With 2 text-figures
Printed in Great Britain

Dilution of gene products in the cytoplasm of heterokaryons in *Coprinus lagopus*

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(*Received* 3 *August* 1966)

1. INTRODUCTION

The analytical potentialities of comparing gene complementation and dominance in a diploid where the genes are in the same nucleus and in a heterokaryon where the genes are separated in different nuclei has been considered and explored by Pontecorvo (1952, 1963). Genes whose action is confined to or concentrated in the nucleus should complement in a diploid but should not complement or only partially complement in a heterokaryon. An allele which is fully recessive in a diploid should be semi-dominant when it is separated from the dominant allele in another nucleus of a heterokaryon. Examples of both types of effect have been found in *Aspergillus nidulans* (Pontecorvo, 1952, 1963; Roberts, 1964; Apirion, 1966).

There is, however, some uncertainty about the interpretation of differences between heterozygous diploids and heterokaryons (Pontecorvo, 1963). At the two extremes, either the genes showing such differences have their activity completely localized in the nucleus, or the primary product of the gene passes from the nucleus to the cytoplasm, as in the majority of genes studied, and the difference is caused by the distance apart of the genes resulting in a dilution of gene products in the cytoplasm. In the example of complementation the two gene products would be completely overlapping in space in the cytoplasm of a diploid but would only rarely overlap except at high dilution in a heterokaryon. The same process would allow a recessive gene to come to expression and hence produce a shift in dominance.

A method of discriminating between the two interpretations, *intra-nuclear activity* or *cytoplasmic dilution*, is possible in the basidiomycete *Coprinus lagopus* in which there are haploids and diploids with one nucleus per cell, dikaryons with two nuclei per cell stringently maintained by a synchronous division, and a heterokaryon with the two types of nuclei irregularly distributed throughout the mycelium. There are pores in the cross walls between cells through which molecules if not organelles can pass.

The present study compares complementation of the alleles of auxotrophic mutants and the recessiveness of a suppressor gene in diploids, dikaryons and heterokaryons.

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2. MATERIALS AND METHODS

(i) *Mutants*

The alleles *ad*-8, *me*-5, *me*-1, *adhi*-1, *adhi*-2 and *chol*-1 impose growth requirements for adenine, methionine, adenine + histidine and choline respectively. *Su*-3 denotes a recessive suppressor of *me*-1. Mutants are linked as follows:

ad-8 *me*-5; *adhi*-1 *adhi*-2 *chol*-1; *me*-1 *su*-3

(ii) *Culture*

The complete and minimal media were those used by Lewis (1961) with the addition of 0·5 g. magnesium sulphate per litre. Minimal medium supplemented with methionine contained 100 mg. DL-methionine per litre. Incubation of cultures was at 37°C.

(iii) *Growth tests*

Growth was estimated by measuring colony radius over a period of 5 days. Data given for each culture represents the mean readings from three colonies.

(iv) *Synthesis of dikaryons, heterokaryons and diploids*

The formation of a dikaryon in *C. lagopus* is determined by two genes *A* and *B* each with a number of alleles. A dikaryon is formed when two strains having different alleles at both loci are placed side by side on an agar plate. It is easily identified by the presence of clamp connexions at all cross walls. If alleles of one or both genes are common to mated cultures the dikaryon cannot be formed. Hyphal anastomosis produces a heterokaryon. Heterokaryons used in the present work were homogenic for the *A* gene and heterogenic for the *B* gene (common *A* heterokaryons). Nuclear migration occurs in these heterokaryons (Swiezynski & Day, 1960) but due to the incompatibility relationship at the *A* locus, division of the different nuclei is not synchronized. Diploids were synthesized from common *A* heterokaryons using the method described by Casselton (1965).

3. RESULTS

(i) *Complementation*

A comparison of growth on complete and minimal medium has been used to measure the efficiency of complementation in diploids, dikaryons and heterokaryons heterogenic for four auxotrophic mutants. Two arrangements of genes have been tested. In the first, the linked mutants *ad*-8, *me*-5 and *adhi*-1, *chol*-1 and their wild-type (+) alleles were in *cis* arrangement and in the second, the same genes were in *trans* arrangement as shown in Table 1. The + alleles in both sets of heterogenotes

were distributed equally between the component nuclei of the dikaryons and heterokaryons.

