the backmutated '*rho*' (*i.e.* '*Rho*') homologues at such a disadvantage in action and replication that their presence cannot be detected.

Only two of the prototrophic mutant nuclei tested are incompatible with the mutant cytoplasm. In association with the w_3 or m_1W_3 nuclei the '*rho*' homologues are extremely difficult to maintain; they were lost from colonies with the m_1W_3 nucleus after only two asexual generations. The '*rho*' : '*Rho*' balance is affected most adversely, however, by nuclei carrying mutations for biochemical requirements. Indeed, the


Parent colonies
Genotype
Nucl.
Cyto.
Phenotype
r>R asexual progeny (%)
0
50
100
Pure breeding cytoplasmic mutant recovered
wild type
r>R red
R>r green
m4
r>R red-brown
" R>r orange
m2
r>R red
" R>r green
m3
r>R red-brown
" R>r pale brown
m5
r>R pale red
" R>r green
w3co
r>R red
" R>r white
w3df
r>R red
" R>r white
m1
r>R pale red
" R>r green
w3
r>R red
" R>r pale pink
W3ad⁻
r>R pink
" R>r green
w3rb⁻
r>R brown
" R>r orange
W3lys⁻
r>R –
" R>r –
not recovered
not recovered
×
✓

'*rho*' homologues could not survive with any of the three nuclei used, w_3rb^-, W_3ad^- and W_3lys^-.

There is, therefore, a very strong interaction between the mutant cytoplasmic determinant '*rho*' and the nucleus with which it is associated. Whether the mutant '*rho*' or normal '*Rho*' homologues are at an advantage in replication and expression depends on the associated nucleus and this can be affected by single major nuclear gene differences. However, is this phenomenon restricted to the ' red ' variant or does it occur in other strains? Can the sorts of nucleo-cytoplasmic interaction described here be obtained with the cytoplasm of any strain or are they dependent on specific determinants in the cytoplasm of Birmingham isolate 1? Only limited information on this is available (Roper, 1958; Srb, 1958; Mahoney and Wilkie, 1962) but evidence from preliminary studies of other presumptive extrachromosomal mutants of *A. nidulans* examined in Birmingham favour the view that the behaviour of the ' red ' variant is unique in several respects. These other mutants have many properties which are similar or identical to those of the ' red ' variant but they have different extrachromosomal changes which are affected in their own particular way by nuclear gene mutations. This is apparent from the behaviour of two persistently segregating mutants of *A. nidulans*, the acriflavine-induced ' minute ' variant (Arlett, 1960; Faulkner and Arlett, 1964) and the spontaneously occurring ' purple ' cytoplasmic variant (Grindle, 1963).

The ' red ' variant carrying the wildtype nucleus, W_3 is phenotypically stable and it can be maintained easily by cloning. The ' minute ' variant, derived from the same wild isolate as that from which the ' red ' variant was obtained, is very unstable with the W_3 nucleus and clones break down rapidly to produce true breeding wildtype colonies. Colonies of the ' red ' variant W_3'*rho*>*Rho*' are slightly larger than wildtype colonies but those of the ' minute '

TEXT-FIG. 6.—The effects of mutant nuclei on the properties of the ' red ' variant. For each class of parental colony the distribution of percentage red, '*rho*>*Rho*' segregants, determined from numerous asexual progenies, is shown by a solid thick black line and the presence or absence of true breeding, homoplasmic mutant colonies is noted. (*E.g.* colonies of the ' red ' variant with wildtype nucleus, W_3'*rho*>*Rho*' give as little as 15 per cent. and as many as 95 per cent. W_3'*rho*>*Rho*' segregants but no homoplasmic mutants, W_3'*rho*', are recovered; m_3W_3'*Rho*>*rho*' colonies give 15-85 per cent. m_3W_3'*rho*>*Rho*' segregants and homoplasmic mutants, m_3W_3'*rho*', are recovered.) Arrows denote the observed (solid lines) and presumed (broken lines) changes in the percentages of '*rho*>*Rho*' segregants obtained during the growth of both '*rho*>*Rho*' and '*Rho*>*rho*' colonies with the appropriate nucleus (*e.g.* the percentage of m_4W_3'*rho*>*Rho*' segregants produced by an actively growing m_4W_3'*rho*>*Rho*' or m_4W_3'*Rho*>*rho*' colony rises to about 95 per cent. then falls to about 80 per cent. where, presumably, the '*rho*' and '*Rho*' homologues are in balanced equilibrium; after introducing the w_3co nuclei into the heteroplasmon '*rho*,*Rho*' only pure breeding red, w_3co'*rho*' colonies were eventually recovered so presumably the '*rho*' and '*Rho*' homologues cannot coexist in the presence of the w_3co nucleus and the '*Rho*' homologues are lost. With the m_1W_3 and w_3rb^- nuclei, for example, the '*rho*' homologues are lost.)

r = '*rho*'; R = '*Rho*'.

variant are much smaller than the wild strain. The ' purple ' variant, derived from IMI strain 16643, is phenotypically more stable than the ' minute ' variant but less stable than the ' red ' variant (plate III, figs. 3 and 4). Thus, the wildtype nuclei favour the replication and expression of the mutant extrachromosomal homologues (in the ' red ' variant) or the normal homologues (in the ' minute ' and ' purple ' variants). These three variants are alike, however, in that not a single pure breeding mutant colony carrying wildtype nuclei has been obtained from any of them.

By introducing a mutation for white spores into the ' red ' variant the '*rho*:*Rho*' balance is shifted substantially in favour of the '*Rho*' homologues and the mutant '*rho*' homologues are difficult to maintain. In contrast, mutations for white or yellow spores in the ' purple ' variant, *WY*'*pi*>*Pi*' have very little effect on the expression or behaviour of the mutant '*pi*' cytoplasmic homologues. Indeed, when '*pi*' homologues from *wY*'*pi*>*Pi*' and *Wy*'*pi*>*Pi*' colonies are reassociated with the wildtype nucleus *WY* by heterokaryotic exchange, purple colonies identical in phenotype and behaviour to the original ' purple ' variant are recovered. It seems, therefore, that the association of the heteroplasmon '*pi*,*Pi*' with mutant nuclei for a period of three months did not affect irreversibly the properties and functions of the '*pi*' homologues.

When viable ascospores have been obtained from colonies carrying '*rho*' homologues, they have given rise to exclusively normal offspring. Similarly, ascospores of the ' minute ' variant give only true breeding wildtype colonies. Thus, the mutant form of some extrachromosomal elements cannot be recovered from the meiotic products (Mather and Jinks, 1958). Perithecia of the ' purple ' variant *WY*'*pi*>*Pi*' and the mutant forms *wY*'*pi*>*Pi*' and *Wy*'*pi*>*Pi*', however, give high percentages of mutant '*pi*>*Pi*' offspring in non-Mendelian proportions. Clearly, the '*pi*' homologues, unlike the '*rho*' homologues, pass through the sexual stage although they do so with irregular frequencies.

Despite the many differences in properties and breeding behaviour of the ' red ' and ' purple ' variants, both the '*rho*' and '*pi*' cytoplasmic determinants are difficult to maintain in the presence of nuclei carrying mutations for biochemical requirements. In view of this, it is possible that a more intensive investigation of the effects of a wide range of biochemical markers on cytoplasmic mutants might lead to a better understanding of the mode of action of cytoplasmic hereditary determinants, of the metabolic processes of the cell in which they are involved and of the interrelationships of nucleus and cytoplasm in these processes.

6. SUMMARY

A U.V.-induced mutant of *Aspergillus nidulans*, the ' red ' variant, is presumed to be an heteroplasmon containing mutant '*rho*' and normal '*Rho*' forms of an hereditary cytoplasmic determinant. In the ' red ' variant with wildtype nuclei '*rho*' is dominant in its action to

'*Rho*' but it is dependent on '*Rho*' for its survival. By introducing nuclei carrying various mutant genes into this variant, however, the replication and expression of either '*rho*' or '*Rho*' can be favoured and, consequently, the phenotype and breeding behaviour of the ' red ' variant can be altered substantially.

The effects on the ' red ' variant of 11 different mutant genes were studied. Two of these mutant genes modify only slightly the appearance and properties of the ' red ' variant but each of the remaining 9 mutations has a considerable effect on both its phenotype and breeding behaviour. In the presence of 4 of the mutant genes the '*rho*' homologues are no longer dependent on '*Rho*' homologues for their survival with the result that pure breeding mutant colonies can be recovered. When associated with any of the other 5 mutant genes, the '*rho*' homologues are either difficult to maintain or they cannot survive.

Properties of the ' red ' variant are compared with those of two other extrachromosomal mutants of *A. nidulans*. The reasons for the proposed cytoplasmic basis for the ' red ' variant are discussed.

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