Growth on complete medium is considered to represent maximal growth. On minimal medium growth depends on complementation. Growth rates obtained in the two tests are given in Table 1.

Table 1. *Radial growth rates of cultures heterogenic for four auxotrophic mutants*

Genotype	Combination	Complete medium (mm./day)	Minimal medium (mm./day)	Reduction in growth rate on minimal medium (%)
(a) Mutants in *cis*				
me-5 *ad*-8 / + +	Dikaryon	15·00	15·00	0·0
+ + / *adhi*-1 *chol*-1	Diploid	7·75	7·25	6·5
	Heterokaryon	8·50	4·50	41·2
(b) Mutants in *trans*				
me-5 + / + *ad*-8	Dikaryon	15·25	14·75	3·3
adhi-1 + / + *chol*-1	Diploid	7·25	6·75	6·9
	Heterokaryon	6·50	2·75	57·5

Agreement between the results of both tests was good showing that gene arrangement in the nuclei has no effect on cytoplasmic interaction of the products. As a result of efficient complementation almost maximal growth was produced on minimal medium by diploids and dikaryons. Thus separation of genes in different nuclei which are close together in the same cell does not affect their ability to interact. On the other hand, there was a marked difference between the growth rates of heterokaryons on the two types of media. Growth rate on minimal medium was approximately half the growth rate on complete medium. This shows that genes complement less efficiently in heterokaryons where the cytoplasmic distance between nuclei is not restricted.

Whilst radial growth is not a measure of total growth it will be seen from Figs. 1 and 2 that growth of all cultures was linear, indicating that this method of assessing complementation is valid.

(ii) *Dominance*

The complementation tests measure the degree of interaction or overlap of dominant gene products, but do not directly test whether the recessive genes can be expressed. If the dilution hypothesis is correct, the products of a dominant allele in a heterokaryon should be diluted sufficiently to allow the expression of a recessive allele. This is prevented in a dikaryon and a diploid because of a strictly controlled gene distribution. Dilution would cause a clear phenotypic difference between heterokaryons and diploids (or dikaryons) where growth is dependent on the expression of a recessive allele, for example a recessive suppressor.

Suppressors of *me*-1 are recessive to their wild-type alleles in both dikaryons and diploids (Lewis, 1961; Casselton, 1965). The recessiveness of one of these suppressor mutants has been tested in a heterokaryon.

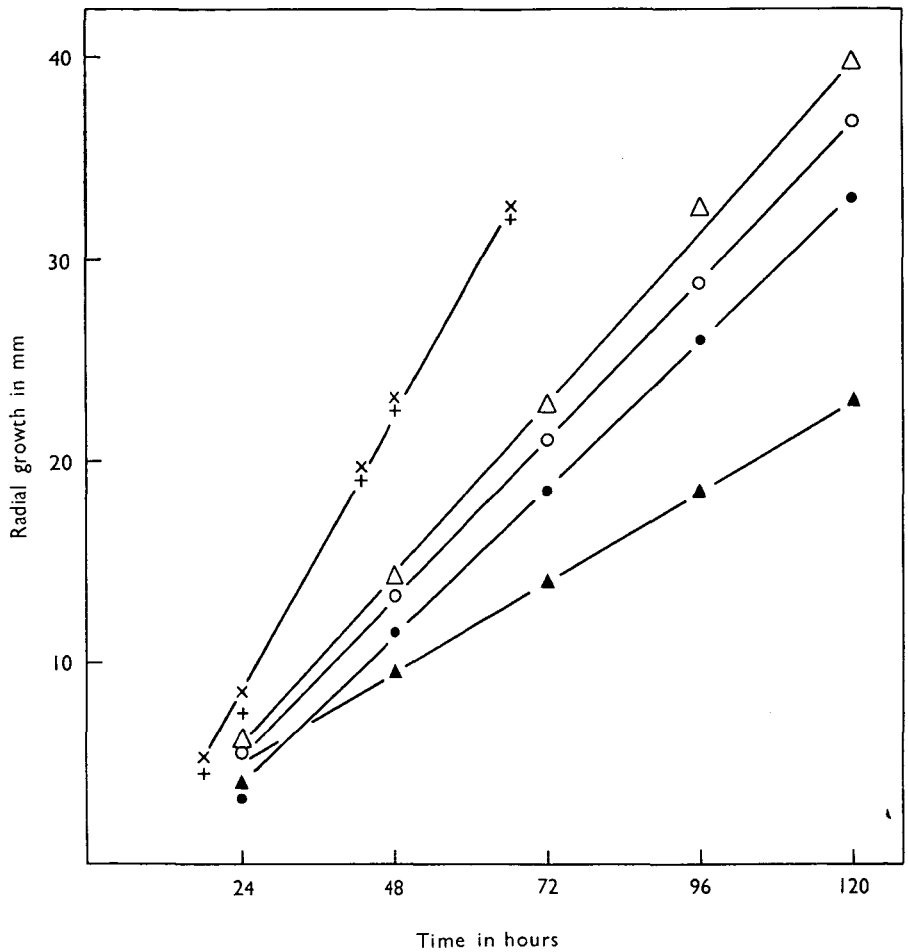

Fig. 1. Growth of cultures heterogenic for linked auxotrophic mutants in *cis* arrangement.
× dikaryon; ○ diploid; △ heterokaryon on complete medium
\+ dikaryon; ● diploid; ▲ heterokaryon on minimal medium

Heterokaryons, heterogenic and homogenic for *su*-3 were tested for growth on minimal medium and minimal medium supplemented with methionine together with comparable diploids and dikaryons. Non-allelic forcing markers in the heterokaryons and diploids, *adhi*-1 and *adhi*-2, impose identical growth requirements and therefore reduce possible effects of differential growth requirement. (Dikaryons do not contain the *adhi*-2 mutant but this would not affect growth response to absence or presence of methionine.) Heterokaryons grew slowly even on medium

with methionine therefore the results given in Table 2 are expressed as total radial growth in 5 days.

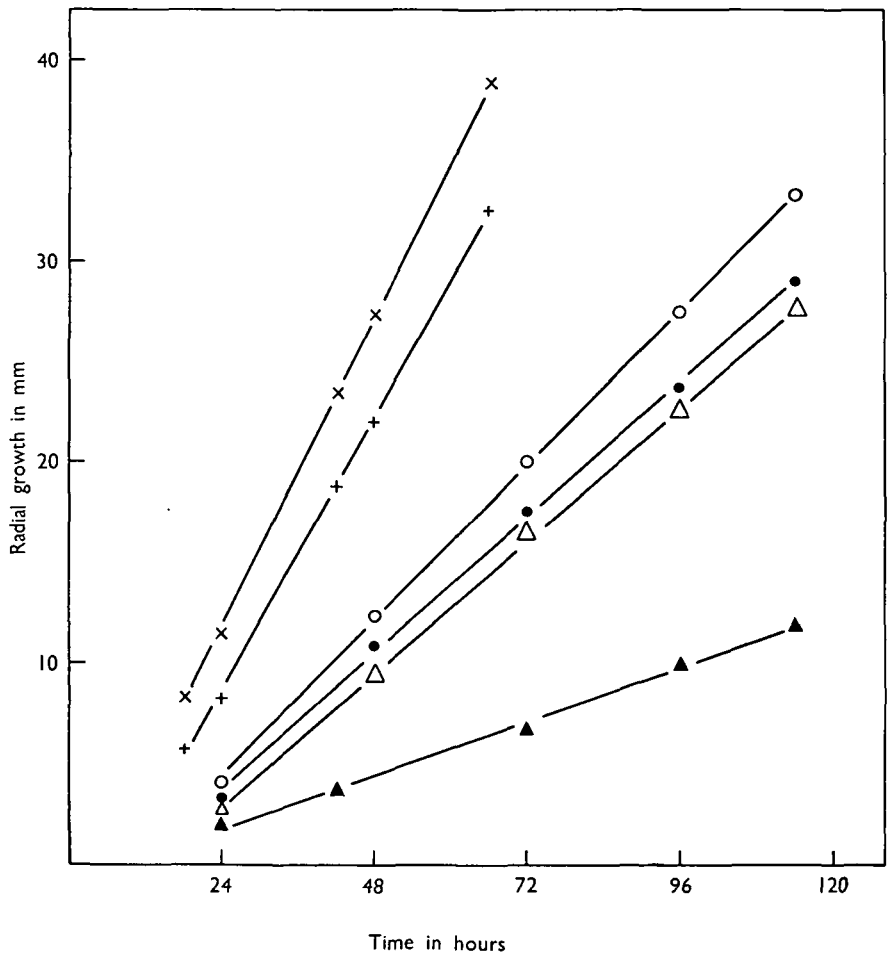

Fig. 2. Growth of cultures heterogenic for linked auxotrophic mutants in *trans* arrangement.
× dikaryon; ○ diploid; △ heterokaryon on complete medium
\+ dikaryon; ● diploid; ▲ heterokaryon on minimal medium

The reason for a pronounced reduction in growth on minimal medium of homogenic *su*-3 cultures is not known. The effect in the dikaryon, which characteristically grows faster than a monokaryon, is to reduce growth to the level of a monokaryon.

Whereas the diploid and dikaryon heterogenic for *su*-3/+ were unable to grow on minimal medium, confirming that *su*-3 is fully recessive, the comparable heterokaryon produced one-third the amount of growth on minimal that it produced on supplemented medium. Moreover, this was equivalent to growth of the homogenic *su*-3 heterokaryon on the same medium. The recessive *su*-3 allele in one nucleus of

a heterokaryon can therefore be expressed in the presence of its dominant + allele in another nucleus. This constitutes dilution of the action of the dominant + allele.

(iii) *Nuclear balance*

Two factors could effect dilution of gene products in heterokaryons: (1) unequal numbers of component nuclei, and (2) equal numbers of component nuclei irregularly distributed in the mycelium such that certain regions contain predominantly one nuclear type.

Table 2. *Radial growth of* 5*-day-old cultures homogenic for* me-1 *and* (*a*) *heterogenic*, (*b*) *homogenic for a recessive suppressor of* me-1. *Dikaryons do not contain the* adhi-2 *mutant*

Genotype	Combination	Minimal medium + methionine (growth in mm.)	Minimal medium (growth in mm.)
(a) Heterogenic suppressor			
adhi-1 + / *me*-1 *su*-3	Dikaryon	59·00	0·00
+ *adhi*-2 / *me*-1 +	Diploid	34·00	2·25
	Heterokaryon	15·00	5·50
(b) Homogenic suppressor			
adhi-1 + / *me*-1 *su*-3	Dikaryon	64·00	30·50
+ *adhi*-2 / *me*-1 *su*-3	Diploid	33·75	24·00
	Heterokaryon	7·75	5·75

It was possible to discriminate between these alternatives by estimating the proportions of the two nuclear types in samples of uninucleate oidia (asexual spores) of relevant heterokaryons. A greater proportion of one oidial type would reflect selection for a particular nucleus in the mycelium and hence unequal numbers of the component nuclei.

Table 3. *Effect of growth medium on the proportion of oidial types in the heterokaryon* (adhi-1 + me-1 su-3/ + adhi-2 me -1 +) *heterogenic for a recessive suppressor*

Growth medium	% *su*-3	% +
Minimal	30·5	69·5 observed
	47·0	53·0 corrected
Minimal + methionine	23·8	76·2 observed
	38·5	61·5 corrected

A heterogenic heterokaryon (*su*-3/ +) was grown on minimal medium and minimal medium supplemented with methionine. If nuclear selection occurs it will be in favour of *su*-3 on minimal medium and in favour of + when methionine is supplied. The proportion of oidia having *su*-3 or + was estimated in each case by sowing oidia

at low density on complete medium and testing all colonies produced (approximately 300).

The percentages of oidial types are given in Table 3. The parental types showed differential viability and a correction has been applied. It will be seen that the proportions of the two oidial types were equivalent when the heterokaryon was grown on minimal medium. The methionine supplement induced only a small shift in favour of the + type oidia. These results indicate that irregular distribution of nuclei is the most likely cause of dilution of gene products in heterokaryons.

4. DISCUSSION

The diploid heterogenic for the genes *me*-5, *ad*-8, *adhi*-1 and *chol*-1, and the dikaryon with the + (active) alleles distributed equally between the two haploid nuclei grow equally well on minimal and complete media. The comparable heterokaryon grows on minimal at only half complete medium rate. In terms of gene complementation the + alleles fully complement in the diploid and dikaryon but only partially complement in the heterokaryon. The diploid and dikaryon homogenic for *me*-1 and heterogenic for *su*-3, which suppresses the effect of *me*-1, is unable to grow without methionine but the comparable heterokaryon grows about one-third as well as on medium with methionine. This means that *su*-3 is fully recessive in the diploid and dikaryon but semi-dominant in the heterokaryon.

The similarity of diploid and dikaryon and the different phenotype of the heterokaryon must be caused by a dilution of gene products in the cytoplasm in the heterokaryon and not due to intra-nuclear gene action. This is to be expected with the genes, *me*-5, *ad*-8, *adhi*-1 and *chol*-1 which are structural genes for enzymes synthesized in the cytoplasm and which are presumably freely diffusable. The biochemical action of the *su*-3 gene is not known but the differential effect in the heterokaryon indicates that it operates in the cytoplasm. Similar differences in *Aspergillus nidulans* between diploids and heterokaryons have been found in the interaction of auxotrophic mutants (Pontecorvo, 1952) and in the methionine gene suppressors (Luig, 1962; Ayling, 1965). In these cases the dikaryon test could not be applied.

The dilution effect in the heterokaryon could be caused by an unbalanced nuclear ratio or by an irregular distribution of the two types of nuclei which are in approximately equal numbers. Although selection can alter nuclear ratios in wild heterokaryons of *Penicillium* (Jinks, 1952), and in auxotrophically balanced heterokaryons in *A. nidulans* (Warr & Roper, 1965), there is evidence that the nuclear ratio in the heterokaryons of *C. lagopus* is not significantly different from equality. It is therefore more probable that the dilution effect is caused by an irregular distribution of the two types of nuclei in different hyphae. This has also been found cytologically in *Aspergillus* by Clutterbuck & Roper (1966).

Regulator genes (Pontecorvo, 1963), structural genes for ribosomal protein and possibly for mitochondrial enzymes (Apirion, 1966) are genes that might have

intra-nuclear activity. Two examples in *A. nidulans* show a complete lack of complementation in heterokaryons and not a partial lack as in the examples discussed. These are acetate mutants (Apirion, 1966) and sorbitol mutants (Roberts, 1964). These may be true examples of genes with intra-nuclear activity. Similar types of mutants in *Coprinus* where the critical dikaryon-diploid comparison can be made would be of interest.

SUMMARY

(*a*) *Coprinus lagopus* has in nature both a monokaryon with one haploid nucleus per cell and a dikaryon with two haploid nuclei per cell. It also has in experiment both a heterokaryon with two types of nuclei distributed irregularly in the mycelium and a diploid monokaryon with one diploid nucleus per cell.

(*b*) Two pairs of linked recessive auxotrophic mutants, *me*-5 *ad*-8 and *adhis*-1 *chol*-1 have been combined in two different sets of multiple heterogenotes so that in the dikaryon and heterokaryon the + (active) alleles of the four genes are distributed equally in the two component nuclei. In one combination the linked genes are in *trans* and in the other they are in *cis* arrangement in diploid, dikaryon and heterokaryon.

(*c*) Complementation between the + genes, as measured by growth on minimal and complete media, was complete in the diploid and dikaryon but was only partial in the heterokaryon.

(*d*) A similar comparison of a recessive suppressor of the mutant *me*-1 revealed that the recessive gene was not expressed and therefore was fully recessive in the diploid and dikaryon but was only partially recessive in the heterokaryon.

(*e*) The exact similarity of complementation of the structural genes of the auxotrophic mutants and the recessiveness of the *me*-1 suppressor in diploids and dikaryons exclude a localization of gene interaction in the nucleus. In a heterokaryon cytoplasmic dilution of the product of a dominant gene can occur and has the effect of producing a phenotypic difference between heterokaryons and diploids.

The award of a Senior Studentship by the Royal Commission for the Exhibition of 1851 to L. A. C. is very gratefully acknowledged.

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